RESEARCH ARTICLE

Haplotype Analysis of *MDR1* and Risk for Cervical Cancer in Northeastern Thailand

Sophida Phuthong[1], Wannapa Settheetham-Ishida[1]*, Sitakan Natphopsuk[2], Dariwan Settheetham[3], Takafumi Ishida[4]

Abstract

Objective: The aim of this study was to investigate the association between genotype and haplotype of *MDR1* (C1236T, G2677T/A and C3435T) and the risk for cervical cancer in Northeastern Thai women. **Methods:** An age-matched case-control study involving squamous cell cervical cancer (SCCA) patients (n=204) and healthy controls (n=204) was enrolled for *MDR1* genotyping by real-time PCR method. **Results:** The genotype distribution of *MDR1* in both patients and controls was not significantly different ($p>0.05$). The haplotype analysis showed that T-T-T was the most common haplotype in this population. Significantly increased risk of cervical cancer was observed in carriers of T-T-C and C-G-T haplotypes with ORs of 1.86 (95%CI=1.02-3.39, $p=0.0416$) and 2.00 (95%CI=1.18-3.40, $p=0.0140$), respectively. Analysis of 2677-3435 haplotype showed increased risk for cervical cancer in G-T (OR=1.55; 95% CI=1.12-2.13, $p=0.0432$) and T-C (OR=1.91; 95%CI=1.05-3.47, $p=0.0325$). **Conclusion:** The results provide evidence that haplotype of *MDR1* may be an important risk factor for cervical cancer development in Northeastern Thai women.

Keywords: *MDR1*- cervical cancer- haplotype- Northeastern Thailand

Asian Pac J Cancer Prev, **18 (7)**, 1815-1819

Introduction

The *multidrug resistance 1* (*MDR1*) gene located on chromosomal region 7q21 encodes P-glycoprotein (P-gp). P-gp is a membrane transporter of an ATP-dependent efflux pump which contributes to protecting the body from environmental toxins and carcinogenic compounds. It is widely expressed on the membrane of normal tissues, such as breast, colon, gastric and cervical tissues (Marzolini et al., 2004; Nicol et al., 2014; Riou et al., 1990).

The *MDR1* is a highly polymorphic gene with at least 50 single nucleotide polymorphisms (SNPs) (Fung and Gottesman, 2009). The most common three SNPs are C1236T, G2677T/A and C3435T in the exon 12, 21 and 26, respectively (Kimchi-Sarfaty et al., 2007(a); Panczyk et al., 2009). C1236T and C3435T are silent mutations that do not change the encoded amino acid, whereas non-synonymous G2677T/A results in an amino acid substitution from alanine to serine or threonine. Polymorphisms of the *MDR1* may lead to inter-individual variation of P-gp expression and function, and may influence inter-individual carcinogenic susceptibility (Wang et al., 2005).

Among the identified *MDR1* SNPs, C3435T was the first SNP correlated with expression levels and function of P-gp (Hitzl et al., 2001; Hoffmeyer et al., 2000). Several studies have reported that genetic variations of the *MDR1* alter the risk of cancer development which is more apparent in haplotype analysis with other two, C1236T and G2677T/A, SNPs (Abuhaliema et al., 2016; Panczyk et al., 2009; Potocnik et al., 2008; Wu et al., 2012). Carriers of the T allele of C3435T were mostly found with the increased risk for cancer in colon, renal and breast (Kurzawski et al., 2005; Siegsmund et al., 2002; Turgut et al., 2007), while some studies reported that carriers of the C allele had an increased risk of colorectal cancer and breast cancer (Abuhaliema et al., 2016; Yue et al., 2013). However, no significant relationships between the polymorphism and increased colorectal cancer (Petrova et al., 2008), breast cancer (Rubis et al., 2012) and gastric cancer (Zebrowska et al., 2014) have also reported.

Since the C3435T polymorphism is in linkage disequilibrium with C1236T and G2677T/A polymorphisms (Kimchi-Sarfaty et al., 2007(b)), haplotype analyses for the three polymorphisms may provide more useful information than simple genotypic analyses. Therefore, our study was designed to determine whether the *MDR1* polymorphisms and their haplotype are associated with the risk for developing cervical cancer among Northeastern Thai women.

