# Relationships between CYP2D6 phenotype, breast cancer and hot flushes in women at high risk of breast cancer receiving prophylactic tamoxifen: results from the IBIS-I trial.

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Clinical Studies

# Relationships between CYP2D6 phenotype, breast cancer and hot flushes in women at high risk of breast cancer receiving prophylactic tamoxifen: results from the IBIS-I trial

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BACKGROUND: Several studies have reported discordant results regarding the impact of the CYP2D6 phenotype on both the effectiveness and the degree of endocrine symptoms associated with tamoxifen. Other studies have suggested that menopausal symptoms may be a predictive factor to tamoxifen response.

METHODS: We investigated the relationship between the CYP2D6-predicted phenotype and tamoxifen response in a nested case–control study among women from the International Breast cancer Intervention Study (IBIS-I), which evaluated tamoxifen in the preventive setting.

RESULTS: In this retrospective analysis of the tamoxifen-treated women in the IBIS-I study, 9 women (16.6%) who developed oestrogen receptor-positive invasive breast cancer had a 2D6 poor or intermediate metaboliser phenotype compared with 45 (20.6%) controls. Adjusted matched logistic regression revealed no significant difference between cases and controls for extensive vs intermediate metaboliser phenotype (OR = 0.81 (0.30–2.23), P = 0.7) or extensive vs poor metaboliser phenotype (OR = 1.02 (0.31–3.32), P = 0.9). Controls in the tamoxifen group with a poor metaboliser phenotype developed nonsignificantly fewer hot flushes compared with those with an extensive metaboliser phenotype (OR = 0.40 (0.12–1.31)), but those with the intermediate phenotype developed nonsignificantly more hot flushes (OR = 1.38 (0.58–3.29)) in an unadjusted analysis.

CONCLUSION: Data from the preventive IBIS-I study did not support an association between the CYP2D6 phenotype and breast cancer outcome or the development of endocrine symptoms in tamoxifen-treated women.

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Treatment efficacy and treatment-induced endocrine symptoms are likely to be at least partly related to the underlying host factors, and specifically to genetic variations involving drug metabolism. Tamoxifen is metabolised through the cytochrome P450 (CYP) 2D6 pathway to 4-hydroxy-tamoxifen and endoxifen. Both of these metabolites are believed to be more potent anti-oestrogens than tamoxifen itself (Mortimer *et al*, 2008). Reports suggest that women with specific alterations in the CYP2D6 enzyme, which correlate with reduced enzyme activity and lower endoxifen levels (Desta *et al*, 2004; Borges *et al*, 2006), may have less benefit from tamoxifen treatment and fewer hot flushes than women with a normal enzyme activity (Goetz *et al*, 2005). Retrospective analyses from European (Schroth *et al*, 2007) (HR = 2.24 (1.16–4.33), P = 0.02) and Asian (Lim *et al*, 2007; Kiyotani *et al*, 2008; Xu *et al*, 2008) (HR = 4.7 (1.1–20.0), P = 0.04) studies have found a strong association between CYP2D6 genotypes and treatment outcomes. A nested case–control study including 46 women with breast cancer and 136 controls was conducted within the Italian tamoxifen

prevention trial (Veronesi *et al*, 2003; Veronesi *et al*, 2007), and it was found that women with the low-metabolising *CYP2D6 \*4/\*4* genotype tended to have higher risk of primary breast cancer. The authors also reported that these women experienced more hot flushes (Bonanni *et al*, 2006). However, this was not seen in the study conducted by Goetz *et al* (2007). Furthermore, two large clinical trials in the adjuvant setting (ATAC, BIG 1–98) have reported no correlation between the *CYP2D6* genotype and recurrence (Rae *et al*, 2012; Regan *et al*, 2012).

