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Table A. 東アジア人、欧米人、アフリカ人の代表的な CYP2D6 遺伝子変異のアリル頻度

CYP2D6 Alleles	Enzyme Activity	Allele Frequencies (%)				
		Japanese (n=206)	Asian Korean (n=400)	Chinese (n=223)	Caucasian (n=589)	African American (n=154)
*1	Normal	43.00	33.25	37.90	36.40	34.70
*2	Normal	12.30	10.13		32.40	26.90
*3	None		0	0.00	2.04	0.30
*4	None	0.20	0.25	0.20	20.70	7.80
*5	None	4.50	6.13	7.20	1.95	6.20
*6	None		0.00	0.00	0.93	
*7	None		0.00		0.08	
*8	None		0.00	0.00	0.00	
*9	Decreased		0.00		1.78	
*10	Decreased	38.10	45.00	51.30	1.53	7.50
*11	None		0.00		0.00	
*12	None		0.00		0.00	
*13	None		0.00		0.00	
*14	None	0.70	0.50	2.00	0.00	
*15	None		0.00		0.08	
*16	None		0.00		0.08	
*17	Decreased		0.00			14.60
*18		0.20	0.00			
*21			0.25			
*27			0.38			
*35			0.13			
*39			0.63			
*41			1.88			
*47			0.13			
Duplication		1.00	1.13	1.30	1.93	1.90
*1×N		0.50	0.13		0.51	
*2×N		0.50	0.50		1.34	
*4×N			0.00		0.08	
*10×N			0.50		0.00	
Undetermined			0.25			

Table B-1. これまでに報告のある CYP2D6*10 変異ホモ保因者の経口投与後の薬物血漿中濃度の AUC 変化

薬剤	分類	野生型からの AUC 変化	参考文献
propranolol	β-遮断薬	2.3	[24]
carvedilol		1.5～2.1	[25]
metoprolol		3.5	[60]
tropisetron	制吐剤	6.3	[11]
tramadol	鎮痛剤	1.4	[27]
loratadine	抗アレルギー薬	2.2	[28]
risperidone	抗精神病薬	3.1	[29]
haloperidol		1.5	[22]
aripiprazole		1.6	[21]
paroxetine	SSRI	3.4	[26]
venlafaxine	SNRI	5.5 , 5.8	[10, 31]
nortriptyline	抗うつ薬	2.2 , 3	[32]
propafenone	抗不整脈薬	2.1	[33]
mexiletine		1.3	[34]

Table B-2. これまでに報告のある CYP2D6*10 変異による *in vitro* 薬物代謝活性の変化

代謝反応	*10 ミクロソーム	活性比	参考文献
Dextromethorphan O-demethylation	バキュロウイルス発現系	50, 100, 164	[12, 13, 17]
Codein O-demethylation		*10 定量限界以下	[12]
Fluoxetine N-demethylation		50	
MDMA demethylatin		123, 135	[13, 17]
p-Methoxy-amphetamine O-demethylation		34	
(-)-Methamphetamine N-demethylation		157	[13]
MPTP N-demethylatin		22	
Dextromethorphan O-demethylation	酵母発現系	16	[14]
Mexiletine p-hydroxylation		1.3	[35]
Mexiletine 2-methyl hydroxylation		1.1	
Bufuralol 1'-hydroxylation		3, 4.2	[14, 18]
Venlafaxine O-demethylation		2.1	[18]
(+)-Bunitrolol 4'-hydroxylation		4.1	
(-)-Bunitrolol 4'-hydroxylation		4.7	[36]
Debrisoquine 4'-hydroxylation		3	
Mexiletine p-hydroxylation	ヒト肝※	3.7, 5.2	[37]
Mexiletine 2-methyl hydroxylation		32	
Bufuralol 1'-hydroxylation		*10 定量限界以下	[14, 15]

