

Allele Frequency of *SLC22A1* Met420del Metformin Main Transporter Encoding Gene among Javanese-Indonesian Population

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Abstract

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BACKGROUND: Genetic variation in the genes that encode metformin transporters has been proven to cause pharmacokinetic variability and various glycemic response to metformin. Organic Cation Transporter (OCT) 1 protein encoded by the *SLC22A1* gene is primarily responsible for the process of metformin influx to the hepatocytes as the target of antihyperglycemic action as well as metformin elimination through the renal. This study aimed to determine the allele frequency distribution of the *SLC22A1* Met420del gene in OCT1 among the Javanese population, the largest ethnic group in Indonesia with T2DM.

METHODS: The research involved 100 adult patients from 9 healthcare facilities in Yogyakarta Province. The PCR-RFLP method was employed as a genotype analysis to detect polymorphism using 5'-AGGTTACGGACTCTGTGCT-3' forward primer and 5'-AAGCTGGAGTGTGCGATCT-3' reverse primer.

RESULTS: No AA variant (wild type) type was found in the *SLC22A1* Met420del gene, and only 4% of the subjects had Aa heterozygote type. The allele frequencies of A and a were 2.0% and 98.0% in all subjects, respectively.

CONCLUSION: The allele frequencies in the Javanese-Indonesian population were almost the same as those in the studies involving Japanese, Chinese-Han, and Asian-American populations. This study recommends further research on the correlation between the influence of methionine deletion at codon 420 on the variability of pharmacokinetic profiles and the glycemic response to metformin as well as the incidence of gastrointestinal intolerance due to metformin administration.

Introduction

The prevalence of diabetes mellitus in Indonesia continues to increase, reaching 2.1% compared to the last 6 years [1]. Meanwhile, the International Diabetic Federation estimates that DM prevalence in Indonesia will reach 14.1 million in 2035 [2]. Therefore, good management of glycemic control is required to prevent as well as reduce morbidity and mortality due to diabetes mellitus [3].

From 2013 to 2017, metformin remained in the list of Indonesia's National Formulary as one of the oral antidiabetic drugs available up to primary

healthcare facilities. The ability of metformin to reduce HbA1c levels in the range of 1.0-2.0% and the low hypoglycemic effects are among its advantages over other oral antidiabetic drugs. However, the glycemic response to metformin use is varied as 35 to 40% of patients have yet to reach the target of fasting blood glucose levels [4]. Variability in patients' response to antidiabetic drugs can result from genome variations that lead to variations in disposition and response to antidiabetic drugs including metformin [5].

Our previous study found a variety in the minimum as well as maximum metformin steady-state concentrations, reaching > 100-fold and 15-fold respectively, in 82 T2DM patients who received metformin at the similar dosage (1000 mg/day) [6]. As

a drug with renal excretion as the primary route of elimination, metformin has > 0.6 rGC (genetic component), indicating that variations in steady-state concentration can result from the involvement of genetic factors during the renal clearance of metformin [7].

As a hydrophilic base existing at physiological pH as a cationic species ($> 99.9\%$), the effectiveness of metformin pharmacokinetics depends on the function of the transporters involved [8]. One of the major transporters known to play an important role in metformin pharmacokinetics to date is Organic Cation Transporter 1 (OCT1), a protein mainly expressed in liver sinusoidal cells, renal basolateral membrane [9], and apical membrane of tubule cells [10], which transports metformin to hepatocytes as the target of its antihyperglycemic action as well as plays a part in the elimination and reabsorption in the renal tubules. Variations in *SLC22A1* gene have led to changes in the function of OCT1 protein, which results in varied plasma concentrations of metformin and decreased amount of metformin in the receptors, making the therapeutic response to metformin decline. Such genetic variations can take the form of methionine deletion at codon 420 located in the ninth transmembrane domain of *SLC22A1*, which is the highest functional variant in the gene. Several studies have found that *SLC22A1* gene variants cause variability in both steady-state concentrations of metformin and glycemic response [11], [12], [13], [14]. Also, recent research showed that genetic variations in the gene are related to the level of gastrointestinal intolerance due to the use of metformin [15], [16].

