Acta Oncologica, 2006; 45: 597–601

Taylor & Francis
Taylor & Francis Group

ORIGINAL ARTICLE

The association of CYP2D6 *10 polymorphism with breast cancer risk and clinico-pathologic characteristics in Chinese women

HONGXIA LI[1], LIYA FENG[1], YE XU[1], LIHUA YAO[1], TAO OUYANG[1], JINFENG LI[1], TIANFENG WANG[1], ZHAOQING FAN[1], BENYAO LIN[1], JIYOU LI [2] & YUNTAO XIE[1]

[1]*Breast Centre, Beijing Cancer Hospital, Peking University School of Oncology, Beijing, P. R. China, and* [2]*Department of Pathology, Beijing Cancer Hospital, Peking University School of Oncology, Beijing, P. R. China*

Abstract

A relatively little is known of whether CYP2D6 *10 (188 C to T) polymorphism mediates susceptibility to breast cancer. In this study the CYP2D6 *10 polymorphism was detected in Chinese women (286 breast cancer patients and 305 healthy women) by a PCR-RFLP assay. We found that women with the 188T/T genotype displayed a slightly increased risk for breast cancer as compared with those with the 188C/C genotype (OR 1.36, CI 0.89–2.1), the association of the 188T/T genotype with breast cancer risk was more pronounced among postmenopausal women (OR 1.49, CI 0.8–2.76), but the association did not reach statistical significance. Furthermore, we found that patients carrying the 188T/T or T/C genotype were more likely to be a positive lymph node status than those with the 188C/C genotype (OR 2.12, CI 1.08–4.18, P = 0.019). Our results suggest that CYP2D6 *10 mutant 188T/T genotype displays a non-significant increased risk for breast cancer. Moreover, patients carrying 188T/T or T/C genotype might exhibit a more aggressive phenotype than those carrying 188C/C genotype, as the observation association of genotype with clinical outcome may be due to chance, therefore, further studies are required to confirm our present findings.

The incidence of breast cancer has been increasing in China for the last decade, particularly in large cities [1]. Thus, identifying new risk factors for breast cancer can have a great impact on the prevention and screening for this disease.

Increasing evidence suggests that breast cancer may result from the interaction between the genetic elements and a variety of environmental factors. The familial breast cancer owing to BRCA1 and/or BRCA2 mutations accounts for a small fraction of the disease [2]. The majority of breast cancer patients are sporadic with no family history of the disease, indicating that these cases can be mainly due to environmentally induced carcinogenesis. The Cytochrome P450 (CYP) catalytic enzymes that participate in the activation and deactivation of diverse chemical carcinogens, therefore, are extremely important [3]. Indeed, the association of CYP with breast cancer risk has been studied extensively where it has been shown that the polymorphisms of the CYP17 and CYP19 are associated with breast cancer risk [4]. On the other hand, the association of the polymorphism of CYP2D6 with breast cancer risk is not firmly established. Previous studies suggested that CYP2D6 mutant alleles may exhibit a low increased risk for breast cancer in Caucasians populations [5–9].

CYP2D6 gene, a member of the CYP superfamily, encodes a phase I enzyme that is highly polymorphic with more than 91 allele variants identified so far (http://www.imm.ki.se/cypalleles/). The most common CYP2D6 variants in Caucasians are variants *3, *4 and *5, resulting in a decreased or absence of enzyme activity, and leading to poor metabolizer phenotypes (PM) [10–12]. However, these mutant alleles are rarely found in the Chinese population [13], the most common polymorphism of CYP2D6 in the Chinese population is variant *10 (formerly defined as CYP2D6 *Ch1*) [13–16], which is relatively rare in Caucasians population. The CYP2D6 *10 polymorphism exhibits a 188 C to T transition in exon 1, resulting in a Proline 34 to Serine amino acid substitution, and leading to an unstable form of the enzyme with lower catalytic activity [15].

Correspondence: Y. Xie or J. Li, Breast Centre, Beijing Cancer Hospital, Peking University School of Oncology, 100036, Beijing, P. R. China. Fax: +86 10 88196362. E-mail: zlxyt2@bjmu.edu.cn or lijiyou@263.net

(Received 17 May 2005; accepted 27 February 2006)

ISSN 0284-186X print/ISSN 1651-226X online © 2006 Taylor & Francis
DOI: 10.1080/02841860600660803

To our best acknowledge, no study so far was focused on the correlation between the CYP2D6 *10 polymorphism and breast cancer risk. Therefore, in this study we detected the CYP2D6 *10 polymorphism in total 591 subjects, our aims were to investigate the potential role of the CYP2D6 *10 polymorphism in breast cancer risk in Chinese women, and furthermore to assess the correlation of CYP2D6 *10 polymorphism with the clinico-pathologic characteristics in this cohort of patients.

