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# Polymorphisms of the Gene Encoding Multidrug Resistance Protein 1 in Taiwanese

**MAY-JEN HUANG[1], YUNG-LIANG CHEN[2], CHANG-YU CHANG[2], YANG-YANG HUANG[1] AND CHING-SHAN HUANG[1,3]***

[1.] *Department of Laboratory Medicine, Cathay General Hospital, Taipei, Taiwan, R.O.C.*

[2.] *Department of Medical Technology and Institute of Biotechnology of Yuan-Pei University of Science and Technology, Hsinchu, Taiwan, R.O.C.*

[3.] *Department of Medical Technology, Fooyin University, 151 Chin-Hsueh Rd., Ta-Liao Township, Kaohsiung County 831, Taiwan, R.O.C.*

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## ABSTRACT

Multidrug resistance protein 1 (MDR1) is one of the most important phase-3 proteins for detoxification. We hypothesized that the allele frequencies of polymorphisms in the *MDR1* gene in Taiwanese are different from those in other ethnic groups. The polymorphisms of A-41G, C-145G, T307C, G1199A, C1236T, G2677T, G2995A, and C3435T for the *MDR1* gene were determined for 110 healthy Taiwanese adults using restriction fragment length polymorphism of PCR product and the enzymes TspR I, Stu I, Hae II, Pst I, Hae III, BseY I, BstZ17 I, and Mbo I. The results showed that all the study subjects carried the wild type at nucleotides 307, 1199, and 2995. The frequencies of the heterozygous and homozygous variations were 17.3% and 0.0%, 15.5% and 2.7%, 50.0% and 39.1%, 56.4% and 28.2%, and 45.5% and 14.5% at nucleotides -41, -145, 1236, 2677, and 3435, respectively. Compared with previous reports, for Taiwanese, the allele frequencies of polymorphisms in the *MDR1* gene were significantly different from Japanese, Caucasians, Africans, Chinese living in Singapore, and Chinese living in Mainland China; except for those of -41G (identical to Japanese), 1236T, and 2677T (identical to Chinese living in Singapore). In conclusion, differences in allele frequencies for polymorphisms in the *MDR1* gene were observed for Taiwanese in comparison with other ethnic groups and Chinese living in Singapore and Mainland China.

Key words: *MDR1* gene, single nucleotide polymorphism, PCR-RFLP

## INTRODUCTION

The three phases of detoxification are oxidation (phase 1), conjugation (phase 2), and transportation (phase 3). The oxidation reaction is mainly catalyzed by cytochrome P (CYP) 450, while UDP-glucuronosyltransferase (UGT) is the major enzyme in the conjugation reaction, with a number of proteins responsible for the transportation of toxic metabolites[1-3]. Of these three detoxification phases, oxidation has been mostly studied, while conjugation and transportation are potential fields of research in the 21st century.

In Taiwan, CYP 450 polymorphism has been studied since 1993[1]; by contrast, research on UGT and transportation has only commenced recently. Since 2000 seven research papers on the *UGT1A1* gene in Taiwanese have been published[4-10]. By contrast, the study of *UGT1A7* gene in Taiwanese is in its infancy[11]. The results of the previous studies indicate that, for the *UGT1A1* gene the frequency of the A$(TA)_7$TAA allele in the promoter area is lower and the rate of variation within the coding region much higher, for Taiwanese as compared with Caucasians[4,6-10,12-14]. Further, the allele frequencies for single nucleotide polymorphisms (SNPs) in *UGT1A7* are different for these two ethnicities[11,15].

The multidrug resistance protein 1 (*MDR1*) gene product, P-glycoprotein, is one of the most important ATP-binding transporters and one of the most important proteins for phase 3 detoxification[16,17]. The *MDR1* gene is located on chromosome 7q21 and consists of 28 exons, encoding a 1280 amino acid transporter[16]. To date, 28 SNPs in the promoter and exons of the *MDR1* gene have been reported and it has been demonstrated that seven of these are associated with variation of P-glycoprotein function[18]. We hypothesized that the allele frequencies for the polymorphisms in the *MDR1* gene in Taiwanese are different from those in other ethnic groups and then performed this study to investigate the eight known SNPs of the *MDR1* gene for Taiwanese. To the best of our knowledge, this is the first report to investigate so many SNPs in the *MDR1* gene in the Chinese ethnic group.

