Impact of *CYP2D6*10* on recurrence-free survival in breast cancer patients receiving adjuvant tamoxifen therapy

Kazuma Kiyotani,[1] Taisei Mushiroda,[1] Mitsunori Sasa,[2] Yoshimi Bando,[3] Ikuko Sumitomo,[2] Naoya Hosono,[4] Michiaki Kubo,[4] Yusuke Nakamura[1,5] and Hitoshi Zembutsu[5,6]

[1]Laboratory for Pharmacogenetics, SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Tokyo 108-8639; [2]Department of Surgery, Tokushima Breast Care Clinic, Tokushima 770-0052; [3]Department of Molecular and Environmental Pathology, Institute of Health Biosciences, The University of Tokushima Graduate School, Tokushima 770-8503, [4]Laboratory for genotyping, SNP Research Center, The Institute of Physical and Chemical Research (RIKEN), Yokohama 230-0045; [5]Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo 108-8639, Japan

(Received December 3, 2007/Revised December 28, 2007; January 14 and 16, 2008/Accepted January 17, 2008/Online publication February 25, 2008)

The clinical outcomes of breast cancer patients treated with tamoxifen may be influenced by the activity of cytochrome P450 2D6 (CYP2D6) enzyme because tamixifen is metabolized by CYP2D6 to its active forms of antiestrogenic metabolite, 4-hydroxytamoxifen and endoxifen. We investigated the predictive value of the *CYP2D6*10* allele, which decreased CYP2D6 activity, for clinical outcomes of patients that received adjuvant tamoxifen monotherapy after surgical operation on breast cancer. Among 67 patients examined, those homozygous for the *CYP2D6*10* alleles revealed a significantly higher incidence of recurrence within 10 years after the operation ($P = 0.0057$; odds ratio, 16.63; 95% confidence interval, 1.75–158.12), compared with those homozygous for the wild-type *CYP2D6*1* alleles. The elevated risk of recurrence seemed to be dependent on the number of *CYP2D6*10* alleles ($P = 0.0031$ for trend). Cox proportional hazard analysis demonstrated that the *CYP2D6* genotype and tumor size were independent factors affecting recurrence-free survival. Patients with the *CYP2D6*10/*10* genotype showed a significantly shorter recurrence-free survival period ($P = 0.036$; adjusted hazard ratio, 10.04; 95% confidence interval, 1.17–86.27) compared to patients with *CYP2D6*1/*1* after adjustment of other prognosis factors. The present study suggests that the *CYP2D6* genotype should be considered when selecting adjuvant hormonal therapy for breast cancer patients. (*Cancer Sci* 2008; 99: 995–999)

Tamoxifen has been widely used for the treatment and prevention of recurrence for patients with estrogen receptor (ER)–positive breast cancer. The clinical benefit of this agent for the treatment of ER-positive early breast cancer is evident by the eminent reduction of recurrence and mortality rates.[1,2] However, 30–50% of patients with adjuvant tamoxifen therapy experience relapse and subsequently die of the disease.[1,2] Despite decades of research, the mechanisms underlying the ineffectiveness to a subset of the patients are not fully understood.

Tamoxifen is a prodrug that requires metabolic activation to elicit its pharmacological activity. It has been reported that its metabolites, 4-hydroxytamoxifen and 4-hydroxy-*N*-desmethyltamoxifen (endoxifen), are the active therapeutic moieties. Compared with the parent drug, these two metabolites have 100-fold greater affinity to ER and 30- to 100-fold greater potency in suppressing estrogen-dependent cell proliferation.[3–5] Thus, interindividual differences in the formation of these active metabolites could be one of important factors affecting variability in the response to tamoxifen. Cytochrome P450 2D6 (CYP2D6) is one of the key enzymes for the generation of 4-hydroxytamoxifen and endoxifen.[6] In the *CYP2D6* gene, many polymorphisms including alleles that alter the function and/or amount of the gene product have been reported (http://www.cypalleles.ki.se/cyp2d6.htm). Subjects with two null alleles are classified as poor metabolizers (PMs) of drugs that are metabolized mainly by CYP2D6, and 5–10% of Caucasians are considered to be PMs.[7] The *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5*, and *CYP2D6*6* are major null alleles that cause the PM phenotype and account for nearly 95% of the PMs in Caucasians.[8] Patients classified as PM were reported to have lower plasma levels of endoxifen and poorer clinical outcomes when treated with tamoxifen.[9–11] Although the frequency of PMs in Asians is much lower (only <1%),[12] the *CYP2D6*10* allele, which causes amino-acid substitutions in the gene product and results in instability of the protein, has been observed as a frequency of 40–50%.[13,14] However, the effects of *CYP2D6*10* on the clinical outcome of adjuvant tamoxifen therapy have not yet been investigated. In this study, we evaluated the association of *CYP2D6*10*, common in the Asian population, with clinical outcome of tamoxifen therapy.