*[1]Department of Physiology, HPV and EBV and Carcinogenesis Research Group, Faculty of Medicine, [3]Department of Environmental Health, Faculty of Public Health, Khon Kaen University, Khon Kaen, [2]Chulabhorn International College of Medicine, Thammasat University, Pathum Thani, Thailand, [4]Department of Biological Sciences, Graduate School of Science, The University of Tokyo, Tokyo, Japan. *For Correspondence: wannapa@kku.ac.th*

Materials and Methods

Study population

This case-control study was conducted in Khon Kaen Hospital and Srinagarind Hospital, Khon Kaen Province, Northeastern Thailand. Participants were 204 women with pathologically diagnosed squamous cell carcinoma of the cervix (SCCA) and 204 healthy women without cervical cancer and/or pre-invasive lesion of the cervix confirmed by cytological and histological examination. The controls were matched to the cases on age within 5 year interval. The study protocol was approved by the Ethics Committee of Khon Kaen University (HE 561382) and a signed informed consent was obtained from each participant.

Detection of MDR1 polymorphism

Genomic DNA was extracted from buffy coat using GF-1 Blood DNA Extraction Kits (Vivantis, USA). The real-time polymerase chain reaction with TaqMan® probe (Applied Biosystems, USA) was used for the detection of *MDR1* polymorphisms. Taqman® probe was designed for specific SNP, VIC fluorescence dye's probe was used for wild type allele (C allele) whereas FAM fluorescence dye's probe was used for mutant type allele (T allele) of C1236T and C3435T. For G2677T/A SNP which provided two types of variant allele (T and A alleles) performed in two assays, one for the detection of G and A alleles and another for the detection of G and T alleles, VIC probe was specific with the G allele and FAM probe was specific with the A allele or T allele. The reactions were set under the following conditions: for C1236T (rs1128503) and C3435T (rs1045642) SNPs, holding stage at 95 °C for 10 minutes, follow by 40 cycles each of denaturation at 95 °C for 15 seconds, annealing and extension at 60 °C for 60 seconds. For G2677T/A (rs2032582) SNP, holding stage at 95 °C for 10 minutes, follow by 50 cycles each of denaturation at 95 °C for 15 seconds, annealing and extension at 60 °C for 60 seconds.

Statistical analyses

Statistical analyses were performed using STATA software. Deviation from Hardy-Weinberg equilibrium (HWE) for the genotype of each SNP was tested by Pearson's chi-square. The haplotypes were inferred using PHASE algorithm version 2.1.1. Association between genotype or haplotype and cervical cancer risk was tested by calculating the odd ratios (OR) with 95% confidence intervals (CI) by logistic regression analyses. *P* values less than 0.05 were considered statistically significant.

Results

The genotype distribution and allele frequency of the *MDR1* polymorphisms (C1236T, G2677T/A and C3435T) are summarized in Table 1. The genotype distribution for each locus among the cases and controls was in agreement with Hardy-Weinberg equilibrium ($p > 0.05$). The genotype distribution of C1236T, G2677T/A and C3435T in both patients and controls were not different and no association was found between these genotype and cervical cancer risk. For the allele distribution, only the C3435T polymorphism showed significantly different distribution between cases and controls (p=0.042), which indicated that the presence of T allele gave a higher risk for cervical cancer.

Table 2 presents the distribution of haplotype for the

Table 1. Genotype Distribution and Allele Frequency of The *MDR1* in Cervical Cancer

Position	Exon	Amino acid change	Genotype/ Allele	Number/Frequency Case[a]	Control[b]
			CC	24	23
1236	12	Gly412Gly	CT	96	97
			TT	84	84
			C	0.35	0.35
			T	0.65	0.65
2677	21	Ala893Ser/Thr	GG	55	59
			GT	84	92
			GA	10	6
			TT	47	32
			TA	7	13
			AA	1	2
			G	0.50	0.53
			T	0.45	0.41
			A	0.05	0.06
3435 [c]	26	Ile1145Ile	CC	47	55
			CT	84	97
			TT	73	52
			C	0.44	0.51
			T	0.56	0.49

a, no deviation from Hardy–Weinberg equilibrium; b, no deviation from Hardy–Weinberg equilibrium; c, allele distribution of Ile1145Ile (C/T) was different between case and control (p=0.042)