※ヒト肝ミクロソームの活性比は、mg ミクロソーム蛋白当たりの CL_{int} 比 (*1/*1／*10/*10)で表している。

Table 1. EM、*10/*10保因者、及びPMの肝ミクロソームを用いた *in vitro* 代謝実験から算出したキニジン非存在下及び存在下の固有クリアランス（CL_{int} 及び $CL_{int,quin}$）（μL/min/mg）

薬剤	EM [&]		*10/*10 [#]		PM [#]	
	CL_{int}	$CL_{int,quin}$	CL_{int}	$CL_{int,quin}$	CL_{int}	$CL_{int,quin}$
desipramine	34.4	4.15	5.97±3.95	ND	2.96±4.95	3.34±4.56
venlafaxine	7.51	1.83	2.80±0.61	1.28±0.33	1.74±0.83	1.68±1.40
propafenone	217	30.7	55.8±7.33	ND	ND[$]	ND[$]
risperidone	24.5	8.77	5.35±0.18	2.91±0.71	10.4[$]	11.4[$]
tropisetrone	2.58	1.13	0.17±0.66	ND	1.03[$]	0.97[$]
metoprolol	3.80	0.05	1.20±0.14	0.87±0.05	0.97±0.35	0.98±0.26

ND： not detected
&: プールド
#: 3例の mean ± SD
$: 1例

Table 2. ヒト肝ミクロソームを用いて算出した CYP2D6 の代謝寄与率（%）、及び EM に対する*10/*10 保因者 CYP2D6 相対活性の比較

薬剤	CYP2D6 寄与率 (%)	*10/*10 の CYP2D6 相対活性※1 (%, mean ± SD)
desipramine	88	24±8
venlafaxine	76	27±7
propafenone	86	26±11
tropisetron	57	20±11
risperidone	64	17±6
metoprolol	99	9±2
dextromethorphan※2	100	21±6
bufuralol※3	98	19±7

※1: 3 人の*10/*10 保因者から調製した肝ミクロソームを用い、CYP2D6 の選択的阻害剤である quinidine 存在下及び非存在下で測定した *in vitro* 代謝クリアランスより算出した。

※2: 代謝物 dextrorphan の生成速度より評価

※3: 代謝物 1'-hydroxybufuralol の生成速度より評価

Table 3. バキュロウイルス発現系の CYP2D6*1 及び*10 ミクロソームを用いて測定した固有クリアランス及びその活性比

基質	CLint,recCYP2D6 （μL/min/pmolP450）		CLint,recCYP2D6 活性比
	*1	*10	*1/*10
propafenone	10.3	0.775	13.3
risperidone	1.00	0.053	18.8
tropoisetron	0.14	N D	10.0
propranolol	8.55	0.161	53.2
paroxetine	8.40	0.576	14.6
nortriptyline	4.52	0.020	228
carvedilol	25.3	0.316	80.1
metoprolol	0.29	0.004	70.3
desipramine	1.25	0.014	90.9
bufuralol	2.24	0.057	39.2

（注） bufuralol は代謝物 1'-hydroxybufuralol の生成速度（pmol product/min/mg）。

Table 4. 種々の異なるミクロソームを用いて算出した野生型に対する*10/*10 保因者における CYP2D6 相対活性の比較（%）

基質	バキュロ	ヒト肝	酵母
propafenone	4.0	26	—
risperidone	2.9	17	—
tropoisetron	<5.4	20	—
propranolol	1	—	—
paroxetine	3.7	—	—
nortriptyline	0.2	—	—
carvedilol	0.7	—	—
metoprolol	0.8	9	—
desipramine	0.6	24	—
bufuralol	1.4	19	12 , 17 [14, 18]
dextromethorphan	0.3, 0.5 , 1.1 [12, 13, 17]	21	3[14]

(注) bufuralol, dextromethorphan は代謝物 1'-hydroxybufuralol, dextrorphan の生成速度。

酵母の各反応及びバキュロウイルスの dextromethorophan の反応は文献値を使用。

Table 5. 各薬剤の血漿中非結合率、及び *in vivo* より算出した肝固有クリアランス($CL_{h.int}$)と、*in vitro* 代謝実験で求めた $CL_{h.int}$ との比較