Materials and Methods

Patients. Among 1764 patients who were pathologically diagnosed with breast cancer and received surgical treatment between 1986 and 2006 at Tokushima Breast Care Clinic, 468 patients were received adjuvant monotherapy of tamoxifen. The patients who came to Tokushima Breast Care Clinic from September to November in 2007 were registered in this study according to the following criteria: (1) they received adjuvant monotherapy of tamoxifen without any chemotherapy, (2) they were ER and/or progesterone receptor (PR) positive, (3) they gave written informed consent. The consecutive 72 patients that participated in this study had been treated with tamoxifen at a dose of 20 mg/body/day for 5 years. We excluded 5 ductal carcinoma *in situ* (DCIS) samples from the 72 samples and used 67 invasive breast cancer samples for the analysis. No patient received selective serotonin reuptake inhibitors. The nuclear grade and status of ER, PR, and Her-2 expression in breast cancer cells were investigated in 53 patents whose paraffin-embedded tissues were available. ER and PR statuses were evaluated by immunohistochemistry according to the Allred system.[15] The cut-off for Her-2 overexpression was defined as 3+ stained in immunohistochemistry.[16] Nodal status was determined according to the International Union Against Cancer tumor-node-metastasis classification. Nuclear grade was classified

[6]To whom all correspondence should be addressed.
E-mail: zembutsh@ims.u-tokyo.ac.jp

doi: 10.1111/j.1349-7006.2008.00780.x

by the criteria of National Surgical Adjuvant Study of Breast Cancer. This study was approved by the Ethical Committee at the Institute of Medical Science, the University of Tokyo, Tokyo, Japan.

Genotyping. Genomic DNA was extracted from peripheral whole blood of each patient using Qiagen DNA extraction kit (Qiagen, Valencia, CA, USA). The alleles of *CYP2D6*4*, *CYP2D6*5*, *CYP2D6*6*, *CYP2D6*10*, *CYP2D6*14*, *CYP2D6*18*, *CYP2D6*21*, and *CYP2D6*41* were genotyped using multiplex polymerase chain reaction (PCR)–based Invader assay and TaqMan assay as reported previously.[17,18] Briefly, we used TaKaRa *Ex Taq* HS (TaKaRa Bio, Shiga, Japan) for PCR amplification using specific primers for the *CYP2D6* gene. PCR was performed on GeneAmp 9700 (Applied Biosystems, Foster City, CA, USA) with a reaction volume of 10 μL. The PCR condition was initiated at 95°C for 2 min followed by 35 cycles at 95°C for 15 s and 68°C for 4 min. After PCR, the products were diluted up to 10-fold and used as templates for the Invader assay. The fluorescent signal was detected using ABI prism 7900HT (Applied Biosystems).

End point and statistical analysis. Recurrence-free survival period was defined as the period between surgical treatment to the recurrence of a breast cancer (i.e. local or distant recurrence, or contralateral breast cancer). Patients without recurrence were censored at the date of the last follow-up inquiry. We calculated risk estimates of an association between genotype and recurrence. An association between genetic variants of the *CYP2D6* and clinical benefit after 10 years was tested with Fisher's exact test. The trends of association between *CYP2D6* genotype and the incidence of recurrence were estimated with Cochran–Armitage test. Recurrence-free survival was analyzed by *CYP2D6* genotype using Kaplan–Meier methods. Statistical significance of a relationship between outcome and genetic polymorphism was assessed by log-rank test. Cox proportional hazard analysis was used to identify significant prognostic clinical factors and to test for an independent contribution of genetic factors to the outcome variable. The significant subsets of variables in the univariate analysis were used in the multivariate analysis. Statistical analyses were carried out using StatView software version 5.0 (SAS Institute, Cary, NC, USA).

Results

Patient characteristics. Table 1 shows the characteristics of 67 patients received adjuvant tamoxifen therapy. Their median age at the time of surgery was 50 years old (range, 34–82 years), and the median follow-up period was 8 years (range, 1.6–21.6 years). The numbers of pre- and postmenopausal patients were 35 and 32, respectively.