Vasomotor symptoms, especially hot flushes, are increased for women taking tamoxifen (Sestak *et al*, 2006; Cuzick *et al*, 2007). Mortimer *et al* (2008) reported on data from the Women's Health Eating and Living study, which showed that women with primary breast cancer treated with tamoxifen who experienced hot flushes at baseline had a significantly lower risk of recurrence compared with women without hot flushes. Similar results were reported with the ATAC trial where an inverse association between the occurrence of vasomotor symptoms and breast cancer recurrence was seen (Cuzick *et al*, 2008).

Here, we have investigated the association between the CYP2D6 phenotype, breast cancer incidence and hot flushes in healthy women taking part in the International Breast cancer

Intervention Study I (IBIS-I) in order to test the hypothesis that poor metabolisers of tamoxifen are more likely to relapse and have fewer symptoms.

## PATIENTS AND METHODS

The IBIS-I is a randomised, double-blind, placebo-controlled study of the effects of 5 years of tamoxifen treatment in women at high risk of developing breast cancer (Cuzick et al, 2002; Cuzick et al, 2007). Detailed study design and baseline characteristics have been described previously (Cuzick et al, 2002). In short, women aged 35–70 years, having at least a two-fold relative risk of developing breast cancer, were eligible to join the trial. Women were randomly allocated to either 5 years of tamoxifen (20 mg per day) or matching placebo, and were followed up every 6 months during the 5 years of treatment and annually thereafter. Median follow-up for this analysis was 96 months. The IBIS-I trial was conducted under the auspices of the UK Coordinating Committee for Cancer Research (now part of the National Cancer Research Network) and was approved by the local ethics committee for each participating centre. The IBIS-I trial is registered as an International Standard Randomised Controlled Trial, number ISRCTN91879928.

We conducted a case–control study in women allocated to tamoxifen, in which those women with an oestrogen receptor (ER)-positive tumour at any follow-up time were included. Women on tamoxifen with ER-negative tumours were excluded from this case–control analysis and also did not serve as potential controls. Purified DNA from whole-blood samples collected at baseline was analysed using the AmpliChip CYP450 Test at the Roche laboratories (Roche Molecular Systems, Inc., Pleasanton, CA, USA), blind to case–control status and all clinical factors. Each CYP2D6 allele was assigned to one of four phenotypic categories according to its associated enzyme function: poor, intermediate, extensive or ultra-rapid metaboliser. CYP2D6 genotypes were then classified into three phenotypic categories, ranked from low to high level of enzymatic function: poor metaboliser, intermediate metaboliser and extensive metaboliser. Any patient possessing two nonfunctioning alleles was designated as a poor metaboliser, any patient possessing at least one decreased function allele with no wild-type allele was designated as an intermediate metaboliser and any patient with at least one normally functioning allele was designated as an extensive metaboliser.

All the side effects reported were graded by a clinical observer (doctor or trained research nurse) at the time of interview. Specific questions about hot flushes were asked at each 6-month follow-up visit, but not at baseline, where only details of menopausal symptoms were recorded. Most hot flushes occurred soon after women started endocrine treatment (Sestak et al, 2006); therefore, we used the reporting of these symptoms (all severities) at the first 6-month follow-up visit as our measure of symptom occurrence. Symptoms reported after 6 months of initiation of the study therapy were not included in this analysis. Data on concomitant medications associated with CYP2D6 inhibition were collected during the 5-year treatment period. The use of strong CYP2D6 inhibitors, namely paroxetine, fluoxetine, quinidine and bupropion, has been evaluated in this analysis. Patients were considered to have taken inhibitory drugs if prescription/use of the drug was recorded at any time during their anti-oestrogen treatment period.

The primary objective was to determine the effect of the CYP2D6 phenotype on the development of ER-positive invasive breast cancer in the tamoxifen arm of the trial. A secondary objective was to evaluate the effect of the CYP2D6 phenotype on the development of endocrine symptoms in the controls. The association between the CYP2D6 phenotype and breast cancer development or endocrine symptoms was determined using conditional logistic regression for case–control sets and logistic regression for endocrine symptoms. All P-values are two-sided and all confidence intervals are at the 95% level. All calculations were performed using STATA (Version 11; StataCorp, College Station, TX, USA).

