Original Artical

Prevalence of *CYP2D6*10* Genotype in Thai Breast Cancer Patients

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Introduction: *Cytochrome P450 2D6 (CYP2D6), one of the most important drug metabolizing enzymes, is involved in the biotransformation of many clinically used drugs, and is known to be genetically polymorphic resulting in a high degree of interindividual variation in drug therapy. The most common polymorphism of CYP2D6 in Asian women is CYP2D6*10, and associated with decreased CYP2D6 activity resulting from the formation of an unstable enzymes, and effects on the efficacy of a drug and on its adverse reactions.* ***Objective:*** *To investigate the frequencies of CYP2D6*10 in breast cancer patients.* ***Setting:*** *Phramongkutklao Hospital, Bangkok.* ***Research design:*** *A retrospective study.* ***Patients:*** *Pre and postmenopausal women with diagnosed breast cancer who were starting tamoxifen (20 mg/day) as standard adjuvant therapy were included.* ***Method:*** *Six-year retro-prospective data, January 2006 - Febuary 2012, were collected from electronic database and medical records of the outpatient breast cancer clinic. The CYP2D6*10 were determined using the TaqMan Allelic Discrimination Assay.* ***Result:*** *Data of 67 breast cancer patients were analyzed. The allele frequency of CYP2D6*1 was 49% and CYP2D6*10 was 51%.* ***Conclusion:*** *Our result showed that the CYP2D6*10 frequency in Thai breast cancer women was nearly the same as general Asian populations.*

Key Words: ● *CYP2D6*10* ● *Prevalence* ● *Breast cancer* ● *Thai*

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Introduction

The cytochrome P450 (CYP) system is a group of primitive heme containing enzymes with diverse metabolic functions. The enzyme system is divided into several families and subfamilies.[1] They have a major role in xenobiotic metabolism including drug metabolism by either inactivation or the activation of pro-drugs.[2] The *CYPD6* gene is CYP 450, family 2, subfamily D, polypeptide 6, and locates on the long arm of chromosome 22.[3] *CYP2D6*, one of the most important drug metabolizing enzymes, is involved in the biotransformation of a large number of drugs approximately 20-25%.[4] There are marked ethnic variations in the frequency of these alleles, with *CYP2D6*2, *3, *4, *5, *6, *10, and *41* being more common in the Caucasians population, *CYP2D6*2* and *17 more frequently observed in Africans and *CYP2D6*10* more prevalent in Asians.[5] According to the activity of the enzyme, classically four phenotypes can be identified: poor metabolisers (PMs), who are

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homozygous for one deficient allele or heterozygous for two different deficient alleles; intermediate metabolisers (IMs), who are heterozygous for one deficient allele or carry two alleles that cause reduced activity; extensive metabolisers (EMs), who have two wild-type alleles; and ultrarapid metabolisers (UMs), who have multiple gene copies.[6] The different phenotypes have profound effects on the efficacy of a drug and on its adverse reactions. Clinical studies demonstrate that breast cancer patients treated with adjuvant tamoxifen who have decreased *CYP2D6* due to genetic polymorphisms or drug interactions may have an increased risk of recurrence and reductions in disease free survival.[7] There is a limited resource of pharmacogenomics information in Thai population. Therefore, we are conducting on comprehensive research program in personalized healthcare in oncology based on Thai population profiles, which are important as base line information in subsequent study in cancer patients.

Objective

The purpose of this study was to investigate the frequency of *CYP2D6*10* in Thai breast cancer patients.

Patients and Methods

Study design

A retrospective study design was used. The protocol was approved by Institutional Review Board, Royal Thai Army, Medical Department, (Bangkok, Thailand). Six years retrospective data, during January 2006 – February 2012 were reviewed using electronic database and medical record.

Patients

Sixty seven eligible women with diagnosed breast cancer who received tamoxifen (20 mg/day) as standard adjuvant therapy were recruited from breast cancer surgery clinic at Phramongkutklao Hospital.