Genotype frequency. We examined DNAs of 67 invasive breast cancer patients for the *CYP2D6*1*, *CYP2D6*4*, *CYP2D6*5*, *CYP2D6*6*, *CYP2D6*10*, *CYP2D6*14*, *CYP2D6*18*, *CYP2D6*21*, and *CYP2D6*41* alleles (Table 2). The allele frequency of *CYP2D6*10* was calculated to be 41.8%. Seven patients carried the *CYP2D6*5* heterozygous variant, indicating its allele frequency to be 5.2%. There was one heterozygous patient for each of the *CYP2D6*4*, *CYP2D6*21*, or *CYP2D6*41* alleles. No *CYP2D6*6*, *CYP2D6*14*, and *CYP2D6*18* allele was observed in this test population. We then focused on patients with the *CYP2D6*10/*10*, *CYP2D6*1/*10*, and *CYP2D6*1/*1* genotypes for the following analysis because the frequencies of the other alleles were too low to be analyzed.

Associations between genotype and clinical outcome. Patients with the *CYP2D6*10/*10* genotype revealed a significantly higher incidence of recurrence than those with the *CYP2D6*1/*1* genotype ($P = 0.0057$; odds ratio, 16.63; 95% confidence interval, 1.75–158.12; Table 3) or the combined patient group carrying at least one *CYP2D6*1* allele (*CYP2D6*1/*1 + *1/*10*) ($P = 0.0079$; odds ratio, 6.65; 95% confidence interval, 1.68–26.38). The tendency of an increase of the incidence of recurrence by an increase of the number of *CYP2D6*10* alleles ($P = 0.0031$ for trend) was also observed. Kaplan–Meier estimates indicated the significantly shorter recurrence-free survival for patients with *CYP2D6*10/*10* than those with *CYP2D6*1/*1* or those with *CYP2D6*1/*1 + *1/*10* ($P = 0.0031$ or $P = 0.0010$; Fig. 1). In the univariate Cox proportional hazard analysis for recurrence-free survival, *CYP2D6* genotype (**10/*10 versus *1/*1*) and tumor size were considered to be significantly associated factors (Table 4). In the multivariate analysis with the significant parameters in the

Table 1. Characteristics of patients

Characteristic	Total ($N = 67$) Number of patients (%)
Age at surgery, years	
Median	50
Range	34–82
Menopausal status	
Premenopausal	35 (52.2)
Postmenopausal	32 (47.8)
Tumor size, cm	
≤2	41 (61.2)
2–5	26 (38.8)
Nodal status	
n0	48 (71.6)
n1	19 (28.4)
Nuclear grade	
1	36 (53.7)
2	9 (13.4)
3	8 (11.9)
Unknown	14 (20.9)
Estrogen receptor status†	
≤2	3 (4.5)
3–6	19 (28.4)
≥7	31 (46.3)
Unknown	14 (20.9)
Progesterone receptor status†	
≤2	7 (10.4)
3–6	29 (43.3)
≥7	17 (25.4)
Unknown	14 (20.9)
Her-2	
Positive‡	3 (4.5)
Negative	48 (71.6)
Unknown	16 (23.9)

†Estrogen receptor and progesterone receptor statuses were shown as Allred score by immunohistochemistry.
‡Score of 3+ in immunohistochemistry.

Table 2. Genotype frequency of *CYP2D6*

CYP2D6 genotype	*N* (%)
**1/*1*	20 (29.9)
**1/*4*	1 (1.5)
**1/*5*	4 (6.0)
**1/*10*	23 (34.3)
**5/*10*	2 (3.0)
**5/*41*	1 (1.5)
**10/*10*	15 (22.4)
**10/*21*	1 (1.5)

doi: 10.1111/j.1349-7006.2008.00780.x

Table 3. Risk of recurrence within 10 years for the *CYP2D6* genotype in breast cancer patients treated with tamoxifen

CYP2D6 genotype	No event, *N* (%) (*N* = 46)	Event[†], *N* (%) (*N* = 12)	*versus* *1/*1		*versus* *1/*1 + *1/*10	
			P-value	Odds ratio [95% CI]	*P*-value	Odds ratio [95% CI]
*1/*1	19 (41.3)	1 (8.3)	–[‡]	1.00[‡]	–[‡]	1.00[‡]
*1/*10	19 (41.3)	4 (33.3)	0.35	4.00 [0.41–39.18]		
*10/*10	8 (17.4)	7 (58.3)	0.0057	16.63 [1.75–158.12]	0.0079	6.65 [1.68–26.38]

[†]Recurrent site.
**1/*1*: one local.
**1/*10*: one contralateral breast, three regional lymph nodes.
**10/*10*: one local, two contralateral breast, three regional lymph nodes, one osseous, and pulmonary.
[‡]Reference category.
CI, confidence interval.

