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Research Article

DISTRIBUTION OF THE SINGLE NUCLEOTIDE POLYMORPHISM C3435T OF MDR1 GENE AMONG PEOPLE IN WESTERN AUSTRALIA, AUSTRALIA

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ABSTRACT

What is known: Ethnic variation as well as the population frequency of the *MDR1* C3435T transition has been observed in the few other populations but not in Australians.

Objective: This study aimed to investigate the distribution of the single nucleotide polymorphism *C3435T* in exon 26 of the *MDR1* gene among people in Western Australia, Australia.

Methods: Blood samples were collected from participants from a tertiary hospital in Western Australia from October 2010 to July 2011. The *MDR1 C3435T* polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: It was found that the frequency of the homozygous *3435TT* allele among the subjects was 30.77% compared to the 21.98% that carries the homozygous *3435CC* allele while almost half of our study subjects are carrying the heterozygous *3435CT* allele. Our study shows similarity with *C3435T* genotype distribution among New Zealander and Asians population which could be attributed to the close geography between Australia and New Zealand with other part of Asia and possibly due to high migration between these countries.

What is New: This study included Australian patients.

Conclusion: This is the first study to illustrate the distribution of *MDR1 C3435T* genotypes for Australians that could be useful in predicting risk for certain disease and beneficial in tailoring a patient`s drug regimen.

Keywords: *MDR1, C3435T,* Polymorphism, P-glycoprotein.

INTRODUCTION

ATP-binding cassette (ABC) transporter super family is the biggest transporter gene family that is essential to transport specific substances including amino acids, sugars, inorganic ions, polysaccharides and peptides across the extra and intracellular lipid membranes [1]. The most widely studied human ABC transporter is *ABCB1*, which encodes P-glycoprotein. P-glycoprotein is a 170-180 kDa adenosine triphosphate (ATP)-dependent efflux protein that was first identified by Juliano and Ling in 1976 in Chinese hamster ovary cells which exhibited colchicines resistance [2]. *ABCB1* was among the first genes to be fully sequenced in the coding and regulatory regions [3,4].

The single nucleotide polymorphisms can be linked to altered oral bioavailability, drug resistance and susceptibility to human disease. Among different *MDR1* variants, the *C3435T* genotype had been extensively studied. Studies have shown that genetic polymorphisms of *MDR1* are associated with alteration in P-glycoprotein expression and function in different ethnicities and subjects [5]. For example, in *MDR1 C3435T*, the Japanese demonstrated similar C-allele frequency to the other Asian populations while higher frequency of the C-allele was found in the African population [6].

Genome-wide association studies have been performed to identify genetic factors that may contribute to these disorders. To date, no literature is available regarding prevalence of *MDR1 C3435T* polymorphism among people in Australia. In this study, we aimed to investigate the distribution of the single nucleotide polymorphism *C3435T* in exon 26 of the *MDR1* gene among people in Western Australia, Australia.

MATERIALS METHODS

i. Participants

All patients who attended the Endoscopy Unit, Sir Charles Gairdner Hospital, Perth, Western Australia from October 2010 to July 2011 for an upper gastroendoscopy were invited to participate in the study. Patients who were unable to converse in English, were unwilling to participate or deemed too unwell by the doctor had been considered ineligible for study. This study was approved by ethics committee at Sir Charles Gairdner Hospital, Nedlands which conforms to the provisions of the World Medical Association's Declaration of Helsinki in 1995 (as revised in Tokyo 2004) followed by human ethics clearance granted by Curtin University Ethics Committee in September 2010.

ii. Sample collection and *MDR1 (C3435T)* genotyping

Whole blood samples were collected by jugular venipuncture into vacutainer tubes with anticoagulant, Ethylene diamine Tetra-acetic Acid (EDTA). The genotyping involved blood DNA preparation from frozen sample, polymerase chain reaction (PCR) for determining P-glycoprotein band, followed by enzyme digestion with Mbo-1 to obtain particular band for *3435CC,3435 TT* and *3435CT* genotype.