Table 2. Association between *MDR1* Haplotypes (1236-2677-3435) and Risk for Cervical Cancer

MDR1 1236-2677-3435	Case n (%)	Control n (%)	OR [95%CI, *p*] T-T-T as standard	OR [95%CI, *p*] in haplotype pair
T-T-T	148 (36.27)	145 (35.54)	1	
T-T-C	36 (8.82)	19 (4.66)	1.86 [1.02-3.39, 0.042*]	
C-G-C	73 (17.89)	90 (22.06)	0.80 [0.54-1.17, 0.241]	1
C-G-T	51 (12.50)	25 (6.13)	2.00 [1.18-3.40, 0.014*]	2.52 [1.42-4.45, 0.021*]
C-A-C	9 (2.21)	17 (4.17)	0.52 [0.22-1.20, 0.120]	1
C-A-T	10 (2.45)	6 (1.47)	1.63 [0.58-4.61, 0.350]	3.15 [0.86-11.49, 0.113]
T-G-C	59 (14.46)	81 (19.85)	0.71 [0.48 – 1.16, 0.103]	1
T-G-T	21 (5.15)	20 (4.90)	1.03 [0.54 - 1.98, 0.932]	1.44 [0.72-2.90, 0.303]
C-T-C	1 (0.26)	0 (0.00)	b	b
C-T-T	0 (0.00)	5 (91.26)	b	b

*p<0.05; b, drop because confidence levels not possible with zero count cells

three loci of *MDR1* (1236-2677-3435). The haplotype T-T-T was most frequently observed both in cases and controls. T-T-C and C-G-T haplotypes significantly increased the risk for cervical cancer compared to T-T-T with OR=1.86 (95%CI=1.02-3.39, *p*=0.042) and OR=2.00 (95%CI=1.18-3.40, *p*=0.014), respectively. Furthermore, carriers of C-G-T had 2.5 times higher risk of cervical cancer compared to the Caucasian and African common C-G-C haplotype (95%CI=1.42-4.45, *p*=0.021).

Discussion

The *MDR1* polymorphisms have been intensively studied so far, mostly focusing on the role of C3435T (Fung and Gottesman, 2009; Wang et al., 2005). C3435T correlated with other SNPs *i.e.* G2677T/A and C1236T that have been shown to be a part of common haplotypes, is the main determinant of functional differences in P-gp (Kimchi-Sarfaty et al., 2007(b); Panczyk et al., 2009). Analysis of *MDR1* haplotype likely provide a better understanding of *MDR1* polymorphism on cancer risk rather than their allelic frequency distribution for each individual SNP.

In our result, the genotype distributions for the three *MDR1* SNPs in the controls were in Hardy-Weinberg equilibrium which confirmed they represented the given population. Among healthy Thais, the commonest allele for each locus was T (C1236T), G (G2677T/A) and C (C3435T) as in other Asian populations but was different from that in European-Americans and Caucasians (Ameyaw et al., 2001; Cascorbi et al., 2001, Kim et al., 2001; Komoto et al., 2006; Li et al., 2007; Tang et al., 2002; Wang et al., 2015), showing inter-ethnic differences.

As for C3435T, risk for several cancers, such as breast, endometrial and colorectal have been documented (Cizmarikova et al., 2010; Mrozikiewicz et al., 2007; Khedri et al., 2011; Wu et al., 2012), whereas in this study, T allele itself showing significantly higher risk for cervical cancer was observed nevertheless genotype was not involved in.

Because of the linkage disequilibrium, the haplotype frequencies were not of the representative of allelic frequencies; T-T-T haplotype was the most common haplotype in our population. It is documented that this haplotype is a predominant haplotype among Asian populations (Fung and Gottesman, 2009; Xu et al., 2008), whereas C-G-C haplotype is a predominant haplotype in Caucasian and African populations (Fung and Gottesman, 2009; Gumus-Akay et al., 2010; Jeannesson et al., 2007); here observed is inter-ethnic differentce, too.