薬剤	血漿中非結合率% ※1	$CL_{h.int}$ (μ L/min/mg micorosme)		
		in vivo※1	*in vitro*	
			ヒト肝	バキュロウイルス ※2
propafenone	11	460	362 (1.3)	60.0 (7.7)
risperidone	11	38.5	34.2 (1.1)	7.79 (4.9)
tropisetron	42	39.4	2.58 (15.3)	0.92 (43)
propranolol	13	96.5	143 (0.7)	79.0 (1.2)
paroxetine	5	135	165 (0.8)	42.0 (3.2)
nortryptyline	8	70.5	51.2 (1.4)	48.5 (1.5)
carvedilol	5	136	213 (0.6)	174 (0.8)
metoprolol	89	13.2	3.80 (3.5)	1.66 (7.9)
venlafaxine	73	23.6	7.51 (3.1)	

(注) *in vitro* の列の ( ) 内は、固有クリアランスの *vivo / vitro* 比

※1：経口投与後の AUC と血漿中非結合率の文献値はインタビューフォームあるいは Goodman & Gilman's the pharmacological basis of therapeutics (9th edition)を参照した。
※2：バキュロウイルス発現系ミクロソームは、CYP2D6 の発現量（5 pmol CYP2D6/mg microsome；文献値　）と寄与率を考慮して、mg ミクロソーム蛋白量当たりの肝固有クリアランスに直した。

Table 6. *In vitro*から予測した*10/*10保因者の野生型に対するAUC上昇率と、*in vivo*報告値

薬剤	*10/*10		*10/*10AUC上昇率のvivo/vitro比	PM
	predicted AUC上昇率 (*vitro*)	observed AUC上昇率 (*vivo*)		observed AUC ratio (*vivo*)
venlafaxine	2.8 ± 0.6	5.5 ± 1.7	2.0	2.3 ± 1.1
propafenone	3.9 ± 0.5	2.1 ± 0.7	0.5	N A
risperidone	4.6 ± 0.2	3.1 ± 1.4	0.7	8.7 ± 4.8, 5.0 ± 0.1
metoprolol	3.2 ± 0.3	3.5 ± 0.5	1.1	5.8 ± 1.0, 4.2 ± 1.0, 3.1 ± 0.8
tropisetron	5.6 ※1	6.3 ± 2.0	1.1	3.1 ※2
desipramine	8.8 ± 7.4	N A	—	6.8 ± 1.5