## MATERIALS AND METHODS

### I. *Study Subjects*

The study subjects, consisted of 110 random-collected healthy Taiwanese adults (55 male and 55 female), representing part of general populations whose ancestors came from Fukien province. Every subject gave written consent to participate in this study.

* Author for correspondence. Tel: +886-2-26360450, E-mail: chsh.huang@msa.hinet.net

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**Table 1.** Primers and mutagenesis primers for PCR of the human *MDR1* gene

| SNP Site | Exon | Primer | |
|---|---|---|---|
| A-41G | Promoter | Forward | 5'-CTCCTAGCCTTTTCAAAGGTG-3' |
| | | Reverse | 5'-CAGGTTGAATTTCCAGGAGG-3 |
| C-145G | Promoter | Forward | 5'-GCTCTCTTTGCCACAGGAGG-3'[a] |
| | | Reverse | 5'-CCTGACACTTGGGAAGGTC-3' |
| T307C | 5 | Forward | 5'-GGTGATATCAATGATACAGCG-3'[a] |
| | | Reverse | 5'-CTAAAAACTATCAAGTGTATTG-3' |
| G1199A | 11 | Forward | 5'-GGAATTCAGAAATGTTCACTGCA-3'[a] |
| | | Reverse | 5'-GGAACTGACTGTTCACTAGG-3' |
| C1236T | 12 | Forward | 5'-TCTATTGAATGAAGAGTTTCTG-3' |
| | | Reverse | 5'-CACTCTGCACCTTCAGGTTC-3' |
| G2677T | 21 | Forward | 5'-TATCCTTCATCTATGGTTGG-3' |
| | | Reverse | 5'-TTTAGTTTGACTCACCTTCCC-3' |
| G2995A | 24 | Forward | 5'-CAGTTCATTTGCTCCTGAGTA-3'[a] |
| | | Reverse | 5'CAGTTGAAACATCAAACTCACC-3' |
| C3435T | 26 | Forward | 5'-GTTTTCAGCTGCTTGATGGC-3' |
| | | Reverse | 5'-CATTAGGCAGTGACTCGATG-3' |

[a]Underscoring indicates mutagenesis site.

**Table 2.** Restriction enzymes for digestion of PCR products

| Region | Restriction enzyme | PCR fragment (base pair) | Restriction band (base pair) |
|---|---|---|---|
| A-41 G | TspR I | 276 | 175; 82; 19 [variant] |
| C-145G | Stu I | 211 | 191; 20 [wild type] |
| T307C | Hae II | 176 | 154; 22 [variant] |
| G1199A | Pst I | 166 | 142; 24 [wild type] |
| C1236T | Hae III | 173 | 150; 23 [wild type] |
| G2677T | BseY I | 155 | 132; 23 [wild type] |
| G2995A | BstZ17 I | 133 | 112; 21 [variant] |
| C3435T | Mbo I | 216 | 159; 57 [wild type] |

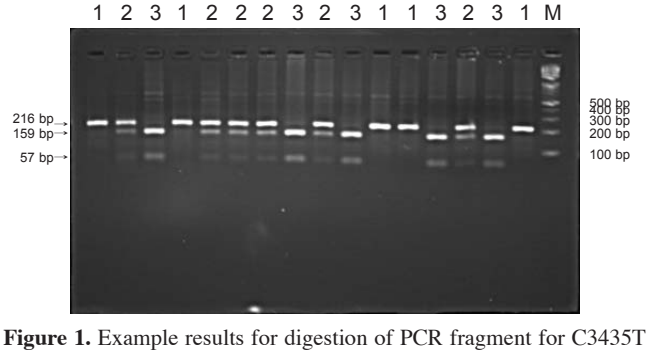


**Figure 1.** Example results for digestion of PCR fragment for C3435T *MDR1* gene. M: DNA size markers; 1: homozygous variation; 2: heterozygous variation; and 3: wild type. The DNA sizes are 216 bp in lane 1; 216 bp (top), 159 bp (intermediate), and 57 bp (bottom) in lane 2; and 159 bp (upper) and 57 bp (lower) in lane 3, respectively.