This research is a part of pharmacogenetic studies of metformin use among the Indonesian population suffering from T2DM. Analysis of genetic variants in the target gene that encodes metformin transporters is important to provide information on the profile of genetic variation in Indonesian population which can then be further researched on the implications for the use of metformin as a first-line antidiabetic drug for T2DM and its safety for the gastrointestinal tract. Therefore, this study aims to determine the allele frequency distribution of *SLC22A1* Met420del gene encoding OCT1 among the Javanese population, the largest ethnic group in Indonesia with T2DM.

Methods

Recruitment of Subjects

T2DM patients were recruited from 9 existing healthcare facilities in Yogyakarta Special Province categorised as Javanese based on their three previous generations from Javanese ethnic. Informed consent was obtained from each patient who was

willing to be involved in the study. The research has obtained ethical clearance from the Ethics Commission of the Faculty of Medicine of Gadjah Mada University.

Genotype Analysis of *SLC22A1* Met420del in OCT1

Genotyping at *SLC22A1* Met420del was carried out using PCR followed by Restriction Fragment Length Polymorphism (RFLP). The PCR primer design used the forward primer 5'-AGGTTACGGACTCTGTGCT-3' and the reverse primer 5'-AAGCTGGAGTGTGCGATCT-3'. The PCR conditions for amplification consisted of initial denaturation at 93°C for 3 minutes followed by 35 denaturation cycles at 93°C for 45 seconds, annealing at 58°C for 35 seconds, and extension at 72°C for 35 seconds as well as a final extension at 72°C for 5 minutes. The amplification products (600bp) were then analysed in 1% agarose gel for 30 minutes at 100 Volt followed by restriction digestion using *BspHI*, incubated for ± 12 hours at 37°C. *BspHI* enzyme cut T-CATGA sequence at the 197th base of DNA template. AA genotype was recognised and digested by the enzyme. PCR products with the T-CATTT sequence would not be recognised by the *BspHI* enzyme, leaving the product undigested. The resulted products were then analysed in 1% agarose gel and quantified using floor safe. Digestion of amplification products resulted in 600 bp fragments for AA (wild type) genotype as well as 403 bp and 197 bp fragments for aa (mutant) genotype, and 600 bp, 403 bp, and 197 bp for heterozygotes (Aa).

The results were presented in percentage using the Hardy-Weinberg principle. Referring to the previous research, the A allele (wild type) showed a GAT base deletion on DNA target sequences, and an allele (mutant) indicated GAT base insertion in DNA sequences [13].

$$A \text{ allele} = \frac{(AA \text{ genotype} \times 2) + Aa \text{ genotype}}{2 \times \text{the number of samples}}$$

$$a \text{ allele} = \frac{(aa \text{ genotype} \times 2) + Aa \text{ genotype}}{2 \times \text{the number of samples}}$$

Results

A total of 100 Javanese-Indonesian patients with T2DM were involved for the genotype analysis of the *SLC22A1* Met420del gene in OCT1. Characteristics of the research subjects are described in Table 1.

The patients involved in this study were mainly female (69%) with an average age and BMI of 52.88 ± 8 years old and 25.47 ± 4.5 kg/m², respectively.

Table 1: Characteristics of subjects for the genotype analysis of the *SLC22A1* Met420del gene in OCT1

Patient Characteristic	Male n	Female n	p-value
Age (years)			0.22
< 50	7	24	
≥ 50	24	45	
BMI (kg/m ²)			0.54
< 30	28	58	
≥ 30	3	11	
<i>SLC22A1</i> Met420del genotype			*
AA	0	0	
Aa	0	4	
aa	31	65	

Presented only in descriptive statistics

Table 1 shows no differences in patient factors of both age and BMI between male and female patients with T2DM ($P > 0.05$). Also, there was no type of AA variant (wild type) found in the *SLC22A1* Met420del gene, and only 4% of the subjects had the Aa variant. The electrophoretic display of the results of the enzyme digestion for detecting polymorphism in *SLC22A1* Met420del is shown in Figure 1.