Materials and methods

Subjects

This case-control study consisted of 286 consecutive breast cancer patients (Stage I to III) and 305 healthy women. The patients were treated at Breast Centre, Peking University School of Oncology from January 2004 to December 2004. Pathological diagnosis was performed for all patients. More than 80% of patients in this cohort received an Anthracyclin-based or Paclitaxel-based neoadjuvant chemotherapy prior to surgery, after completion of the chemotherapy, patients received a modified radical mastectomy or breast conserving surgery depending of the primary tumour size, the axillary lymph nodes were routinely dissected at least level I and II, and whether the lymph node metastasis or not was determined based on the histological examination. The control subjects were selected from the healthy women who regularly went to hospital for medical examination.

All patients and controls were Han nationality and residents of Beijing city and its surrounding area. The informed consent was obtained from all cases and controls. This study was approved by the Research and Ethics committee of Peking University School of Oncology.

Immunohistochemistry

Immunostaining was performed as described elsewhere [17]. The following panel of monoclonal antibodies was applied: Anti-oestrogen receptor (ER) Mab (clone 1D5, dilution 1:50, Dako); anti-progesterone receptor (PR) Mab (clone 1A6, dilution 1:100, Novocastra); anti-C-erbB2 Mab (clone CB11, dilution 1:40, Zymed, S. Francisco, CA. USA). For ER and PR staining, cells were considered to be positive only when distinct nuclear staining was identified. ER and PR were considered positive when $\geq 25\%$ of tumour cells showed positive staining. For C-erbB2 staining, only the membrane staining was considered as positive staining. The score for C-erbB2 staining was graded as follow: No staining or membrane staining observed in $<10\%$ of tumour cells was given a score 0; faint/barely perceptible membrane staining detected in $>10\%$ of tumour cells was scored as 1+; a moderate or strong complete membrane staining observed in $>10\%$ of tumour cells was graded 2+ or 3+, respectively. A score of 0 and 1+ was considered negative, whereas 2+ and 3+ were considered positive.

DNA extraction and genotyping

Blood samples were collected from each patient at the time of diagnosis, and genomic DNA was extracted from peripheral blood lymphocytes using phenol-chloroform extraction. CYP2D6 *10 polymorphism was detected by using a PCR-RFLP technique. The following primers were used, forward primer 5′- TCA ACA CAG CAG GTT CA -3′ and reverse primer 5′- CTG TGG TTT CAC CCA CC -3′, as previously described by Sachse et al. [10]. PCR was performed in 20 μl reaction mixture containing 100 ng of genomic DNA template, 2 μl 10X PCR buffer, 0.8 mM dNTP, 2.5 mM $MgCl_2$, 0.5 μM primers, and 1 unit AmpliTaq DNA polymerase (Promega). The reaction condition employed were initial denaturation at 94°C for 2 min, followed by 35 step cycles of denaturation at 94°C for 30 s, annealing 56°C for 45 s, and extension 72°C for 30 s followed by a terminal extension time of 10 min. Ten μl of PCR product was digested with Hph I restriction enzyme (New England Biolabs Inc.) for 1 h at 37°C. The digestion products were then resolved on a 2.5% agarose gel containing ethidium bromide. The homozygous 188 C/C genotype was identified by two bands (362bp and 71bp), the homozygous 188 T/T genotype produced three bands (262bp, 100bp and 71bp), and heterozygous 188C/T genotype displayed four bands (362bp, 262bp, 100 and 71bp).

Statistical analysis

The correlation between CYP2D6 *10 polymorphism and breast cancer risk was assessed using a logistic regression method to ascertain the Odds Ratio (OR) and 95% confidence interval (CI). The difference of allele frequency between the cases and controls, and the correlation between the genotype variants and clinico-pathologic characteristics were determined using Pearson's X^2 test. Two-sided p-values less than 0.05 were considered as statistically significant. All statistical analyses were performed using SPSS 10.0 software.