II. *Determination of MDR1 Gene*

The A-41G, C-145G, T307C, G1199A, C1236T, G2677T, G2995A, and C3435T polymorphisms were determined for the *MDR1* gene using method of polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Total genomic DNA was isolated from the blood cells using a blood DNA isolation kit (Maxim Biotech Inc; San Francisco, USA). The primers and mutagenesis primers used for PCR to determine these eight variants are presented in Table 1. The amplification reaction mixture (100 $\mu$L) contained 1 $\mu$g of DNA in 10 mM Tris-HCl (pH = 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 200 $\mu$M of each dNTP, 100 ng of each primer, and 2U of Dynazyme DNA polymerase (Finnzymes OY; Espoo, Finland). The reaction was performed using a DNA thermal cycler (Perkin-Elmer Cetus; Norwalk, CT, USA) for 35 cycles of: denaturation for 60 sec at 94˚C, annealing for 60 sec at 55˚C, primer extension for 60 sec at 72˚C, with a final 10-min extension at 72˚C. The PCR product was digested with the appropriate restriction enzymes (TspR I, Stu I, Hae II, Pst I, Hae III, BseY I, BstZ17 I, and Mbo I; Table 2), and analyzed on 3% agarose gel (NuSieve 3:1, Cambrex Bio Science Rockland Inc.; Rockland, ME, USA) containing ethidium bromide.

III. *Statistical Analysis*

The allele frequencies of the *MDR1* genotypes for our study subjects were compared with those for other ethnic groups and with Chinese living in Singapore and Mainland China[18-23] using the chi-square test. A $p$ value < 0.05 was defined as statistically significant.

## RESULTS

The PCR-RFLP method identified wild type, heterozygous or homozygous variation at the eight polymorphic sites. For example (Figure 1), the Mbo I digestion of the PCR fragment from the homozygous variation at nucleotide 3435 yielded one band of 216 base pair (bp), while the Mbo I digestion of the PCR products from individuals carrying wild type and heterozygous variations resulted in two (159 and 57 bp) and three bands (216, 159 and 57 bp), respectively.

Table 3 shows the number and frequency of genotypes of the *MDR1* gene determined for the study sample. All

110 individuals carried the wild type at nucleotides 307, 1199, and 2995. Less than 20% of the subjects bore variations at nucleotides -41 and -145, while variation at nucleotide 3435 was determined for 60% of the sample, and more than 80% were carriers of variants for nucleotides 1236 and 2677. All 50 subjects with heterozygous variation at nucleotide 3435 also carried heterozygous C1236T and G2677T variations, and all 16 with homozygous C3435T bore homozygous C1236T and G2677T variations (data not shown in Tables). The allele frequency of a variant was calculated by: (2 × number of subjects with homozygous variation) plus (1 × number of subjects with heterozygous variation) and then divided by (2 × 110). For example, allele frequency of 3435T: [(2 × 16) + (1 x 50)]/220 = 82/220 = 37.3%. Table 4 compares the allele frequencies for Taiwanese and other ethnic groups and the Chinese living in Singapore and Mainland China. Between Taiwanese and Japanese, the allele frequencies for -145G, 1236T, 2677T, and 3435T ($p < 0.05$) were significantly different, but not for -41G (P = 0.88). Comparing allele frequencies with Taiwanese, highly significant differences ($p < 0.001$) were demonstrated for Caucasians for 1236T, 2677T and 3435T and for Africans for 3435T. In comparison with Chinese living in Singapore and Mainland China, the allele frequencies of 1236T and 2677T for Taiwanese were not significantly different, while that for 3435T was.