Figure 1: Result of analysis of the cutting region in *Bsp*HI restriction enzyme of *Met420del* polymorphism in *SLC22A1* gene; aa homozygous/mutant (lane 7a-11b: 403bp and 197bp), Aa heterozygous variants (lane 6a and 6b: 600bp, 403bp, and 197bp). Note: lane M = marker/ladder 1 kbp; lane 6a and 6b = samples of heterozygote type in; lane 7a-11b = samples of mutant type; lane C = negative control; lane U = undigested sample

Also, to examine the allele frequencies in *SLC22A1* for both male and female patient groups, a descriptive analysis was employed with the results presented in Table 2.

Table 2: Allele frequency in the *SLC22A1* gene and *SLC47A1* gene according to gender

Allele Variant	Male (%)	Female (%)
A allele	0	2.90
a allele	100	97.10

Table 2 shows the highest proportion of allele in OCT1-Met420del of this study, namely mutant allele a (> 95%) in both male and female patients, and even in the male patient group, 100% of them had typed a mutant allele.

Discussions

No AA variant type was found in the *SLC22A1*

Met420del gene in this study. Such non-existence of AA type was similar to the results of studies that involved 116 patients of Japanese, Chinese-Han, and Asian-American populations [17], [18], [19]. Meanwhile, among African-American and European-American populations, each with 200 research subjects, the frequencies of the mutant allele was found to be 2.9% and 18.5%, respectively [19]. In contrast, in studies involving 117 Iranian T2DM patients [20], 232 healthy Caucasian subjects [21], and 103 healthy Caucasian subjects in another study [10], as well as 246 T2DM patients in Latvian population [22] and 361 Danish patients [12], the frequency of wild-type genotype (AA) was higher than that of mutant genetic variant (aa) and heterozygote (Aa). Although infrequently conducted, pharmacogenomics studies that involve Indonesian population tend to find genetic profiles that are similar to those of Southeast Asian populations [23] and other Asian populations such as Chinese population [24] when compared to the genetic profiles of other populations. Different frequencies of Met420del genetic variants in OCT1 were also found in this study. This has certainly reinforced the importance of genetic profiles as a consideration in personal drug selection, effective dosage for a population/human race that is rarely involved in research into the safety and efficacy of novel drugs, such as in Indonesia.

Despite being performed only on experimental animal models, there were no differences in the expression of OCT1 in renal cells based on gender [25], [26]. Therefore, the difference in sex-type proportions in a pharmacogenomics study involving OCT1 transporters can be ignored, or no matching technique is needed in the data analysis for this patient-factor.

Several studies have been conducted to analyse the association of genetic variation in the *SLC22A1* gene with its effects, such as the variability of expression, disposition, and therapeutic response of a drug. A study of liver tissue samples from subjects of the Caucasian population identified the genetic variation as a critical factor of OCT1 hepatic expression [27]. This could lead to changes in the function of OCT1 as a protein transporter for several drugs that have liver as the action target, such as metformin. Expert studies of OCT1 distribution showed that such protein is found in the stomach, small intestine, kidney, and skeletal muscles in human, and is mainly expressed through the liver [28], [29]. Although early studies reported that OCT1 is found in the basolateral membrane [30], some other studies reported that the apical surface of intestinal epithelial cells also becomes the location of OCT1 [31], [32], [33]. To date, studies of the reduced function of OCT1 transporter in the intestine has been more associated with the level of gastrointestinal intolerance of metformin use because of the possibly higher effect on increasing local concentrations of metformin in the intestine (lumen and enterocytes)

when compared to the level of metformin transported to the blood [15]. Thus, genetic variation in SLC22A1 as an OCT1 encoding gene affects not only the absorption of metformin but also the function of OCT1 involved during the distribution to hepatocytes as the primary action target of such antidiabetic drug as well as during the reabsorption in the renal tubules. This analysis has been justified in several studies that found the effects of polymorphism on the pharmacokinetic and pharmacodynamic variability of metformin.

