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Relationship of *ZNF423* and *CTSO* with breast cancer risk in two randomised tamoxifen prevention trials

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Abstract

Purpose—A case-control study from two randomised breast cancer prevention trials of tamoxifen and raloxifene (P-1 and P-2) identified single nucleotide polymorphisms (SNPs) in or near genes *ZNF423* and *CTSO* as factors which predict which women will derive most anti-cancer benefit from selective oestrogen receptor modulator (SERM) therapy. In this article we further examine this question by using blood samples from two randomised tamoxifen prevention trials: the International Breast Cancer Intervention Study I (IBIS-I), and the Royal Marsden trial (Marsden).

Methods—A nested case-control study was designed with 2:1 matching in IBIS-I and 1:1 matching in Marsden. The OncoArray was used for genotyping, and included two SNPs previously identified (rs8060157 in *ZNF423* and rs10030044 near *CTSO*), and 102 further SNPs within the same regions. Overall there were 369 cases and 662 controls, with 148 cases and 268 controls from the tamoxifen arms. Odds ratios were estimated by conditional logistic regression, with Wald 95% confidence intervals.

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Ethical standards

The IBIS-I trial was approved by the local ethics committee of each centre. The Marsden trial was approved by the Royal Marsden Hospital ethics committee. They are registered at controlled-trials.com as ISRCTN91879928 (IBIS-I) and ISRCTN07027313 (Marsden).

Conflict of interest statement

JC reports research funding from AstraZeneca. MD reports a consultant/advisory role with Radius, GTx. All other authors declare they have no conflict of interest.

Results—In the tamoxifen arms the per-allele odds ratio for rs8060157 was 0.99 (95%CI 0.73–1.34), and 1.00 (95%CI 0.76–1.33) for rs10030044. In the placebo arm, the odds ratio was 1.10 (95%CI 0.87-1.40) for rs8060157 and 1.01 (95%CI 0.79-1.29) for rs10030044. There was no evidence to suggest other SNPs in the surrounding regions of these SNPs might predict response to tamoxifen.

Conclusions—Results from these two prevention trials do not support the earlier findings. rs8060157 in *ZNF423* and rs10030044 near *CTSO* do not appear to predict response to tamoxifen.

Keywords

Breast Cancer; Randomised Prevention Trials; Single Nucleotide Polymorphisms; Stratified Medicine; Tamoxifen response

Introduction

Breast cancer is the most common cancer in women worldwide, with an estimated 1.7 million cases diagnosed and 500,000 deaths in 2012[1]. Selective oestrogen receptor modulators (SERMs), including tamoxifen and raloxifene, have been shown to reduce the risk of breast cancer: a combined analysis of almost 84 thousand women in nine trials estimated that over ten years approximately two in five breast cancers were prevented in women who had been randomised to receive a SERM[2]. Tamoxifen has been licensed for prevention in women at an elevated risk of breast cancer in the United States of America and approved for this indication by the national institute for clinical excellence (NICE) committee in the United Kingdom (UK), but uptake has been modest[3]. Improved risk estimates for response to SERMs for an individual woman could have a significant impact on the utility and acceptability of these preventive treatments.

Ingle and colleagues reported that two single nucleotide polymorphisms (SNPs), rs8060157 in *ZNF423* and rs10030044 near *CTSO*, appear to predict response to tamoxifen and raloxifene[4]. Their findings were from a genome-wide association study using DNA from the NSABP P-1 and P-2 (STAR) breast cancer prevention trials[5,6]. Our objective was to assess the value of these SNPs in the IBIS-I and Marsden trials[7,8,9].

Methods

Patients

Healthy women with an increased risk of breast cancer mostly from their family history were recruited to IBIS-I and Marsden trials [7,8,9]. Both trials were double-blind with women randomised to receive tamoxifen (20 mg/day) or placebo for 5 years in IBIS-I, and 5-8 years in Marsden. Cases were ascertained during treatment by clinic visits and thereafter by clinic visits or questionnaires. Full details on the trials have been described previously [7,8,9]. They are registered at controlled-trials.com as ISRCTN91879928 (IBIS-I) and ISRCTN07027313 (Marsden).