## DISCUSSION

P-glycoprotein transports a wide range of lipophilic and amphiphatic substances, such as anticancer agents, cardiac drugs, HIV protease inhibitors, immuno- suppressants, and β-adrenoceptor antagonists[16-18]. This protein was found in 1976 and its gene (*MDR1*) was cloned in 1986, with the first SNP (C3435T) described in 2000[24]. Recent experimental findings strongly implicate P-glycoprotein as: a determinant of homeostatic interaction between bacteria and host in humans, a factor in the pathogenesis of inflammatory bowel disease[25], playing an important role in the tumorigenesis of colon cancer[26], a susceptibility factor for development of renal epithelial tumors[27], and a genetic factor determining earlier onset of Parkinson's disease symptoms[28]. The results of studies exploring the relationship between *MDR1* polymorphisms and clinical outcome have demonstrated that: AIDS patients with the 3435 TT genotypes have superior outcomes relative to patients carrying 3435 CT or CC[29], acute-lymphoblastic-leukemia patients bearing the 3435 CT/TT genotypes have a significantly lower rate of CNS relapse compared to the CC group[30], and presence of two T alleles at position 3435 was associated with a higher risk for occurrence of postural hypotension in nortriptyline-treated patients in comparison with those patients with the 3435 CC/CT genotypes[31]. Very recently, several studies

**Table 3.** Number and frequency (%) for genotypes of the *MDR1* gene in the 110 subjects

| SNP | Heterozygous variation | | Homozygous variation | | Wild type | |
|---|---|---|---|---|---|---|
| | Number | Frequency | Number | Frequency | Number | Frequency |
| A-41G | 19 | 17.3 | 0 | 0.0 | 91 | 82.7 |
| C-145G | 17 | 15.5 | 3 | 2.7 | 90 | 81.8 |
| T307C | 0 | 0.0 | 0 | 0.0 | 110 | 100.0 |
| G1199A | 0 | 0.0 | 0 | 0.0 | 110 | 100.0 |
| C1236T | 55 | 50.0 | 43 | 39.1 | 12 | 10.9 |
| G2677T | 62 | 56.4 | 31 | 28.2 | 17 | 15.4 |
| G2995A | 0 | 0.0 | 0 | 0.0 | 110 | 100.0 |
| C3435T | 50 | 45.5 | 16 | 14.5 | 44 | 40.0 |

**Table 4.** The comparison for allele frequencies of *MDR1* gene between Taiwanese and other ethnic groups using the chi-squared test (*P*-value)

| Ethnicity | Number of chromosome | Allele frequency (%) | | | | |
|---|---|---|---|---|---|---|
| | | -41G | -145G | 1236T | 2677T | 3435T |
| Taiwanese | 220 | 8.6 | 10.5 | 64.1 | 56.4 | 37.3 |
| Japanese[19] | 200 | 9.4 | 1.0 | 35.4 | 41.7 | 49.0 |
| *P*-value | | 0.88 | < 0.001 | < 0.001 | 0.004 | 0.02 |
| Caucasians[23] | 922 | | | 41.0 | 41.6 | 53.9 |
| *P*-value | | | | < 0.001 | < 0.001 | < 0.001 |
| Africans[22] | 412 | | | | | 17.0 |
| *P*-value | | | | | | < 0.001 |
| Chinese in Singapore[20] | 192 | | | 71.9 | 50.0 | 53.1 |
| *P*-value | | | | 0.09 | 0.41 | 0.002 |
| Chinese in Singapore[21] | 196 | | | | | 54.0 |
| *P*-value | | | | | | < 0.001 |
| Chinese in Mainland [22] | 264 | | | | | 46.6 |
| *P*-value | | | | | | 0.04 |

have revealed that the *MDR1* gene is highly associated with therapeutic response in some life-threatening diseases. Patients with drug-resistant epilepsy were found to be more likely to have the CC genotype of the *MDR1* gene than the TT genotype[32]. In kidney transplant recipients, C3435T and G2677T/A polymorphism of the *MDR1* gene decreased the risk for steroid-induced osteonecrosis of the femoral head[33], and tacrolimus dose requirement was 40% higher for those with homozygous C2677T in comparison with patients bearing the wild-type variant for this SNP[34]. In a sample of irinotecan-treated individuals, there was a significant association between carriage of C1236T, G2677T and C3435T and reduced renal clearance of those compounds[35].