Results

*The correlation between the CYP2D6 *10 polymorphism and breast cancer risk*

The age distribution between the cases (range 23 to 84 years, mean age 52 years, SD 12 years) and controls (range 22 to 76 years, mean age 50 years, SD 12 years) was not significantly different. No significant difference was found for menopausal status, parity, and age of first live birth in this case-control study (data not shown).

The frequency of CYP2D6 *10 genotypes among the cases and controls is presented in Table I. The most common variant was mutant homozygous 188T/T genotype, and the frequency of the T/T genotype was higher among the breast cancer patients than that of the controls (0.49 versus 0.45), however, the difference did not reach statistical significance (OR 1.36, CI 0.89–2.1, p = 0.15, Table I). We then further analysed the correlation of the CYP2D6 *10 polymorphism with breast cancer risk according to the menopausal status, we found that postmenopausal women with the T/T genotype showed a moderate increased risk for breast cancer (OR 1.49, CI 0.8–2.76, Table I), this correlation again did not reach statistical significance (p = 0.205, Table I). On the other hand, the frequency of genotypes and correlation of the T/T genotype with breast cancer risk among the premenopausal women were similar to that observed for the entire population (Table I).

*The association between the CYP2D6 *10 genotypes and clinico-pathologic characteristics*

No correlation was found between the CYP2D6 *10 genotypes and ER or PR status, tumour size, C-erbB2 status or clinical stage (data not shown). However, the CYP2D6 *10 polymorphism was significantly associated with lymph nodes metastases. Patients with the T/T or T/C genotype were more likely to be a positive lymph node status than those with the C/C genotype (35.2% or 46.6% versus 23.6%, p = 0.019, Table II). As compared with patients with the C/C genotype, patients with the T/T or T/C genotype had 2.1-fold increased risk to be a positive lymph node status (OR 2.1, CI 1.08–4.18).

Discussion

Previous studies have shown that CYP2D6 polymorphism is associated with carcinogenesis in a wide range of tumours [4,18–21]. Nevertheless, when the association of CYP2D6 polymorphism with breast cancer risk exists, such association is modest [5–8]. The CYP2D6 *10 variant is the most common polymorphism in the Chinese population, in which the mutant homozygous 188T/T genotype represents around 50% of the population [13–15], and this was demonstrated in the present study. The 188T/T genotype produces an unstable enzyme with shorter half-life and lower activity [15]. *In vitro* experiments have shown that the catalytic activity of the 188T/T genotype is 1/40th of the activity of wild type 188C/C genotype [15], thus, it is reasonable to assume CYP2D6 *10 polymorphism may influence breast cancer risk in Chinese women. In the present study, we found women with the 188T/T genotype exhibited a slightly increased risk for breast cancer (OR 1.36) as compared with subjects with the 188C/C genotype, and the association of the 188T/T genotype with breast cancer risk was more pronounced among postmenopausal women (OR 1.49), however, it also did not reach statistical significance. Our observation was, at least in part, in agreement with several previous studies that subjects carrying

Table I. Association between the CYP2D6 *10 polymorphism and breast cancer risk

CYP2D6 genotype	Cases n (%)	Controls n (%)	OR (95%CI)	p-value
Total subjects	286	305		
C/C	56 (19.6)	73 (23.9)	1	
C/T	89 (31.1)	97 (31.8)	1.20 (0.76–1.88)	0.437
T/T	141 (49.3)	135 (44.3)	1.36 (0.89–2.07)	0.150
Premenopausal	141	168		
C/C	30 (21.3)	41 (24.4)	1	
C/T	45 (31.9)	54 (32.1)	1.14 (0.62–2.11)	0.678
T/T	66 (46.8)	73 (43.5)	1.24 (0.69–2.20)	0.472
Postmenopausal	145	137		
C/C	26 (17.9)	32 (23.4)	1	
C/T	44 (30.3)	43 (31.4)	1.26 (0.65–2.45)	0.497
T/T	75 (51.7)	62 (45.2)	1.49 (0.80–2.76)	0.205

OR: odds ratio.
95%CI: 95% confidence interval.

Table II. Association between the CYP2D6 *10 polymorphism and lymph node status in 286 breast cancer patients.

Characteristics	Total (n)	C/C (n%)	C/T (n%)	T/T (n%)	p-value
Lymph node status					0.019
Positive	103	13 (23.6)	41 (46.6)	49 (35.2)	
Negative	179	42 (76.4)	47 (53.4)	90 (64.8)	
Unknown	4	1	1	2	

defective CYP2D6 alleles display a non-significant increased risk for breast cancer in Caucasians populations [5–8]. The present study demonstrated that CYP2D6 polymorphism was not significantly associated with breast cancer risk in a Chinese population.