In our study, genomic DNA was extracted from venous blood using home-made lysis buffer as described in Omar et al [7]. Basically, the frozen blood had been thawed in the room temperature upon the laboratory work. The thawed blood then was decanted into a sterile conical centrifuge tube and red cell lysis buffer was added. The mixture was mixed gently by inversion for several times at room temperature before being centrifuged at 2000 x g for 10 minutes. The supernatant was discarded and a fresh red cell lysis buffer was added again to the conical centrifuge tube followed by inversion and centrifuging. That step was repeated three to four times. Following the final centrifugation step, the supernatant was discarded and remaining pellet was vortexed. White cell lysis buffer was added to the dispersed pellet. The lysis buffer and pellet were mixed gently by repeated aspiration with a pipette, before being transferred to a fresh sterile conical centrifuge tube and incubated for 10 minutes at 40 ^{o}C. A Proteinase K 10 mg/ml was added to each conical centrifuge tube and the incubation continued at 55 ^{o}C for 60 minutes. A 10M ammonium acetate solution was added to each conical centrifuge tube, followed by vortex then centrifuging process at 2000 x g for 10 minutes. The supernatant was then decanted into an amount of absolute ethanol in a fresh small conical tube. The solution was mixed by gentle inversion until the DNA becomes visible as white

strands or clumps. The tubes were left for several hours or overnight at -20 °C. All the conical tubes containing the DNA strands were then centrifuged at 2000 x g for 10 minutes. The supernatant was discarded and the pellet was washed with small amount of 70% ethanol before being transferred to an eppendorf tube. The eppendorf tube was then centrifuged at 12 000 RPM for 10 minutes. The 70% ethanol was discarded carefully by inverting the eppendorf tubes on an absorbent paper and each sample was rehydrated with Tris-EDTA buffer

The DNA quantification was read by using Biospec-Nano (Shimadzu Biotech, New South Wales) at 0.712 mm with Tris-EDTA buffer as blank solution. The PCR analysis for determining P-glycoprotein band was carried out in a 25 uL of reaction volume consists of 1 uL of genomic DNA and 24 uL of reaction mixture containing 10× PCR buffer, 25mM $MgCl_2$, 5mM dNTPs and 5 mM of each oligonucleotide primers (forward 5`-TCTTTTCAGCTGCTTGATGG-3` and reverse 5`-AAGGCATGTATGTTGGCCTC-3`) in PTC-100™ Programmable Thermal Controller (MJ Research) [8]. Thermo-stat Taq DNA polymerase was added to each reaction volume. The Caco-2 cell line was used as a positive control. To determine P-glycoprotein band, about 10 µL of amplified samples were electrophoresed on 2% agarose gel in TAE buffer for 60 minute at 80 volt. The gel was stained with 30 µL gel red in 100 µL of TAE buffer and the amplified bands were visualised under ultraviolet light.

A 20 µL of reaction consisted of 10x buffer, 2 units of Mbo-1 enzyme (BioLabs Inc), pure water and 10 µL of PCR product obtained from previous step was set on ice. After incubation overnight at 37°C, the amplification of DNA was analysed by agarose gel electrophoresis using standard procedures where 10 µL of amplified samples were electrophoresed on 2.5% agarose gel in TAE buffer for about 60 minute at 80 volt. The gel was stained with gel red (30 µL in 100 µL TAE buffer) and the amplified bands were visualised under ultraviolet light. The final genotype bands were examined by two independent investigators. Enzymatic digestion with 2 units of Mbo-1 that had been performed to analyse *C3435T* polymorphism would be yielded products of one band (197 bp) for homozygous TT, 158 bp (3435C) and two bands (197 and 158 bp) for heterozygous CT genotyope [9].

Statistical analysis

Standard descriptive statistics (frequencies and percentages for categorical variables) were used to summarize the demographic data for the study participants. SAS® software (SAS Institute Inc.) was used in this study and in all analyses, p-value less than 0.05 was taken to indicate a statistically significant association.

RESULT

In this study, a total of 91 participants had been recruited and their demographic is outlined in **Table 1**. Most of our subjects were from age group of 40 to 49 years old. Only 16.48% of the subjects were found with peptic ulcer disease after the endoscopy procedure. The frequency of the homozygous *3435TT* allele among our subjects was 30.77% compared to the 21.98% that carries the homozygous *3435CC* allele. Meanwhile, almost half of our study subjects are carrying the heterozygous *3435CT* allele. There were no statistically significant differences observed in frequency distribution between three genotypes.