（比較としてPMの野生型に対するAUC上昇率の*in vivo*報告値を右列に載せた）

*10/*10保因者あるいはPMとの比較, mean±SD
NA: not available

※1) 3例中のうち1ロットのクリアランスがN.Dであったので、*10/*10の固有クリアランスは残りの2ロットの平均を使用
※2) 6時間後の血漿中濃度比

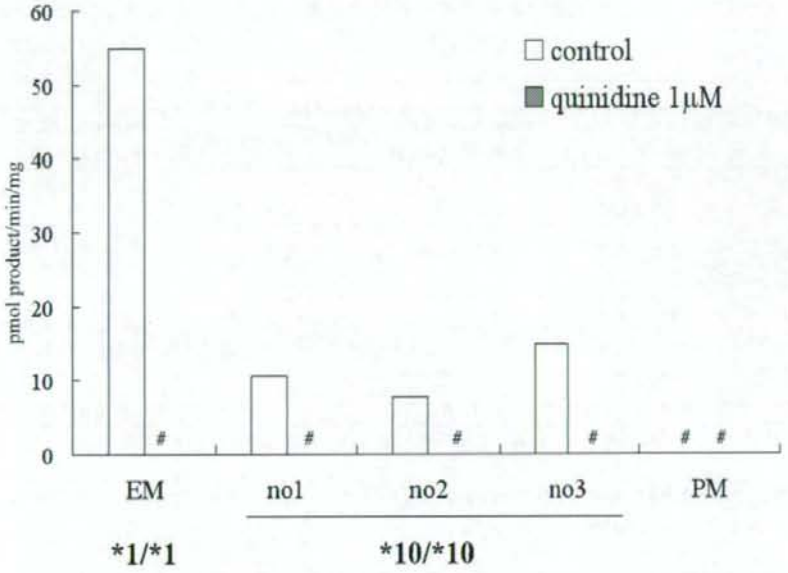


Fig. 1　ヒト肝ミクロソームにおける dextromethorphan O-demethylation 活性
#: quinidine 添加のサンプルの活性は全て検出されなかった。
duplicate のインキュベーションの平均を示す。

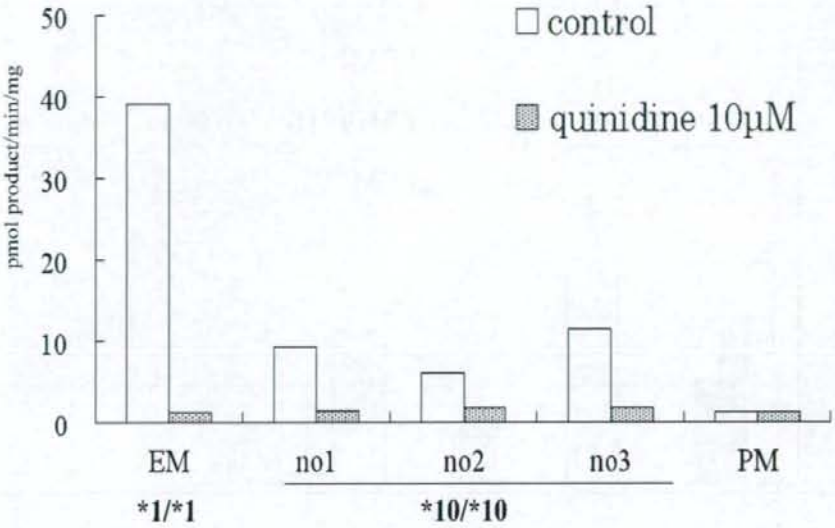


Fig. 2　ヒト肝ミクロソームにおける bufuralol 1'-hydroxylation 活性
duplicate のインキュベーションの平均を示す。

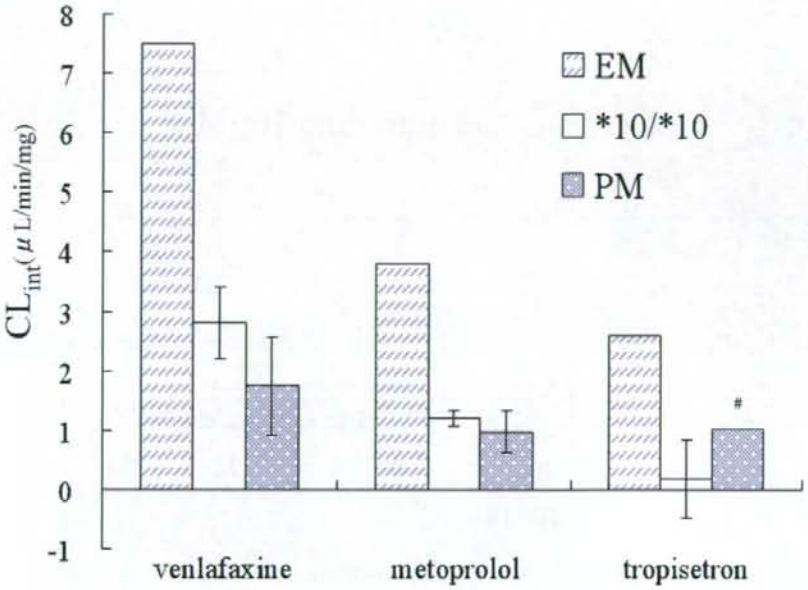
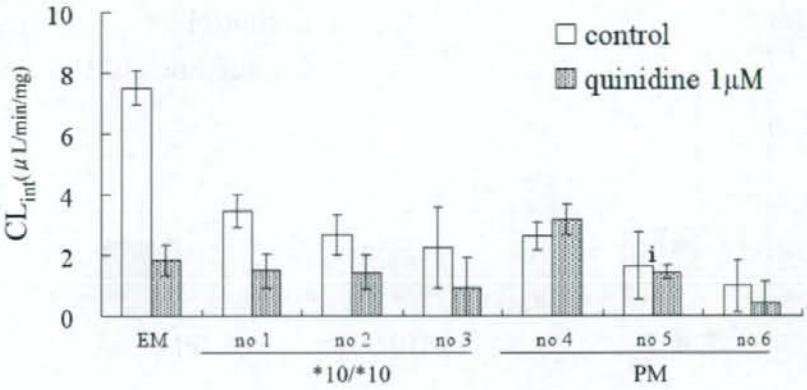