An interesting finding was CYP2D6 *10 polymorphism was associated with lymph node metastasis, patients carrying one or two mutant alleles, 188T/T or T/C, were more likely to be a positive lymph node status than those carrying wild type alleles (188C/C). It is generally accepted that lymph node involvement is an independent prognostic factor in multivariate analysis [22,23]. This phenomenon is very intriguing; however the underlying mechanisms are largely unknown. We raise several possibilities. First, we could not exclude the possibility that the association of the CYP2D6 *10 polymorphism with lymph-node metastasis was due to chance. Second, since the majority of patients in this cohort received neoadjuvant chemotherapy (Anthracyclin-based or Paclitaxel-based chemotherapy) prior to surgery, it could be possible that the mutant 188T/T genotype might influence the therapeutic outcome of chemotherapy and result in a high frequency of a positive lymph-node status. A recent study demonstrates that CYP2D6 genotype is significantly associated with the efficiency of tamoxifen treatment in a Caucasian population [24]. Our present findings may have some clinical implications, as CYP2D6 genotype may also affect the efficiency of chemotherapy treatment, thus the patients with mutant CYP2D6 genotypes may less benefit from chemotherapy. Third, CYP2D6 may deactivate carcinogens in relation to breast cancer development. Studies indicated that CYP2D6 did not express in normal breast tissue [25,26], hence, a potential carcinogen for breast cancer development might be catalysed in the liver, these specific endogenous or exogenous carcinogens may not be deactivated properly in patients with the CYP2D6 *10 mutant alleles, in turn leading to the tumour cells exhibiting an aggressive phenotype.

This is the first study to investigate the association between the CYP2D6 *10 polymorphism and breast cancer risk in Chinese women, we found the CYP2D6 *10 mutant genotype (188T/T) displays a non-significant increased risk for breast cancer. Moreover, CYP2D6 *10 mutant genotypes (T/T or T/C) are associated with a positive lymph node status, suggesting that patients with CYP2D6 *10 mutant alleles may exhibit an aggressive phenotype. As the observation association of genotype with clinical outcome may be due to chance, therefore, further studies are required to confirm our present findings.

Acknowledgements

This work was supported by a grant (No. 03–06) from the Beijing Cancer Hospital, Peking University School of Oncology.

References

[1] Gao YT, Shu XO, Dai Q, Potter JD, Brinton LA, Wen W, et al. Association of menstrual and reproductive factors with breast cancer risk: results from the Shanghai Breast Cancer Study. Int J Cancer 2000;87:295–300.

[2] Nathanson KL, Wooster R, Weber BL. Breast cancer genetics: what we know and what we need. Nat Med 2001; 7:552–6.

[3] Gonzalez FJ, Gelboin HV. Role of human cytochromes P450 in the metabolic activation of chemical carcinogens and toxins. Drug Metab Rev 1994;26:165–83.

[4] Dunning AM, Healey CS, Pharoah PD, Teare MD, Ponder BA, Easton DF. A systematic review of genetic polymorphisms and breast cancer risk. Cancer Epidemiol Biomarkers Prev 1999;8:843–54.

[5] Pontin JE, Hamed H, Fentiman IS, Idle JR. Cytochrome P450dbl phenotypes in malignant and benign breast disease. Eur J Cancer 1990;26:790–2.

[6] Ladero JM, Benitez J, Jara C, Llerena A, Valdivielso MJ, Munoz JJ, et al. Polymorphic oxidation of debrisoquine in women with breast cancer. Oncology 1991;48:107–10.

[7] Huober J, Bertram B, Petru E, Kaufmann M, Schmahl D. Metabolism of debrisoquine and susceptibility to breast cancer. Breast Cancer Res Treat 1991;18:43–8.

[8] Ladona MG, Abildua RE, Ladero JM, Roman JM, Plaza MA, Agundez JA, et al. CYP2D6 genotypes in Spanish women with breast cancer. Cancer Lett 1996;99:23–8.

[9] Topic E, Stefanovic M, Ivanisevic AM, Petrinovic R, Curcic I. The cytochrome P450 2D6 (CYP2D6) gene polymorphism among breast and head and neck cancer patients. Clin Chim Acta 2000;296:101–9.