Since the importance of *MDR1* gene variations has been proven, studies of this gene have been performed in several ethnic groups. Ethnic differences in the allele frequency of the variant *MDR1* gene have been observed. For example, the frequency of individuals homozygous for the C and T allele at nucleotide 3435 in European and American Caucasians, respectively, is approximately 25% for each genotype and the TT genotype has a frequency of only 6% in Africans[22]. However, similar examples for other SNPs are limited. The frequency of the 2677T allele is reportedly 42%, 41.7%, and 13% in Germans, Japanese, and African Americans, while the frequency of Caucasians homozygous for the 1236TT is about one-third the value of Japanese (13.3% versus 37.5%)[19,23]. Moreover, some variations are only found in single ethnic groups. For instance, the SNP at nucleotide 3421 has only been observed in Ghanaian populations and African Americans (1.2% and 4.3%, respectively), and not Caucasians[36], while those at nucleotides -41 and -145 in exon 1 have been found in Japanese (7.3% and 1.0% for A-41G and C-145G, respectively), but not Caucasians and Africans[19].

To date, there have been three investigations of the *MDR1* gene in Chinese, one of C3435T, C1236T, and G2677T for Chinese living in Singapore[20], and the other two of C3435T for Chinese living in Singapore[21] and Chinese living in Mainland China[22]. In this study, we investigated eight SNPs of the *MDR1* gene, including six of the seven SNPs affecting P-glycoprotein function[18], as well as the two promoter-area SNPs found in Japanese[19]. Our results indicate that, in Taiwanese the allele frequencies of polymorphisms in the *MDR1* gene are significantly different from those in Japanese, Caucasians, and Africans, except for that for -41G (identical to Japanese). When compared with Chinese living in Singapore and Mainland China, our data show that the allele frequency of 3435T is different while those for 1236T and 2677T are identical. The findings may be explained, at least partially, by that the specimens for the investigation of *MDR1* gene for the Chinese living in Mainland China were collected from Chengtu (in Szechwan province) populations[22] and the origin of Chinese living in Singapore is mainly from Canton province, while our Taiwanese study subjects whose ancestors came from Fukien province of Mainland China. The most important findings are that the frequency (14.5%) of 3435TT in Taiwanese is in between that in Africans (6%) and in Americans (25%) and the frequency of 3435T carriage for Taiwanese varies from that reported for other ethnic groups. We can conclude, therefore, that significant inter-ethnicity differences exist in the *MDR1* gene, as confirmed by other authors[19,22,23,36]. We also found that C3435T is in linkage disequilibrium with C1236T and G2677T, in line with other reports[18,19,28,33-36]. Inter-ethnic variation in the incidence of *MDR1* polymorphisms may contribute to racial differences in drug response and disease susceptibility, distinguishing Taiwanese from individuals of other ethnic origins.

Most of the P-glycoprotein substrates are also metabolized by CYP3A4, the major drug-eliminating enzyme[20]. P-glycoprotein and CYP3A4 work in tissues, such as small intestine and liver, in a coordinate fashion in order to prevent entry of orally ingested xenobiotics into the body. Interestingly, CYP3A4 polymorphisms are rare in Asian populations (< 1.8% in Chinese, Japanese, Malays, and Indians)[20]. On the other hand, the results of several studies[20-22] as well as our own show that Chinese carry a relatively higher frequency (up to 71.9%) of *MDR1* polymorphisms. Those differences may indicate that, in Chinese, the variant *MDR1* gene plays a more important role than the variant CYP3A4 gene in terms of elimination of xenobiotics. It appears reasonable to suggest, therefore, that the relationship between SNPs of the *MDR1* gene and the metabolism of its substrates in Taiwanese merits further study. A research program of this type is ongoing at our laboratory.

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