## RESULTS

Cases were women who developed ER-positive (ER +) invasive breast cancer in the tamoxifen arm of the IBIS-I breast cancer prevention trial. Cases were matched according to personal breast cancer risk (Tyrer et al, 2004), age and follow-up time with four controls who also received tamoxifen but did not develop breast cancer (one case was matched to only three controls). For a total of 54 cases and 215 controls, Cytochrome P450 (CYP) 2D6-predicted phenotypes were analysed. For four controls, the CYP2D6 phenotype could not be determined, leaving 49 cases with 4 controls and 5 cases with 3 controls for analysis.

### CYP2D6 phenotype and risk of breast cancer

Nine (16.6%) women who developed ER + breast cancer had a poor or intermediate metaboliser phenotype compared with 45 (18.0%) controls (Table 1). There were no significant differences in phenotypes between cases and controls. Unadjusted matched logistic regression revealed no significant difference between cases and controls for extensive vs intermediate metaboliser phenotype (OR = 0.81 (0.30–2.23), P = 0.7) or extensive vs poor metaboliser phenotype (OR = 1.02 (0.31–3.32), P = 0.9). No difference was seen when poor and intermediate metaboliser phenotypes were combined vs extensive metaboliser (OR = 1.07 (0.50–2.31), P = 0.9). When the analysis was adjusted for previous hormone replacement therapy, smoking status and menopausal status, similar results were seen as in the unadjusted analysis for poor vs extensive metaboliser phenotype (OR = 0.84 (0.26–2.78), P = 0.8) (Table 1).

Only 25 (11.6%) women (5 cases and 20 controls) who had an extensive metaboliser phenotype used a strong CYP2D6 inhibitor either at entry or during follow-up. The results remained unchanged when these women were excluded (data not shown).

### CYP2D6 phenotype and risk of hot flushes

We investigated the relationship between the CYP2D6 phenotype and development of hot flushes at the 6-month visit in controls receiving tamoxifen (N = 211). In all, 50.3% of women with an extensive metaboliser phenotype developed hot flushes at the 6-month visit, whereas 49.7% did not (P = 0.7). For controls with an intermediate metaboliser phenotype, 58.3% developed a hot flush compared with 41.7% who did not (P = 0.4) (Table 2). Only four women (28.6%) with a poor metaboliser phenotype developed hot flushes at the 6-month visit, whereas ten women (71.4%) with this phenotype did not (P = 0.01).

Those with an intermediate metaboliser phenotype had a small nonsignificant increased risk of developing hot flushes compared with those with an extensive metaboliser phenotype (OR = 1.38 (0.58–3.29)) in an unadjusted analysis. In contrast, those with a poor metaboliser phenotype developed nonsignificantly fewer hot flushes at the 6-month visit compared with those with an extensive metaboliser phenotype (OR = 0.40 (0.12–1.31)), and no trend across phenotype groups was observed ($P_{trend}$ = 0.3). When the analysis was adjusted for hormone replacement therapy, smoking status and menopausal status, similar results were found (Table 2).

**Table I** Distribution of CYP2D6 phenotype (%) according to case–control status in women receiving tamoxifen

| CYP2D6 phenotype | Cases (N = 54) | Controls (N = 211) | OR (95% CI) | OR (95% CI) adjusted* |
|---|---|---|---|---|
| Extensive | 45 (83.3%) | 173 (82.0%) | Reference | Reference |
| Intermediate | 5 (9.3%) | 24 (11.4%) | 0.81 (0.30–2.23) | 0.88 (0.31–2.47) |
| Poor | 4 (7.4%) | 14 (6.6%) | 1.02 (0.31–3.32) | 0.84 (0.26–2.78) |

*Adjusted for hormone replacement therapy, smoking status and menopausal status.