Fig. 3 各種ヒト肝ミクロソームの固有クリアランス　(低クリアランス薬物)
Triplicate のインキュベーションの平均±標準偏差
n = 1



i）PM lot no1： 3A5 plus

Fig. 4　ヒト肝ミクロソーム代謝実験における venlafaxine の固有クリアランス
各ロットのインキュベーションの平均±標準偏差

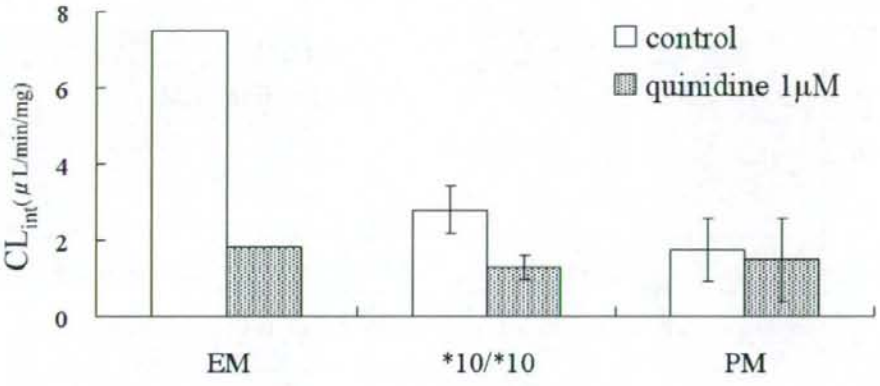


Fig. 5 ヒト肝ミクロソーム代謝実験における venlafaxine の固有クリアランス
*10/*10 , PM ともに 3 ロットの平均±標準偏差

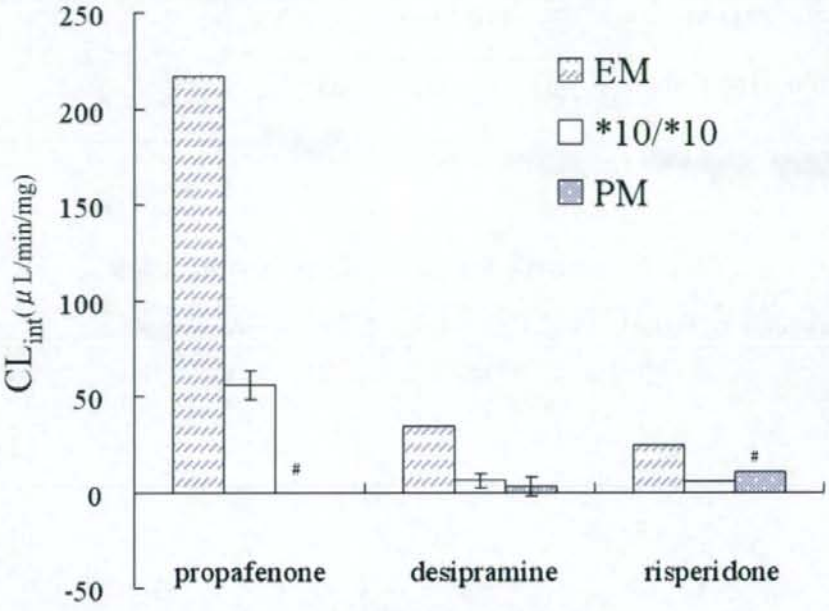


Fig. 6 ヒト肝ミクロソーム代謝実験における固有クリアランス　(高クリアランス薬物)