Participation of *MDR1* polymorphisms in the alteration of risk for cervical cancer might be due to the change of P-gp function which is likely influenced by the 2677-3435 haplotype (Kwon et al., 2009, Ni et al., 2015, Pang et al., 2014). A study of 2677-3435 haplotype demonstrated that G-T haplotype contributed to diffuse large B cell lymphoma development (Ni et al., 2015) which was in agreement with our findings; significant increased risk for cervical cancer was observed in carriers of G-T haplotype (C-G-T and T-G-T) with OR 1.546 (95%CI=1.12-2.13, *p*=0.043) compared to T-T haplotype. A significant increased risk for cervical cancer was also observed in T-C haplotype (OR=1.908 95% CI=1.05-3.47, *p*=0.033); however, A-T haplotype did not show an increased risk. It is suggested that the presence of T allele in these two loci may alter susceptibility to cervical cancer. The presence of variant alleles has been associated with lower mRNA expression and less production of P-gp which results in carcinogen accumulation (Lamba et al., 2006, Johne et al., 2002). In addition, a possible explanation for different P-gp function observed within the *MDR1* haplotype may result from the interaction of C1236T, G2677T/A and C3435T leading to the presence of rare codon. Alteration of codon influences the co-translational folding time and insertion of P-gp into the membrane, thereby change of P-gp structure and substrate specificity (Kimchi-Sarfaty et al., 2007(b)).

In conclusion, our finding demonstrates the association between haplotype of *MDR1* and cervical cancer risk in Northeastern Thai women. The haplotype testing to identify genetic susceptibility may provide more information for the cancer prevention.

Acknowledgements

The authors thank all the staff and the patients who took part in this study. This study was partly supported by (a) a Research Fund for Supporting Lecturer to Admit High Potential Student to Study and Research on Expert Program Year 2015 (581H218), Graduate School, Khon Kaen University, (b) an Invitation Research Grant from Faculty of Medicine (IN60214), Khon Kaen University, (c) a Research Fund from Khon Kaen University, and (d) JSPS Core University Program.

References

Abuhaliema AM, Yousef AM, El-Madany NN, et al (2016). Influence of genotype and haplotype of *MDR1* (C3435T, G2677A/T, C1236T) on the incidence of breast cancer-a case-control study in Jordan. *Asian Pac J Cancer Prev*, **17**, 261-6.

Andersen V, Vogel U, Godiksen S, et al (2013). Low ABCB1 gene expression is an early event in colorectal carcinogenesis. *PloS One*, **8**, e72119.

Brambila-Tapia AJ (2013). *MDR1* (ABCB1) polymorphisms: functional effects and clinical implications. *Rev Invest Clin*, **65**, 445-54.

Cascorbi I, Gerloff T, Johne A, et al (2001). Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter *MDR1* gene in white subjects. *Clin Pharmacol Ther*, **69**, 169-74.

Cizmarikova M, Wagnerova M, Schonova L, et al (2010). *MDR1* (C3435T) polymorphism: relation to the risk of breast cancer and therapeutic outcome. *Pharmacogenomics J*, **10**, 62-9.

Fung KL, Gottesman MM (2009). A synonymous polymorphism in a common *MDR1* (ABCB1) haplotype shapes protein function. *Biochim Biophys Acta*, **1794**, 860-71.

Gaikovitch E, Mrozikievicz P, Wagner F, Roots I (2004). Association of C3435T and G2677T/A polymorphisms of multidrug resistance (*MDR1*) gene with colorectal cancer risk. *Clin Pharmacol Ther*, **75**, P17.

Gumus-Akay G, Rustemoglu A, Karadag A, Sunguroglu A

(2010). Haplotype-based analysis of *MDR1*/ABCB1 gene polymorphisms in a Turkish population. *DNA Cell Biol*, **29**, 83-90.

Hitzl M, Drescher S, van der Kuip H, et al (2001). The C3435T mutation in the human *MDR1* gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD56(+) natural killer cells. *Pharmacogenetics*, **11**, 293-8.