Methionine deletion at codon 420 (Met420del) located in the ninth transmembrane region of the SLC22A1 gene, the polymorphism target of this research, has been the most commonly studied functional variant. In contrast to the majority of other functional SLC22A1 variants that are population specific, the Met420 deletion can be found in some populations in different regions in the world [34], [35], [36], [37]. Such polymorphism causes a decrease in the activity of metformin transporter, leading to a reduced antihyperglycemic response [38]. This is also proven by a study of 20 healthy subjects receiving metformin as much as 1850 mg/day that indicates the presence of polymorphism, one of which is Met420del, causing the variant allele group to have higher AUC of plasma metformin concentration but lower volume of distribution in oral administration compared to the wild-type group [39]. Therefore, the metformin concentration transported to the hepatocytes as its action target is reduced, resulting in a decreased antihyperglycemic response [24]. Also, a study of 108 Iranian patients newly diagnosed T2DM and using metformin for 12 weeks also showed that the Met420del variant causes lower FBG reduction compared to the wild-type group [20]. Other studies also found a variation in the scores of metformin clearance in the kidney and the metformin uptake to the liver which will eventually affect blood glucose levels followed by an effect on the appropriate dose to administer to T2DM patients [37], [40]. In contrast, the research involving 1531 patients in GoDART study revealed that 420del does not affect A1C reduction in T2DM patients receiving metformin [41]. These contradictory differences require further research using more improved methods.

It is widely acknowledged that the effect of 420del polymorphism in SLC22A1 on the expression of OCT1 in the apical membrane of renal tubule cells can reduce re-absorption in the renal tubule, leading to a decrease in the plasma metformin concentration, including its steady-state concentrations [42]. A significant reduction in the minimum steady-state concentration of metformin also occurs in patients with a heterozygous deletion of rs72552763 (Met420del) when compared to the wild-type group (P 0.06) [12].

As previously studied, OCT1 is also found in the basolateral membrane of intestinal cells, so polymorphism in OCT1 causes a decrease in the amount of metformin absorbed into the systemic

circulation and increased its concentration in enterocytes. This has been believed to contribute to the occurrence of metformin intolerance [15]. A prospective study involving 92 newly diagnosed T2DM patients who received metformin found that Met420del variant in the group of patients with R61C (rs12208357) variant has twice higher OR to experience gastrointestinal side effects [43] and even 4 times higher OR in the group of patients who have 2 alleles of OCT1 functional variant including Met420del [44], but different types of polymorphism are found in another study involving 246 T2DM patients [22].

Also, although contradictory findings remain to appear, particularly related to the effect of Met420del polymorphism in SLC22A1 gene on the glycemic response to metformin and gastrointestinal intolerance, the high frequency of mutant alleles in Javanese-Indonesian population requires further research. This is in line with the minimum involvement of the Indonesian population in the development of new drugs. Additionally, OCT1 is also an important transporter for several other drugs. Therefore, further studies of pharmacokinetic variability and therapeutic response to the use of other drugs that also require OCT1 in their pharmacokinetics, such as oxaliplatin, sorafenib, and lamivudine, are recommended [27], [45].

In conclusion, the results of allele frequency study on OCT1 involving the Javanese-Indonesian population is a novelty in the initial study of pharmacogenetics of metformin use which has never been conducted. The frequency of an allele in SLC22A1 Met420del among the Javanese population in Indonesia is reasonably high (> 95%). Therefore, further studies are suggested to investigate the effect of genetic variation of these polymorphisms on the pharmacokinetic profile and glycemic response to metformin in Indonesian patients with T2DM.

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