Triplicate のインキュベーションの平均±標準偏差
n = 1

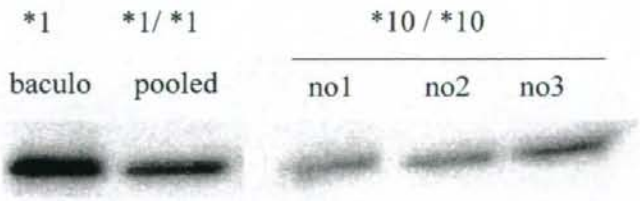


Fig. 7　ウェスタンブロットによる各種ヒト肝ミクロソーム中の CYP2D6 発現量

（左）　0.02pmol CYP2D6*1 バキュロウイルス発現系ミクロソーム（control）

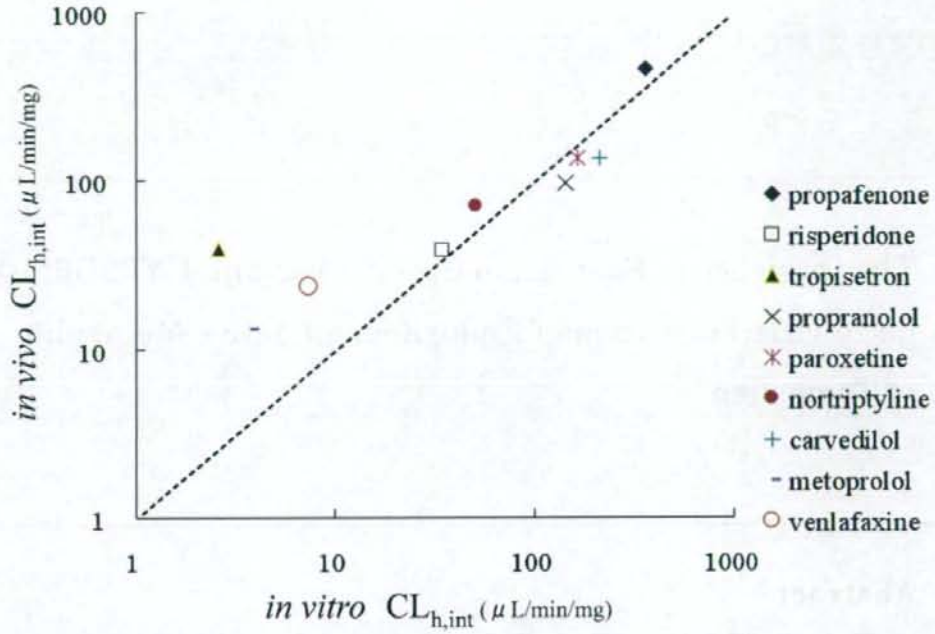


Fig. 8　EM の肝ミクロソームを用いた *in vitro* 代謝実験より求めた肝固有クリアランスと *in vivo* より算出した $CL_{h,int}$※との相関関係

* 経口投与後の AUC 及び血漿中蛋白非結合分率の文献値より算出

資料 2.

