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BRIEF RESEARCH ARTICLES

CYP2D6*4 allele and breast cancer risk: Is there any association?

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Abstract
Background CYP2D6 is an important cytochrome P450 enzyme. These enzymes catalyse the oxidative biotransformation of about 25% of clinically important drugs as well as the metabolism of numerous environmental chemical carcinogens. The most frequent null allele of CYP2D6 in European populations, CYP2D6*4, has been studied here in order to elucidate whether a relationship exists between this allele and the risk of developing breast cancer in a Spanish population.
Materials and methods Ninety-six breast cancer Spanish patients and one hundred healthy female volunteers were genotyped for the CYP2D6*4 allele using AmpliChip CYP450 Test technology.
Results Homozygous CYP2D6*4 frequency was significant lower in breast cancer patients than in the control group (OR=0.22, p=0.04). The heterozygous CYP2D6*4 group also displayed lower values in patients than in controls but the difference was not significant (OR=0.698, p=0.28). Therefore, the presence of the CYP2D6*4 allele seems to decrease susceptibility to breast carcinoma in the selected population.
Conclusions A possible decreased transformation of procarcinogens by CYP2D6*4 poor metabolisers could result in a protective effect against carcinogens.

Keywords CYP2D6 cytochrome · CYP2D6*4 allele · Breast cancer · Poor metaboliser

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Introduction

Candidate genes of low-penetrance breast carcinoma susceptibility include those encoding for xenobiotic metabolising enzymes (XMEs) involved in carcinogen metabolism and detoxification [1]. CYP2D6 is an important member of cytochrome P450 enzymes, which belong to XMEs, specifically to phase I enzymes that activate potentially carcinogenic forms [2]. On the other hand, CYP2D6 catalyses the oxidative antidepressants, neuroleptics, antiemetics and drugs for endocrine therapy in breast cancer treatment such as tamoxifen. The role of CYP2D6 in tamoxifen therapy for breast cancer has also been addressed within the context of breast cancer prevention, because of the role of CYP2D6 in tamoxifen's metabolic activation and efficacy [3].

The CYP2D6 gene is highly polymorphic and the distribution of its allelic variants' frequencies varies among different populations. European samples are characterised by the highest frequency of poor metabolisers when samples from different continents are compared [4]. Among Caucasians the most frequent inactivating polymorphism in CYP2D6 is the CYP2D6*4 allele, which generates a G→A transition at nucleotide 1846, leading to a disruption of the splice acceptor site in intron 3 and to a truncated non-functional gene product [5]. The CYP2D6*4 allele has shown higher frequencies in Europeans (12–21%) than in other samples from different geographical origins, for instance, Asians (1%) or Black Africans (2%) [6].

CYP2D6 polymorphisms have been evaluated in many disorders in order to find an association between specific alleles or genotypes with a genetic predisposition. Controversial findings on cancer risk have been published and no clear relation with breast cancer has been demonstrated. On the one hand, a few studies have reported statistical significance whereas no significant association has been reported by several studies [7]. The aim of this study is to provide more data about the relationship between CYP2D6*4 allele and the risk of developing breast cancer in a Spanish population.

Table 1 Association between the CYP2D6*4 polymorphisms and breast cancer

	Cases	Controls	OR [95% CI]	*p*-value
–/–	72 (0.75)	64 (0.64)	1	
–/*4	22 (0.23)	28 (0.28)	0.698 [0.366–1.334]	0.28
*4/*4	2 (0.02)	8 (0.08)	0.22 [0.052–0.967]	0.044

Genotype frequencies are shown between parentheses
–, non-CYP2D6*4 allele

Materials and methods

Ninety-six Spanish female patients of Caucasian origin with newly diagnosed breast cancer were included in this study. One hundred healthy female volunteers from the same origin served as the control group. Both patients and the control group were from different areas of Spain, representing an unbiased sample of the total Spanish population. The average age was 51.92 (SD=10.13, range 30–79) in the breast cancer patients and 55.00 (SD=10, range 30–83) in the control group. Sixty-nine percent of women included in this study were premenopausal. Different types of tumour were found among patients: 51.7% infiltrating ductal carcinoma, 29.9% ductal carcinoma in situ, 11.5% infiltrating lobular carcinoma and 6.9% both infiltrating ductal carcinoma and ductal carcinoma in situ. Of the patients, 67% were premenopausal while the remaining 33% were postmenopausal. The study protocol was approved by the Ethics Committee at the Universidad Europea de Madrid and written informed consent to participate in the study was obtained from all participants.