[10] Sachse C, Brockmoller J, Bauer S, Roots I. Cytochrome P450 2D6 variants in a Caucasian population: allele frequencies and phenotypic consequences. Am J Hum Genet 1997;60:284–95.

[11] Hersberger M, Marti-Jaun J, Rentsch K, Hanseler E. Rapid detection of the CYP2D6*3, CYP2D6*4, and CYP2D6*6 alleles by tetra-primer PCR and of the CYP2D6*5 allele by multiplex long PCR. Clin Chem 2000;46:1072–7.

[12] Marez D, Legrand M, Sabbagh N, Guidice JM, Spire C, Lafitte JJ, et al. Polymorphism of the cytochrome P450 CYP2D6 gene in a European population: characterization of 48 mutations and 53 alleles, their frequencies and evolution. Pharmacogenetics 1997;7:193–202.

[13] Ji L, Pan S, Marti-Jaun J, Hanseler E, Rentsch K, Hersberger M. Single-step assays to analyze CYP2D6 gene polymorphisms in Asians: allele frequencies and a novel *14B allele in mainland Chinese. Clin Chem 2002;48:983–8.

[14] Garcia-Barcelo M, Chow LY, Chiu HF, Wing YK, Lee DT, Lam KL, et al. Genetic analysis of the CYP2D6 locus in a Hong Kong Chinese population. Clin Chem 2000;46: 18–23.

[15] Johansson I, Oscarson M, Yue QY, Bertilsson L, Sjoqvist F, Ingelman-Sundberg M. Genetic analysis of the Chinese cytochrome P4502D locus: characterization of variant CYP2D6 genes present in subjects with diminished capacity for debrisoquine hydroxylation. Mol Pharmacol 1994;46: 452–9.

[16] Wang SL, Huang JD, Lai MD, Liu BH, Lai ML. Molecular basis of genetic variation in debrisoquin hydroxylation in Chinese subjects: polymorphism in RFLP and DNA sequence of CYP2D6. Clin Pharmacol Ther 1993;53:410–8.

[17] Nilsson G, Skytting B, Xie Y, Brodin B, Perfekt R, Mandahl N, et al. The SYT-SSX1 variant of synovial sarcoma is associated with a high rate of tumor cell proliferation and poor clinical outcome. Cancer Res 1999;59:3180–4.

[18] Agundez JA, Martinez C, Ladero JM, Ledesma MC, Ramos JM, Martin R, et al. Debrisoquin oxidation genotype and susceptibility to lung cancer. Clin Pharmacol Ther 1994;55: 10–4.

[19] Agundez JA, Ledesma MC, Benitez J, Ladero JM, Rodriguez-Lescure A, Diaz-Rubio E, et al. CYP2D6 genes and risk of liver cancer. Lancet 1995;345:830–1.

[20] Wolf CR, Smith CA, Gough AC, Moss JE, Vallis KA, Howard G, et al. Relationship between the debrisoquine hydroxylase polymorphism and cancer susceptibility. Carcinogenesis 1992;13:1035–8.

[21] Agundez JA. Cytochrome P450 gene polymorphism and cancer. Curr Drug Metab 2004;5:211–24.

[22] Sant M, Allemani C, Berrino F, Coleman MP, Aareleid T, Chaplain G, et al. Breast carcinoma survival in Europe and the United States. Cancer 2004;100:715–22.

[23] Woo CS, Silberman H, Nakamura SK, Ye W, Sposto R, Colburn W, et al. Lymph node status combined with lymphovascular invasion creates a more powerful tool for predicting outcome in patients with invasive breast cancer. Am J Surg 2002;184:337–40.

[24] Stearns V, Johnson MD, Rae JM, Morocho A, Novielli A, Bhargava P, et al. Active tamoxifen metabolite plasma concentrations after coadministration of tamoxifen and the selective serotonin reuptake inhibitor paroxetine. J Natl Cancer Inst 2003;95:1758–64.

[25] El-Rayes BF, Ali S, Heilbrun LK, Lababidi S, Bouwman D, Visscher D, et al. Cytochrome p450 and glutathione transferase expression in human breast cancer. Clin Cancer Res 2003;9:1705–9.

[26] Modugno F, Knoll C, Kanbour-Shakir A, Romkes M. A potential role for the estrogen-metabolizing cytochrome P450 enzymes in human breast carcinogenesis. Breast Cancer Res Treat 2003;82:191–7.