Specimen characteristics

Blood samples taken at baseline from all women in IBIS-I were stored at −70C. Baseline material at the Marsden was destroyed in a fire and new blood samples were obtained and stored at −70C. Blood samples were not obtainable for 38 women from Marsden that developed invasive breast cancer and for these paraffin-embedded tissues samples that were obtained from the cancer diagnosis were analysed and used in a sensitivity analysis.

Assay methods

Genomic DNA was quantified using the Picogreen protocol (Quant-iT PicoGreen dsDNA Products, Invitrogen, P-7589) and read on SpectraMAX GeminiXS Spectrophotometer. The Illumina OncoArray was used with the HTS method for the microarray data, as described by the manufacturer's protocol (Illumina, San Diego). An Illumina Hybridization oven was used for incubating amplified DNA (37C) and for BeadChips hybridization (48C). A Hybex incubator was used for the fragmentation (37C) and the denaturation (95C) steps. The X-stain step was carried out with a Tecan Freedom evo robot with a Te-Flow module. Arrays were scanned by an Illumina iScan Reader. Data analysis was performed with the Genotyping module (version 1.9.4) of the GenomeStudio software (Illumina; version 2011.1) using Consortium-OncoArray_15047405_A.bpm manifest. Two trios (two parents and their child) of CEPH (Centre de'Etude du Polymorphism Humain) samples were used in continuous rotation as assay controls, and two internal controls were used per plate. The assay controls were used to monitor assay quality and possible sample mismatch between the planned wells and those actually on the plate.

The main focus was on SNPs rs8060157 and rs10030044 that were identified by [4]. We also considered other SNPs on the OncoArray that were in the same regions, with 24 near *CTSO* (between rs7684248 and rs4555581), and 80 in *ZNF423* (between rs10852596 and rs12935130).

Study design

The primary endpoint was diagnosis of invasive breast cancer or ductal carcinoma in situ (DCIS). A nested case-control study matched all cases with available DNA by trial, follow-up duration, treatment arm (placebo or tamoxifen) and age at entry (±2 years). Samples from the two trials were combined to increase power. Two controls were matched to each case in IBIS-I, and one in Marsden. IBIS-I recruited from 1992-2001, and Marsden from 1986-1996. The end of follow-up for the present article was 2014 and 2010 respectively; median follow-up in IBIS-I was 16.6 years and 8.4 years in Marsden.

Statistical analysis methods

The number of failed SNPs in *ZNF423* and near *CTSO* was examined for quality control of the assay. Hardy-Weinberg equilibrium in cases and controls was tested by assessing the observed number of homozygotes against expected using a binomial distribution. The Tyrer-Cuzick model [11] (v7.02) was used to estimate risk at entry to each trial. This and other baseline characteristics were summarized in a Table, and differences between cases and controls were tested by a likelihood-ratio test from a conditional logistic regression model.

The main analysis estimated the per-allele odds ratios of rs8060157 and rs10030044 via conditional logistic regression, with Wald 95% confidence intervals. Secondary analysis examined the distribution of likelihood-ratio P-values for all nearby SNPs, and for these the observed P-value was plotted against the expected under a null hypothesis of no effect for any SNP and tested using a Kolmogorov-Smirnov test. Subgroup analyses were used to check heterogeneity by treatment randomisation, trial and other risk factors at baseline, through a likelihood-ratio test of interaction. Analysis was undertaken using the statistical software R 2.15.1 [12].

Results

Figure 1 shows a CONSORT diagram. A total of 1,276 women from both trials were initially selected for the case-control study. For 169 of these there was not enough DNA available for assay, so some controls were re-allocated to maintain matching for all cases. For the 80 SNPs in *ZNF423* and 24 near *CTSO,* 35 (89.7%) tissue samples failed more than 5/104 SNPs compared with only 27 (2.6%) of blood samples (Supplementary Table S1). Therefore all tissue samples were excluded from the primary analysis, but a sensitivity analysis was conducted when they were included (Supplementary Table S5). This left a total of 369 cases and 662 controls for the main analysis, and the distribution between the trials is shown in Figure 1.