DNA extraction and *CYP2D6*10* genotyping

Genomic DNA was extracted from the peripheral whole blood of each patient using Qiagen DNA extraction kit (Qiagen, Hilden, Germany). The *CYP2D6* genotype was determined using the TaqMan Allelic Discrimination Assay (Applied Biosystems Inc., USA). The primers and probes for this assay are commercial available (TaqMan Drug Metabolism Genotyping Assay, Assay ID: C_11484460_40 for C100T, Applied Biosystems Inc.) TaqMan PCR and fluorescence measurements were performed using the StepOnePlus™ Real time PCR Systems (Applied Biosystems Inc., Foster City, CA USA) following the manufacturer's instructions. The reaction mix contains TaqMan Drug Metabolism Genotyping Assay Mix, TaqMan Universal PCR Master Mix, No AmpErase UNG, and DNase-free water. The final reaction volume per well is 20 μL in a 96-well plate. The reaction mixtures were 2 mL of genomic DNA (10 ng/mL), 10 mL of 2 x TaqMan Universal Master Mix (Applied Biosystems Inc.), 1 mL of 20 x primer/probe mix, and 7 mL of ddH_2O in a volume of 20 mL.

Data Analysis

Demographic data were determined and presented as mean, median, percentage or frequency where appropriate for qualitative or quantitative variables. Prevalence of *CYP2D6*10* was assessed by Hardy-Weinberg Equilibrium (HWE). Statistic tests provided two-sided p value, and a significant level $p < 0.05$ was used. Statistical analyses were determined by using the SPSS version 17.0 (SPSS Co., Ltd., Bangkok Thailand).

Results

Demographic data

A total of 67 breast cancer patients who received adjuvant tamoxifen 20 mg/day were eligible. Seventy

one point six percent (48 of 67) was premenopausal and 28.4% (19 of 67) was postmenopausal women. Patient's characteristic was shown in Table 1.

The frequency of *CYP2D6*10*

Among the three genotypes group, 32.8% of the patients was *CYP2D6* homozygous variant genotype *(CYP2D6*10/*10)*, 37.3% was *CYP2D6* heterozygous genotype *(CYP2D6*1/*10)*, and 29.9% was *CYP2D6* homozygous wild type genotype *(CYP2D6*1/*1)*. The allele frequency of *CYP2D6*1* was 49% and *CYP2D6*10* was 51%, as shown in Table 2.

Table 1. Demographic data of patients.

Descriptive data	N	%
Number of patients	67	100
Age (year),		
Mean ± SD	48.1 ± 8.4	
Height (cm.)		
Mean ± SD	156.7 ± 4.3	
Weight (kg.)		
Mean ± SD	55.3 ± 8.2	
Menopausal status		
Premenopausal	48	71.6
Postmenopausal	19	28.4
Stage		
I	17	25.4
II	40	59.7
III	10	14.9
Tumor size, cm		
≤ 2	28	41.8
>2	39	58.2
Nodal status		
N0	36	53.7
N1	23	34.3
N2	8	11.9
ER status		
Positive	60	89.6
Negative	7	10.4
PR status		
Positive	56	83.6
Negative	11	16.4

Abbreviations: ER = estrogen receptor; PR = progesterone receptor

Table 2. Prevalence of *CYP2D6* genotype

(67 patients x 2 alleles)				Genotypes	Observed	%	Predicted
Alleles	**N=134**	**%**	**95%CI**		**N=67**		**(HWE)**
***1**	65	49	45.5-52.5	***1/*1**	20	32.8	16
				***1/*10**	25	37.3	34
***10**	69	51	47.5-54.5	***10/*10**	22	29.9	17

Chi-square = 4.853; p = 0.088

Prevalence of *CYP2D6*10* calculation

Allelic frequencies of *CYP2D6* genotypes were in Hardy-Weinberg Equilibrium (HWE), $p = 0.088$. The calculation if allelic frequencies were in HWE:

The number of the *1 allele

= (20 x 2) + (25 x 1) = 65 alleles

The number of the *10 allele

= (22 x 2) + (25 x 1) = 69 alleles

The frequency of the *1 allele

= p = 65 / (65 +69) = 0.49

The frequency of the *10 allele

= q = 69 / (65 + 69) = 0.51

The proportion of expected **1/*1, *1/*10* and **10/*10* genotypes could be predicted from HWE: p+q = 1 and $(p + q)^2 = 1$ or $p^2 + 2pq + q^2 = 1$

$p^2 = 0.49 \times 0.49 = 0.2401$

$2pq = 2 \times 0.49 \times 0.51 = 0.4998$

$q^2 = 0.51 \times 0.51 = 0.2601$

The total number of patients included to this study was 67

Expected number of **1/*1*

= 0.2401 x 67 = 16.09 ≈ 16

Expected number of **1/*10*

= 0.4998 x 67 = 33.49 ≈ 34

Expected number of **10/*10*

= 0.2601 x 67 = 17.43 ≈ 17

The observed number of **1/*1* = 20

The observed number of **1/*10* = 25

The observed number of **10/*10* = 22

Chi-square = 4.853, $P = 0.088$. Therefore, we would accept the null hypothesis that the observed and expected values are not significantly different, and that our population is indeed in Hardy Weinberg equilibrium.