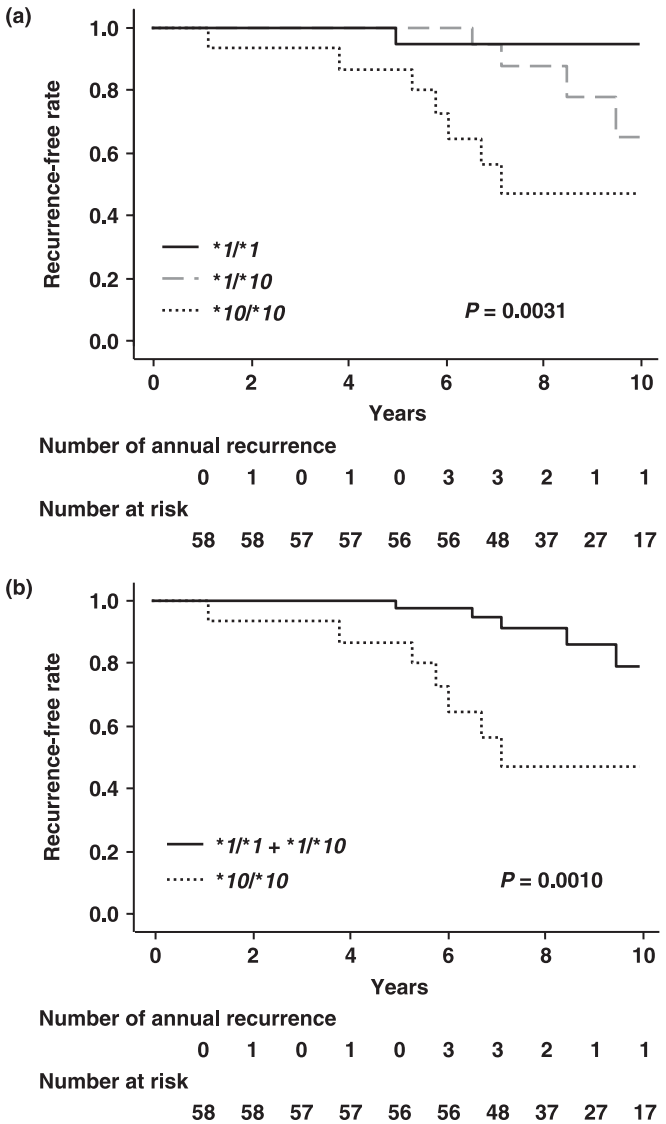

Fig. 1. Kaplan–Meier estimates of recurrence-free survival rate for patients with the *CYP2D6*10* genotype. (a) Comparison among *CYP2D6*1/*1*, **1/*10* and **10/*10* patients. (b) Comparison between *CYP2D6*1/*1* + **1/*10* and **10/*10* patients.

univariate analysis, the *CYP2D6* genotype still remained an independent indicator of recurrence after adjustment for tumor size ($P = 0.036$; adjusted hazard ratio, 10.04; 95% confidence interval, 1.17–86.27 for *CYP2D6*10/*10*).

Discussion

Some years of adjuvant tamoxifen treatment substantially improves the 10-year survival of women with ER-positive tumors (including unknown ER status), with the significant reductions in breast cancer recurrence and in mortality.[1] Although aromatase inhibitors (AIs) have demonstrated their superiority to tamoxifen as an adjuvant therapy for early breast cancer in postmenopausal women, there are some reports indicating that AIs are associated with a higher risk of osteoporosis than tamoxifen.[19,20] In addition, the cost of tamoxifen therapy is significantly lower than that of AIs. Considering many years of administration of these drugs, the effect on the medical cost is not small. Therefore, tamoxifen will still play a major therapeutic role in ER-positive breast cancer. In this study, we investigated the association of the *CYP2D6*10* allele, which encodes the unstable protein (hence, the enzymatic activity is low) and is most frequently found in Asians, including Japanese people, with clinical outcomes of breast cancer patients treated with adjuvant tamoxifen monotherapy. We here demonstrated that patients with *CYP2D6*10/*10* showed a significantly higher incidence of recurrence than patients with *CYP2D6*1/*1*.