Hoffmeyer S, Burk O, von Richter O, et al (2000). Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variations and correlation of one allele with P-glycoprotein expression and activity in vivo. *Proc Natl Acad Sci U SA*, **97**, 3473-8.

Jeannesson E, Albertini L, Siest G, et al (2007). Determination of ABCB1 polymorphisms and haplotypes frequencies in a French population. *Fundam Clin Pharmacol*, **21**, 411-8.

Johne A, Kopke K, Gerloff T, et al (2002). Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein *MDR1* gene. *Clin Pharmacol Ther*, **72**, 584-94.

Khedri A, Nejat-Shokouhi A, Salek R, et al (2011). Association of the colorectal cancer and *MDR1* gene polymorphism in an Iranian population. *Mol Biol Rep*, **38**, 2939-43.

Kim RB, Leake BF, Choo EF, et al (2001). Identification of functionally variant *MDR1* alleles among European Americans and African Americans. *Clin Pharmacol Ther*, **70**, 189-99.

Kimchi-Sarfaty C, Marple AH, Shinar S, et al (2007a). Ethnicity-related polymorphisms and haplotypes in the human ABCB1 gene. *Pharmacogenomics*, **8**, 29-39.

Kimchi-Sarfaty C, Oh JM, Kim IW, et al (2007b). A "silent" polymorphism in the *MDR1* gene changes substrate specificity. *Science*, **315**, 525-8.

Komoto C, Nakamura T, Sakaeda T, et al (2006). *MDR1* haplotype frequencies in Japanese and Caucasian, and in Japanese patients with colorectal cancer and esophageal cancer. *Drug Metab Pharmacokinet*, **21**, 126-32.

Kurzawski M, Drozdzik M, Suchy J, et al (2005). Polymorphism in the P-glycoprotein drug transporter *MDR1* gene in colon cancer patients. *Eur J Clin Pharmacol*, **61**, 389-94.

Kwon WS, Rha SY, Jeung HC, et al (2009). G-T haplotype (2677G>T/A and 3435C>T) of ABCB1 gene polymorphisms is associated with ethnic differences to paclitaxel sensitivity in cancer cells with different gene expression pattern. *Cancer Lett*, **277**, 155-63.

Li D, Zhang GL, Lou YQ, et al (2007). Genetic polymorphisms in *MDR1* and CYP3A5 and *MDR1* haplotype in mainland Chinese Han, Uygur and Kazakh ethnic groups. *J Clin Pharm Ther*, **32**, 89-95.

Marzolini C, Paus E, Buclin T, Kim RB (2004). Polymorphisms in human *MDR1* (P-glycoprotein): recent advances and clinical relevance. *Clin Pharmacol Ther*, **75**, 13-33.

Mrozikiewicz PM, Seremak-Mrozikiewicz A, Semczuk A, et al (2007). The significance of C3435T point mutation of the *MDR1* gene in endometrial cancer. *Int J Gynecol Cancer*, **17**, 728-31.

Ni Y, Xiao Z, Yin G, et al (2015). The single nucleotide polymorphism and haplotype analysis of *MDR1* in Chinese diffuse large B cell lymphoma patients. *Biomed Pharmacother*, **73**, 24-8.

Nicol MR, Fedoriw Y, Mathews M, et al (2014). Expression of six drug transporters in vaginal, cervical, and colorectal tissues: Implications for drug disposition in HIV prevention. *J Clin Pharmacol*, **54**, 574-83.

Panczyk M, Balcerczak E, Piaskowski S, et al (2009). ABCB1 gene polymorphisms and haplotype analysis in colorectal cancer. *Int J Colorectal Dis*, **24**, 895-905.

Pang L, Word B, Xu J, et al (2014). ATP-Binding cassette genes genotype and expression: A potential association with pancreatic cancer development and chemoresistance? *Gastroenterol Res Pract*, **41**, 4931.

Petrova DT, Nedeva P, Maslyankov S, et al (2008). No association between *MDR1* (ABCB1) 2677G > T and 3435C > T polymorphism and sporadic colorectal cancer among Bulgarian patients. *J Cancer Res Clin Oncol*, **134**, 317-22.