The Influence of East Asian Specific Variant, CYP2D6*10, on *in vitro* Formation of Endoxifen, an Active Metabolite of Tamoxifen

Abstract

Tamoxifen (TAM) is widely used in the breast cancer adjuvant therapy, and the most of its efficacy is ascribable to a secondary metabolite, endoxifen (EDX), which is generated from TAM by hepatic cytochrome P450s including CYP2D6. In the present study, we evaluated the *in vitro* metabolizing activity of CYP2D6*10/*10, an East Asian abundant variant with decreasing activity, to form EDX. *N*-Desmethyltamoxifen, a primary metabolite of TAM, was incubated with human liver microsomes from variants of CYP2D6*1/*1 (wild type), *4/*4 (typical genotype of "poor metabolizer" in Caucasians), and *10/*10. The formation rate of EDX by *10/*10 microsome was 23% of the *1/*1 microsome whereas no EDX was produced in the *4/*4 microsome. Based on these data, together with reported data, the steady state plasma concentrations of EDX in *10/*10 and *4/*4 subjects were predicted to be 36% and 17% of wildtype, respectively. Due to the decreased EDX plasma concentration, the relative plasma antiestrogenic potency during the TAM therapy was estimated to be reduced to 49% in*10/*10 subjects and 34% in *4/*4 subjects, respectively. These predictions indicated that the individualized TAM therapy based on CYP2D6 genotype would be clinically beneficial not only in Caucasians but also in East Asians.

Introduction

Tamoxifen (TAM) is a selective estrogen receptor modulator, which has been widely used for breast cancer around the world since its approval in 1973. Its label dosage is 20-40 mg/day orally, and this drug is administered for generally 5 years.

TAM and its most abundant primary metabolite, *N*-desmethyltamoxifen (NDM), share similar *in vitro* antiestrogenic activities, whereas both of another primary metabolite, 4-hydroxy-tamoxifen (4-OH), and a secondary metabolite, endoxifen (EDX, 4-hydroxy-*N*-desmethyltamoxifen), exert 100-fold higher activity than TAM and NDM (Fig. 1 and Refs. 1 and 2). Considering the lower plasma concentration of 4-OH than EDX, EDX should be the dominating efficacious component in the systemic circulation (2).

It is considered that a hepatic enzyme, CYP2D6, plays a key role in the generation of EDX from TAM (Fig. 1). Particularly, the conversion from NDX to EDX is exclusively mediated by CYP2D6 (2). CYP2D6 is one of the most polymorphic CYP enzymes and its variant frequency is highly dependent on ethnicity. Allele frequencies of inactive variants (*4 and *5) are higher in Caucasians than East Asians, whereas *10 with the decreased activity is East Asian specific. Although CYP2D6*10 homozygote is classified as the intermediate metabolizer (IM) in general, the blood concentration of some drugs were increased markedly in this population (3, 4), which is more evident than Caucasian IMs having genotypes of *1/*4 or *1/*5.

Jin *et al.* (5) have reported that the plasma EDX concentration in Caucasian poor metabolizers of CYP2D6 (PM; *4/*4) was reduced to 26% of the wildtype subjects. Furthermore, Goetz *et al.* (6) have reported that CYP2D6 PM had worse relapse-free time and disease-free survival compared to extensive metabolizers (EM; *1/*1) in the North Central Treatment Group (NCCTG) randomized Phase III trial. In view of these results, in October 2006, the FDA Clinical Pharmacology Subcommittee-Advisory Committee had recommended changes in TAM label so that patients can benefit equally from TAM based on genotype of CYP2D6.

Considering high allele frequency of *10 variants (40-50%) (7, 8), and increasing numbers of breast cancer patients in East Asian countries, there is a serious need to clarify the clinical significance of having the *10 allele(s) in the overall TAM therapy. However, no literature exists on the plasma concentration of EDX or the metabolizing activity to generate EDX in East Asian CYP2D6*10/*10 carriers to our knowledge. Therefore, we evaluated the metabolizing activity of the East Asian specific variant CYP2D6*10 on EDX formation from NDM using *10/*10 human hepatic microsome to predict the influence of the variant on the TAM therapy.