In clinical studies in Caucasians, patients with the *CYP2D6*4/*4* genotype, classified as PMs, had poorer clinical outcomes with shorter recurrence-free and disease-free survivals, and it was also shown that the 5-year recurrence-free survival rate for *CYP2D6*4/*4* patients was only 54%, compared with 83% for those who were not carriers of the *CYP2D6*4* allele.[10] In this study, the 10-year recurrence-free survival rates between the *CYP2D6*1/*1* and *CYP2D6*10/*10* groups were significantly different (95% and 53.3%, $P = 0.0057$). The plasma concentrations of endoxifen in patients with *CYP2D6*10/*10* was reported to be as low as for PMs.[9,21] It is also reported that the conversion rate of endoxifen from *N*-desmethyltamoxifen by mutant enzyme encoded by *CYP2D6*10* was only 7.4% of that of the wild-type protein in *in vitro* analysis.[22] These lines of evidence imply that *CYP2D6*10* remarkably reduces the plasma levels of active metabolites, and influences the clinical outcomes in adjuvant tamoxifen therapy, although the degree of its effects are not conclusive due to the small sample size.

The 5-year recurrence-free survival rate of patients with *CYP2D6*10/*10* (86.7%) tended to be lower than that with *CYP2D6*1/*1* (100%), without statistical significance ($P = 0.18$; data not shown), although 10-year recurrence-free survival rates were significantly different between the two genotypes. This result can be explained by the lower recurrence rate within the first 5 years for all patients in this study compared with a previous report[2] (Fig. 2). This observation might be caused by a limited number of samples and a limited registration period (3 months). Some of the patients with early recurrence were obviously unable to participate in this study because

Table 4. Cox proportional hazard analysis for recurrence-free survival in breast cancer patients treated with tamoxifen

Variables	Univariate		Multivariate	
	P-value	Hazard ratio [95% CI]	*P*-value	Hazard ratio [95% CI]
CYP2D6 genotype				
**1/*10*	0.49	2.19 [0.24–19.79]		
**10/*10*	0.044	8.67 [1.06–71.09]	0.036	10.04 [1.17–86.27]
Age	1.00	0.99 [0.94–1.06]		
Menopausal status	0.97	0.96 [0.31–3.04]		
Tumor size	0.0090	2.24 [1.22–4.09]	0.023	2.15 [1.11–4.16]
Nodal status	0.39	1.71 [0.51–5.69]		
Nuclear grade	0.41	1.37 [0.65–2.87]		
ER	0.40	1.15 [0.83–1.61]		
PR	0.45	0.90 [0.68–1.18]		
Her-2	0.46	2.19 [0.28–17.38]		

Note: Hazard ratio for **1/*10* or **10/*10* relative to **1/*1* is shown. Menopausal status, post *versus* premenopausal; and nodal status, n1 *versus* n0; Her-2, positive *versus* negative; were analyzed as binary variables. The others were analyzed as ordinal variables. CI, confidence interval; ER, estrogen receptor status; PR, progesterone receptor.

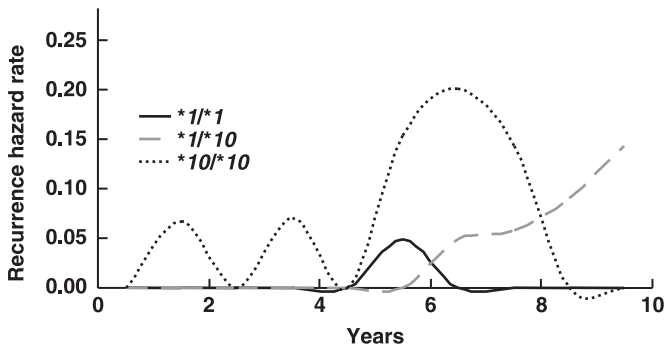

Fig. 2. Annual hazard rates for recurrence-free survival comparing patients with the *CYP2D6*1/*1*, **1/*10* and **10/*10* genotypes.

they could not visit the clinic due to their poor health condition or death. Therefore, further analysis using a large number of registered patients with longer entry periods is required for verification of our results and investigation of the effect of *CYP2D6*10* on overall survival.