Potocnik U, Glavac D, Dean M (2008). Common germline *MDR1*/ABCB1 functional polymorphisms and haplotypes modify susceptibility to colorectal cancers with high microsatellite instability. *Cancer Genet Cytogenet*, **183**, 28-34.

Qiu H, Dong H, Pan S, Miao K (2012). The single nucleotide polymorphism and haplotype analysis of *MDR1* in Jiangsu Han population of China. *Biomed Pharmacother*, **66**, 459-63.

Riou GF, Zhou D, Ahomadegbe JC, et al (1990). Expression of multidrug-resistance (*MDR1*) gene in normal epithelia and in invasive carcinomas of the uterine cervix. *J Natl Cancer Inst*, **82**, 1493-6.

Rubis B, Holysz H, Barczak W, et al (2012). Study of ABCB1 polymorphism frequency in breast cancer patients from Poland. *Pharmacol Rep*, **64**, 1560-6.

Siegsmund M, Brinkmann U, Schaffeler E, et al (2002). Association of the P-glycoprotein transporter *MDR1*(C3435T) polymorphism with the susceptibility to renal epithelial tumors. *J Am Soc Nephrol*, **13**, 1847-54.

Tanabe M, Ieiri I, Nagata N, et al (2001). Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther*, **297**, 1137-43.

Tang K, Ngoi SM, Gwee PC, et al (2002). Distinct haplotype profiles and strong linkage disequilibrium at the *MDR1* multidrug transporter gene locus in three ethnic Asian populations. *Pharmacogenetics*, **12**, 437-50.

Taheri M, Mahjoubi F, Omranipour R (2010). Effect of *MDR1* polymorphism on multidrug resistance expression in breast cancer patients. *Genet Mol Res*, **9**, 34-40.

Turgut S, Yaren A, Kursunluoglu R, Turgut G (2007). *MDR1* C3435T polymorphism in patients with breast cancer. *Arch Med Res*, **38**, 539-44.

Wang D, Johnson AD, Papp AC, Kroetz DL, Sadee W (2005). Multidrug resistance polypeptide 1 (*MDR1*, ABCB1) variant 3435C>T affects mRNA stability. *Pharmacogenet Genomics*, **15**, 693-704.

Wang DX, Sadee W (2006). Searching for polymorphisms that affect gene expression and mRNA processing: Example ABCB1 (*MDR1*). *AAPS J*, **8**, 515-20.

Wang F, Huang Z, Zheng K, Zhao H, Hu W (2015). Two SNPs of ATP-binding cassette B1 gene on the risk and prognosis of colorectal cancer. *Int J Clin Exp Pathol*, **8**, 3083-9.

Wang LH, Song YB, Zheng WL, Jiang L, Ma WL (2013). The association between polymorphisms in the *MDR1* gene and risk of cancer: a systematic review and pooled analysis of 52 case-control studies. *Cancer Cell Int*, **13**, 46.

Wu H, Kang H, Liu Y, et al (2012). Roles of ABCB1 gene polymorphisms and haplotype in susceptibility to breast carcinoma risk and clinical outcomes. *J Cancer Res Clin Oncol*, **138**, 1449-62.

Xu P, Jiang ZP, Zhang BK, Tu JY, Li HD (2008). Impact of *MDR1* haplotypes derived from C1236T, G2677T/A and C3435T on the pharmacokinetics of single-dose oral digoxin in healthy Chinese volunteers. *Pharmacology*, **82**, 221-7.

Yin G, Xiao Z, Ni Y, et al (2016). Association of *MDR1* single-nucleotide polymorphisms and haplotype variants with multiple myeloma in Chinese Jiangsu Han population. *Tumour Biol*, **37**, 9549-54.

Yue AM, Xie ZB, Zhao HF, et al (2013). Associations of ABCB1 and XPC genetic polymorphisms with susceptibility to colorectal cancer and therapeutic prognosis in a Chinese population. *Asian Pac J Cancer Prev*, **14**, 3085-91.

Zebrowska M, Salagacka A, Jelen A, et al (2014). Is the ABCB1 gene associated with the increased risk of gastric cancer development?-preliminary research. *Pathol Res Pract*, **210**, 872-8.