Table 1: Demographic characteristic of study subjects

Characteristic		N	Percentage (%)	p-value
Gender	Male	42	46.2	0.48
	Female	49	49	
Age group (years)	20-29	7	7.69	0.78
	30-39	9	9.89	
	40-49	21	23.1	
	50-59	16	17.58	
	60-69	20	21.98	
	70-79	11	12.09	
	80-99	7	7.6	
Endoscopy finding	Peptic ulcer disease	15	16.48	0.03
	Others	76	83.52	
MDR1 (C3435T) genotype	*3435CC*	20	21.98	0.33

Table 2: Comparison between Australians and other ethnic groups regarding the allele frequencies for the *MDR1 C3435T* polymorphism

Population		Sample size	Genotype frequency (%)			Reference
			CC	TT	CT	
Australians		91	22%	31%	47%	Present study
Asians	Malay	99	25%	28%	46%	[16]
	Chinese	98	25%	32%	44%	
	Indians	93	18%	43%	39%	
Chinese		265	32%	20%	48%	[17]
Iranians		126	53%	61%	42%	[18]
Indians		147	12%	52%	36%	[19]
Ecuadorians		317	25%	22%	53%	[20]
Romanians		102	25%	22%	54%	[21]
Singaporean		224	28%	22%	50%	[22]
New Zealander		160	21%	27%	52%	[23]
Japanese		114	35%	12%	53%	[24]
Polish		122	42%	17%	41%	[25]
French		81	35.8%	42%	22.2%	[26]
Spanish		408	26%	22%	52%	[27]

DISCUSSION

MDR1C3435T polymorphism has been associated with numerous diseases including gastric cancer [8] and breast cancer [11]. Schwab et al [12] reported a strong association between *MDR1 C3435T* polymorphisms and ulcerative colitis in a group of German subjects although no effect for that particular gene polymorphism was observed in Crohn`s disease. Similarly, Ho et al [13] reported that in

Scottish subjects, the *3435TT* genotype was found more commonly in subjects with ulcerative colitis, however there was no association seen with *G2677T* and inflammatory bowel disease. In another study, Iranian subjects with ulcerative colitis also showed higher frequency of the *3435T* allele [14]. On the other hand, an association between *MDR1 C3435T* with Crohn`s disease was observed in a group of Spanish subjects, suggesting that the *MDR1* gene may be a risk factor for inflammatory bowel disease [15].

Table 2 illustrates the comparison between Australians recruited from our study and other ethnic groups from the world regarding the allele frequencies for the *MDR1 C3435T* polymorphism. To the best of our knowledge, there is no other study available for the *MDR1 C3435T* genotype distribution for Australians. Interestingly, similar *C3435T* genotype distribution also been observed in New Zealander and Asians (Malay and Chinese) population. Close geography between Australia and New Zealand with other part of Asia might explain this pattern possibly due to high migration between these countries. The Singaporean also showed the same pattern of *MDR1 C3435T* genotype distribution, where half of the studied population carries either heterozygous or homozygous allele but the *CC* frequency was lower than *TT*. However, that study did not represent all ethnics from Singapore as only Chinese ethnic were recruited which the Chinese in Singapore are predominantly descendents of migrants from south China. [22]

The described distribution of a functional SNP in the human *MDR1C3435T* gene within the different populations might be helpful for individualized pharmacotherapy. For instance, Tahara et al [10] examined the influences of *MDR1* gene polymorphism on the risk of gastric cancer on 157 Japanese patients with gastric cancer and 104 patients without gastric cancer as the control group. Using polymerase chain reaction - restriction fragment length polymorphism, it was shown that the *MDR1 3435 TT* genotype had a significantly higher frequency in control group compared to gastric cancer patients (OR = 0.43; 95% CI = 0.23-0.79) and there were no significant differences of the *CT* and *CC* genotype frequencies between study and control group. Their data suggested that *3435T/T* polymorphism of *MDR1* was associated with a reduced risk of gastric cancer in the Japanese population. Recently, Salagacka et al [28] suggested that having the *CT* and *TT* genotype increased the likehood of *H. pylori* infection compared to CC genotype. Additionally, Gawronska-Szklarz et al [29] reported that there was significantly higher prevalence of subjects with *TT* genotype in Caucasian group of patients who succeed *H. pylori* eradication after the first cycle of the therapy. Interestingly, higher eradication rate was observed in their subjects who received pantoprazole, amoxicillin and metronidazole regimen compared to omeprazole, amoxicillin and metronidazole, supporting to the fact that pantoprazole has lowest potential for metabolic interaction among other proton pump inhibitors [30].

CONCLUSION

Our study shows the comparison for *MDR1 C3435T* polymorphism among Australians and other ethnic groups in the world. With the rising evidences of *MDR1 C3435T* that might influence in drug pharmacokinetic and pharmacotherapy, it is beneficial to recognize this pattern of genetic polymorphism that can be used to tailor a patient`s drug regimen for better drug response.

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Conflict of interest

Marhanis Omar, Andrew Crowe and Jeffery Hughes declare that they have no conflict of interest.

Statement

The experiments comply with the current laws of the country in which they were performed.

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