The allelic frequency of *CYP2D6*10* has been reported to be 38.6% (41.8% in this study) in the Japanese population.[23] Approximately 15% of Japanese people are estimated to have the *CYP2D6*10/*10* genotype. Although the PM frequency in Japanese is lower than in Caucasians, several PM- or intermediate (IM)-related polymorphisms in the *CYP2D6* gene were reported (i.e. **4*, **5*, **14*, **18*, **21*, **41*, **44*),[21–24] and their combined frequency was 11.4%.[23–26] Hence, the frequency of heterozygote for **10* and either of these alleles was suspected to be 8–9%. Therefore, the frequency of subjects classified as IM-related to the *CYP2D6*10* allele was estimated to be 23–24%. Patients heterozygous for the *CYP2D6*10* allele and the other null allele also revealed the IM phenotype, which might have a higher risk of recurrence at a level almost the same as for PMs.[27] These data indicate that *CYP2D6*10* is one of the most important determinants for clinical outcomes in adjuvant tamoxifen therapy, especially in the Asian population. The application of pharmacogenomic/pharmacogenetic information to clinical treatment is expected to contribute to the prediction of drug efficacy and/or toxicity of individual patients. The genotyping of *CYP2D6*, including *CYP2D6*10*, should become an important predictor for the efficacy of tamoxifen treatment for individual breast cancer patients.

Acknowledgments

We thank Ms. Pei-Chieng Cha and Ms. Yuka Kikuchi for technical assistance. We thank all members of the SNP Research Center, the Institute of Physical and Chemical Research; the Institute of Medical Science, the University of Tokyo; and Tokushima Breast Care Clinic, for their contribution to the completion of our study.

References

1 Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Tamoxifen for early breast cancer: an overview of the randomised trials. *Lancet* 1998; **351**: 1451–67.
2 Early Breast Cancer Trialists' Collaborative Group (EBCTCG). Effects of chemotherapy and hormonal therapy for early breast cancer on recurrence and 15-year survival: an overview of the randomised trials. *Lancet* 2005; **365**: 1687–717.
3 Borgna JL, Rochefort H. Hydroxylated metabolites of tamoxifen are formed *in vivo* and bound to estrogen receptor in target tissues. *J Biol Chem* 1981; **256**: 859–68.
4 Lien EA, Solheim E, Lea OA, Lundgren S, Kvinnsland S, Ueland PM. Distribution of 4-hydroxy-*N*-desmethyltamoxifen and other tamoxifen metabolites in human biological fluids during tamoxifen treatment. *Cancer Res* 1989; **49**: 2175–83.
5 Johnson MD, Zuo H, Lee KH *et al*. Pharmacological characterization of 4-hydroxy-*N*-desmethyl tamoxifen, a novel active metabolite of tamoxifen. *Breast Cancer Res Treat* 2004; **85**: 151–9.
6 Desta Z, Ward BA, Soukhova NV, Flockhart DA. Comprehensive evaluation of tamoxifen sequential biotransformation by the human cytochrome P450 system *in vitro*: prominent roles for CYP3A and CYP2D6. *J Pharmacol Exp Ther* 2004; **310**: 1062–75.
7 Broly F, Gaedigk A, Heim M, Eichelbaum M, Morike K, Meyer UA. Debrisoquine/sparteine hydroxylation genotype and phenotype: analysis of common mutations and alleles of *CYP2D6* in a European population. *DNA Cell Biol* 1991; **10**: 545–58.
8 Sachse C, Brockmöller J, Hildebrand M, Müller K, Roots I. Correctness of prediction of the CYP2D6 phenotype confirmed by genotyping 47 intermediate and poor metabolizers of debrisoquine. *Pharmacogenetics* 1998; **8**: 181–5.
9 Borges S, Desta Z, Li L *et al*. Quantitative effect of *CYP2D6* genotype and inhibitors on tamoxifen metabolism: implication for optimization of breast cancer treatment. *Clin Pharmacol Ther* 2006; **80**: 61–74.
10 Goetz MP, Rae JM, Suman VJ *et al*. Pharmacogenetics of tamoxifen biotransformation is associated with clinical outcomes of efficacy and hot flashes. *J Clin Oncol* 2005; **23**: 9312–8.
11 Goetz MP, Knox SK, Suman VJ *et al*. The impact of cytochrome P450 2D6 metabolism in women receiving adjuvant tamoxifen. *Breast Cancer Res Treat* 2007; **101**: 113–21.
12 Nakamura K, Goto F, Ray WA *et al*. Interethnic differences in genetic polymorphism of debrisoquin and mephenytoin hydroxylation between Japanese and Caucasian populations. *Clin Pharmacol Ther* 1985; **38**: 402–8.