Discussion

In Thai populations, there was only few studies on *CYP2D6*10* frequency. Tassaneeyakul W[9] reported that the frequency of *CYP2D6*10* in Thai subjects to be 37.8%. Nakmahachalasint P[23], reported that the frequency of *CYP2D6*10* in Thai subjects to be 69.5%. Our result showed the frequency of *CYP2D6*10* to be closed to several earlier studies in general Asians populations, and confirmed that the prevalence is much higher than in Caucasians which was reported to be 1-2% only.[5,10-22]

Conclusion

In summary, the prevalence of *CYP2D6*10* found in Thai breast cancer women was 51% which was similar to general Asian populations.

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ความชุกของยีน *CYP2D6*10* ในผู้ป่วยไทยที่เป็นมะเร็งเต้านม

ภิชญาภา รุ่งวานนท์ชัย, ดวงจิตต์ พนมวัน ณ อยุธยา, ณัฏฐดา อารีเปี่ยม, นรินทร์ วรวุฒิ และ สุขไชย สาทถาพร

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***บทนำ**: CYP2D6 เป็นเอนไซม์ที่สำคัญในการเปลี่ยนแปลงยาที่ใช้มากในทางคลินิก และเอนไซม์นี้มีความหลากหลายทางพันธุกรรม จึงทำให้ผลการรักษาด้วยยามีความแตกต่างกันมากในแต่ละบุคคล ภาวะพหุสัณฐานของยีน CYP2D6*10 พบมากในชาวเอเชีย CYP2D6*10 เป็นยีนที่มีการทำงานของเอนไซม์ CYP2D6 ลดลง จึงมีผลต่อประสิทธิภาพในการออกฤทธิ์ของยา และอาการไม่พึงประสงค์จากการใช้ยา **วัตถุประสงค์:** เพื่อศึกษาหาความชุกของยีน CYP2D6*10 ในผู้ป่วยไทยที่เป็นมะเร็งเต้านม **สถานที่ที่ทำการศึกษา:** โรงพยาบาลพระมงกุฎเกล้า กรุงเทพมหานคร **รูปแบบการวิจัย:** การศึกษาแบบย้อนหลัง **ผู้ป่วยที่ได้ทำการศึกษา:** ผู้ป่วยในวัยก่อนและหลังหมดประจำเดือนที่ได้รับการวินิจฉัยจากแพทย์ว่าเป็นโรคมะเร็งเต้านมและได้รับการรักษาด้วยยาทาม็อกซิเฟนใน ขนาด 20 มิลลิกรัมต่อวัน **วิธีการศึกษา:** รวบรวมข้อมูลผู้ป่วยที่มารับการรักษาที่คลินิกผู้ป่วยนอกโรคมะเร็งเต้านม โรงพยาบาลพระมงกุฎเกล้าในระหว่างเดือนมกราคม 2549 ถึง กุมภาพันธ์ 2555 จากฐานข้อมูลคอมพิวเตอร์และเวชระเบียน และตรวจวัดตรวจภาวะพหุสัณฐานของยีน CYP2D6*10 ด้วย TaqMan Allelic Discrimination Assay **ผลการศึกษา:** ข้อมูลจากผู้ป่วยโรคมะเร็งเต้านมชาวไทยจำนวน 67 ราย พบความชุกของยีน CYP2D6*1 จำนวนร้อยละ 49 และ CYP2D6*10 จำนวนร้อยละ 51 **วิจารณ์และสรุป:** จากการศึกษาได้แสดงผลว่า ผู้ป่วยหญิงไทยที่เป็นมะเร็งเต้านมมีความชุกของยีน CYP2D6*10 ใกล้เคียงกันกับความชุกของยีน CYP2D6*10 ของชาวเอเชียทั่วไป*

Key Words: ● *CYP2D6*10* ● *Prevalence* ● *มะเร็งเต้านม* ● *ไทย*

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