**Table 2** CYP2D6 phenotype, hot flushes at 6 months and corresponding OR (95% CI) in controls on tamoxifen

| CYP2D6 phenotype | Hot flushes | No hot flushes | OR (95% CI) | OR (95% CI)* |
|---|---|---|---|---|
| Extensive (N = 173) | 87 (50.3%) | 86 (49.7%) | Reference | Reference |
| Intermediate (N = 24) | 14 (58.3%) | 10 (41.7%) | 1.38 (0.58–3.29) | 1.19 (0.48–2.95) |
| Poor (N = 14) | 4 (28.6%) | 10 (71.4%) | 0.40 (0.12–1.31) | 0.40 (0.12–1.36) |
| P-trend | | | 0.3 | 0.09 |

Abbreviations: CI = confidence interval; OR = odds ratio. *Adjusted for hormone replacement therapy, smoking status, and menopausal status.

Very similar results were found if the analysis included both cases and controls (data not shown).

## DISCUSSION

We found no association between the CYP2D6 phenotype and ER + breast cancer occurrence in high-risk women receiving tamoxifen as a preventive agent. These results are in contrast to those reported by Bonanni et al (2006) in the preventive setting and Goetz et al (2005, 2007) in the adjuvant treatment setting.

Results similar to that of our study have been reported in the adjuvant treatment setting by Nowell et al (2005), Wegman et al (2005), Rae et al (2012), Regan et al (2012), who did not find an association between CYP2D6 and tamoxifen response/breast cancer outcome. Despite early suggestions of an impact of the CYP2D6 genotype and breast cancer (Brauch and Jordan, 2009; Higgins et al, 2010), no association has been found in these recent studies.

We found a weak nonsignificant relationship between the CYP2D6 phenotype and endocrine symptoms. Women with a CYP2D6 poor metaboliser phenotype developed fewer hot flushes than women with an extensive metaboliser phenotype. However, women with the intermediate phenotype had nonsignificantly more hot flushes. Stronger results were seen in a prospective cohort study by Henry et al (2009), where women with an intermediate metaboliser phenotype developed significantly more hot flushes compared with women with an extensive metaboliser phenotype. Given the lack of statistical significance of our findings, the role of CYP2D6 polymorphisms in the development of endocrine symptoms in women taking tamoxifen remains an open question.

There are several limitations to this analysis. First, this was a retrospective unplanned analysis and the study was not designed to look at the relationship between CYP2D6 phenotype, hot flushes and breast cancer outcome. Data on hot flushes were not specifically collected; rather, a set of questions was used to gather information about several side effects at each follow-up visit. Furthermore, we do not have information on endocrine symptoms before study entry, and hence we cannot be certain if hot flushes reported at the 6-month visit are indeed tamoxifen-induced symptoms or not. Although the sample size was limited and we could not rule out a two-fold risk for cancer development, it was larger than the Bonanni et al, 2006 study, which did find a positive relationship. For this sample size and phenotype frequencies seen here in the controls, there would be 74% power to see a two-fold increase in cancers in the poor and intermediate groups combined vs the extensive metabolisers (with a two-sided significance level of 5%).

Although we looked at concomitant medications, the numbers of women using these drugs were small and they are unlikely to notably influence these results. Azoulay et al (2011) investigated the concurrent use of tamoxifen and CYP2D6 inhibitors in a nested case–control study using data from the UK General Practice Research Database. They found that the concurrent use was not associated with an increased risk of breast cancer recurrence, and furthermore reported that type or strength of the CYP2D6 inhibitor did not affect the results.

In conclusion, data from the IBIS-I study in the preventive setting could not confirm earlier reports showing an association between the CYP2D6 phenotype and breast cancer outcome. More research is needed to understand the factors included in tamoxifen-induced endocrine symptoms and to investigate the relationship between endocrine symptoms and breast cancer outcome.

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