doi: 10.1111/j.1349-7006.2008.00780.x

13 Yokota H, Tamura S, Furuya H *et al*. Evidence for a new variant *CYP2D6* allele *CYP2D6J* in a Japanese population associated with lower *in vivo* rates of sparteine metabolism. *Pharmacogenetics* 1993; **3**: 256–63.
14 Nakamura K, Ariyoshi N, Yokoi T *et al*. CYP2D6.10 present in human liver microsomes shows low catalytic activity and thermal stability. *Biochem Biophys Res Commun* 2002; **293**: 969–73.
15 Harvey JM, Clark GM, Osborne CK, Allred DC. Estrogen receptor status by immunohistochemistry is superior to the ligand-binding assay for predicting response to adjuvant endocrine therapy in breast cancer. *J Clin Oncol* 1999; **17**: 1474–81.
16 Moeder CB, Giltnane JM, Harigopal M *et al*. Quantitative justification of the change from 10% to 30% for human epidermal growth factor receptor 2 scoring in the American Society of Clinical Oncology/College of American Pathologists guidelines: tumor heterogeneity in breast cancer and its implications for tissue microarray based assessment of outcome. *J Clin Oncol* 2007; **25**: 5418–25.
17 Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome–wide association studies. *J Hum Genet* 2001; **46**: 471–7.
18 Hosono N, Kubo M, Tsuchiya Y *et al*. Multiplex PCR-based real-time invader assay (mPCR-RETINA): a novel SNP-based method for detecting allelic asymmetries within copy number variation regions. *Hum Mutat* 2007; **29**: 182–18.
19 Howell A, Cuzick J, Baum M *et al*. Results of the ATAC (Arimidex, Tamoxifen, Alone or in Combination) trial after completion of 5 years' adjuvant treatment for breast cancer. *Lancet* 2005; **365**: 60–2.
20 Coates AS, Keshaviah A, Thürlimann B *et al*. Five years of letrozole compared with tamoxifen as initial adjuvant therapy for postmenopausal women with endocrine-responsive early breast cancer: update of study BIG 1-98. *J Clin Oncol* 2007; **25**: 486–92.
21 Lim HS, Ju Lee H, Seok Lee K, Sook Lee E, Jang IJ, Ro J. Clinical implications of CYP2D6 genotypes predictive of tamoxifen pharmacokinetics in metastatic breast cancer. *J Clin Oncol* 2007; **25**: 3837–45.
22 Kusama M, Hisaka A, Hibino Y, Suzuki H. The influence of Asian specific cariant, CYP2D6*10 on *in vitro* formation of endxifen, an active metabolite of tamoxifen. *In Proceedings of the 6th JSSX Meeting*, 2006: 229.
23 Kubota T, Yamaura Y, Ohkawa N, Hara H, Chiba K. Frequencies of *CYP2D6* variant alleles in a normal Japanese population and metabolic activity of dextromethorphan *O*-demethylation in different *CYP2D6* genotypes. *Br J Clin Pharmacol* 2000; **50**: 31–4.
24 Yokoi T, Kosaka Y, Chida M *et al*. A new *CYP2D6* allele with a nine base insertion in exon 9 in a Japanese population associated with poor metabolizer phenotype. *Pharmacogenetics* 1996; **6**: 395–401.
25 Chida M, Yokoi T, Nemoto N, Inaba M, Kinoshita M, Kamataki T. A new variant *CYP2D6* allele (*CYP2D6*21*) with a single base insertion in exon 5 in a Japanese population associated with a poor metabolizer phenotype. *Pharmacogenetics* 1999; **9**: 287–93.
26 Yamazaki H, Kiyotani K, Tsubuko S *et al*. Two novel haplotypes of *CYP2D6* gene in a Japanese population. *Drug Metab Pharmacokinet* 2003; **18**: 269–71.
27 Schroth W, Antoniadou L, Fritz P *et al*. Breast cancer treatment outcome with adjuvant tamoxifen relative to patient CYP2D6 and CYP2C19 genotypes. *J Clin Oncol* 2007; **25**: 5187–93.