Venous blood (1 ml) was obtained from each subject and DNA was isolated from peripheral leukocytes using a QIAamp DNA Blood Mini Kit® (Qiagen, Madrid, Spain), according to the manufacturer's guidelines. DNA concentrations were measured and adjusted to 2–20 ng/μl. The AmpliChip CYP450 Test was used to test for CYP2D6*4 allele in the studied samples. The AmpliChip CYP450 Test microarray allows analysis of both sense and antisense strands of an amplified target DNA sample.

The correlation between CYP2D6*4 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 patients and controls was determined using Pearson's chi-square test. A *p*-value of 0.05 or less was regarded as significant.

Results and discussion

The frequency of CYP2D6*4 genotypes among the 96 breast cancer patients and 100 healthy controls is presented in Table 1. Genotypes were in Hardy–Weinberg equilibrium. CYP2D6*4 allele frequencies were 0.14 and 0.22 in patients and controls, respectively. The frequency of the *4/*4 genotype was lower among the breast cancer patients than the controls (0.02 vs. 0.08, respectively) and the correlation of the homozygous CYP2D6*4 genotype and breast cancer risk reached statistical significance (OR=0.220, CI=0.052–0.967, p=0.044, Table 1). The correlation of heterozygous CYP2D6*4 genotype with breast cancer risk was then further analysed, and in this case a moderate decrease risk was observed, but it was not significant (OR=0.698, CI=0.366–1.334, p=0.28, Table 1).

The frequencies of genotypes and alleles of CYP2D6*4 in the breast cancer patients studied match with other Caucasian populations, for instance, Italian [3], Greek [8] or Czech [9]. Several studies of CYP2D6 worldwide genetic variation have shown that the CYP2D6*4 allele displays its maximum frequencies in Caucasian populations (0.12–0.21) [6]. Those values decrease with distance from Europe, suggesting that this region is probably where these haplotypes originated [10]. In this study, there is no association between the presence of CYP2D6*4 and specific types of cancer (χ^2, p=0.57), in contrast to what has been described in other Spanish samples [11].

The cytochrome P4502D6 gene has been extensively studied because of the putative association with cancer risk. The first association between CYP2D6 enzyme activity and cancer risk was described by Ayesh et al. [12] in lung cancer patients. The cytochrome P450 enzymes are generally regarded as detoxifying enzymes that protect the organism from toxic products. However, it could be possible that, in many cases, this metabolic activity forms toxic intermediate compounds that could be more harmful than the original substrate, before further transformation is carried out by additional enzymatic systems. The association of CYP2D6 polymorphisms with breast cancer has been studied by other authors, although this association seems modest, at times contradictory and even dependent upon the geographical origin of the population. On the other hand, many of the published studies are too small to detect a rare allele homozygote relative risk <2.5 [6]. Our results showed a significant association between CYP2D6 wild type vs. CYP2D6*4 homozygous and breast cancer risk, as other authors have previously reported [13]. Conversely, Ladona et al. [11] reported a positive association between breast cancer risk and the heterozygous CYP2D6*4 genotype in a Spanish population. Brazilian women did not display a significant association between CYP2D6*4 allele and breast cancer risk [14]. On the other hand, a Chinese population showed a non-significant increased risk for

breast cancer in homozygous women for the most common null allele in Asians, CYP2D6*10 [15].

The poor metaboliser genotype for the CYP2D6 gene has also been associated with a protective effect against other types of tumours, such as papillary thyroid cancer [16]. This could be due to a possible role of this gene in the metabolic activation of putative environmental chemical carcinogens, as it has also been reported for lung cancer and leukaemia, for which chemical carcinogens are known contributors [17]. The CYP2D6*4 allele did not appear to influence bladder cancer susceptibility or gastric carcinoma risk [18, 19]. In the case of liver cancer, carcinoma of head and neck, and premalignant colorectal adenomas, significant increased risk was detected in individuals who were homozygous for functional CYP2D6 genes [20, 21]. The role of CYP2D6 polymorphism in melanoma has been investigated by different research groups with consistent results that indicate that individuals with defective genes are also at increased risk [22]. Similar results have been observed in patients with pituitary tumours where CYP2D6*4 allele frequency was significantly lower than in control groups [23].

In conclusion, our findings suggest that the homozygosis of the CYP2D6*4 null allele seems to decrease susceptibility to breast carcinoma in the Spanish population. This could be explained by an effect of CYP2D6 on the metabolic activation of putative environmental chemical breast cancer carcinogens or, perhaps, by linkage to another cancer-causing gene. A decreased transformation of pro-carcinogens by poor metabolisers could result in a protective effect against these carcinogens in the case of breast cancer. Further studies in this field may allow the identification of metabolic risk factors and contribute towards a better understanding of molecular carcinogenic mechanisms.

Conflict of interest The authors declare that they have no conflict of interest relating to the publication of this manuscript.

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