Baseline characteristics for cases and controls are shown in Table 1. In brief, 60% of samples were from women randomised to placebo and the remaining 40% from those randomised to tamoxifen. The majority of breast cancer samples came from ER-positive disease (74%). Age (median 50 years) and body mass index (BMI) were balanced between cases and controls. Approximately half of the women were postmenopausal, but cases were slightly more likely to be pre-menopausal (P=0.06). As expected, cases had a higher Tyrer-Cuzick breast cancer risk at entry compared with controls (P<0.001). The characteristics of the IBIS-I and Marsden sets were broadly similar and therefore are combined for the primary analysis. Trial specific demographics are shown in Supplementary Table S2 and results in Supplementary Table S3.

The SNP genotype results are shown in Table 2. Hardy-Weinberg equilibrium was verified in cases and controls (smallest P = 0.2). There were 3/1031 (0.3%) failed samples for rs8060157 and 13/1031 (1.3%) for rs10030044. The minor allele frequency (MAF) for rs8060157 was 44% in cases and controls (Table 2), compared with 39% for cases and 47% for controls from the Ingle study[4]; the MAF for and rs10030044 was 41% in cases and controls, compared with 45% and 36% from the Ingle study[4]. This similarity suggests that the two populations are comparable.

Our results show little evidence for an association between the SNPs and case-control status in either the tamoxifen or placebo arms (Table 2). In tamoxifen-treated women, we observed a per-major-allele OR of 0.99 (95%CI 0.73-1.34) for rs8060157, compared with the previously reported OR of 0.70 (95%CI 0.60-0.81) for the same SNP [4]. Similarly, no evidence of tamoxifen benefit was observed in our case-control study for rs11076499

(OR=0.98, 95%CI 0.73-1.32), whereas the Ingle study [4] reported a benefit associated with this allele.

Supplementary Tables S3a and S3b show results for the SNP analysis for each trial separately. Very similar results between the trials were observed, with no evidence of an association between SNPs and case-control status ($P_{het} > 0.4$, Supplementary Table S4).

Ingle and colleagues suggested using rs8060157 and rs10030044 in combination for individualised breast cancer prevention [4]. However, in our data the per-allele effect estimates in a joint model were 1.00 (95% CI 0.74 - 1.36) for rs8060157 and 1.00 (95% CI 0.75 - 1.32) for rs10030044.

A secondary analysis for all nearby SNPs is shown in Figure 2. In total 64 *ZNF423* SNPs and 19 in *CTSO* showed some variation (MAF ≥1%), but there was no evidence of a difference between cases and controls in these regions (tamoxifen P=0.14, untreated P=0.71). Subgroup analyses were used to explore whether there were differences by randomisation arm, trial and other risk factors at baseline. No significant interactions with any baseline factors were found except for parity with *ZNF423* and this would not be sustained after adjustment for multiple testing (Table S4). Finally, a sensitivity analysis that included all tissue samples from the Marsden trial had very similar results to those that only included blood samples (Supplementary Table S5).

Discussion

Ingle and colleagues hypothesized that rs8060157 and rs10030044 could be used to predict response to tamoxifen and raloxifene [4]. The hypothesis was driven by a genome-wide association study from the P-1 and P-2 trials [5,6]. When we examined this issue further in the IBIS-I and Marsden prevention trials [7,8,9], we could not replicate their results. Per-allelle odds ratios were close to unity in both placebo and tamoxifen arms.

A major strength of our study is that two randomised tamoxifen prevention trials were used to assess the hypothesis. In contrast with the Ingle study [4], participants of the placebo arm were also genotyped, which helped to assess whether the SNPs are risk or tamoxifen-response predictors. Another strength of this study is that it is hypothesis driven, and less affected by over-fitting due to multiple comparisons than for the earlier analysis.

A potential weakness of the study is that the ability to detect SNP effects was limited by sample size. Although the confidence intervals rule out effects as large as those observed in [4], a 30% increase or decrease could not be excluded. It is notable that in the hypothesis generating study [4] neither of the SNPs met the criteria for significance in a genome-wide study but rather were considered of likely biological significance because of the additional evidence from biological studies that showed a SNP-dependent variation in estrogen-dependent induction of the expression of *BRCA1.*

In conclusion, this study from the IBIS-I and Marsden tamoxifen-prevention trials has failed to find evidence to support the use of rs8060157 and rs10030044 as biomarkers for

tamoxifen response. Other SNPs and biomarkers for response to tamoxifen might be better prioritised for future research, including mammographic breast density [10].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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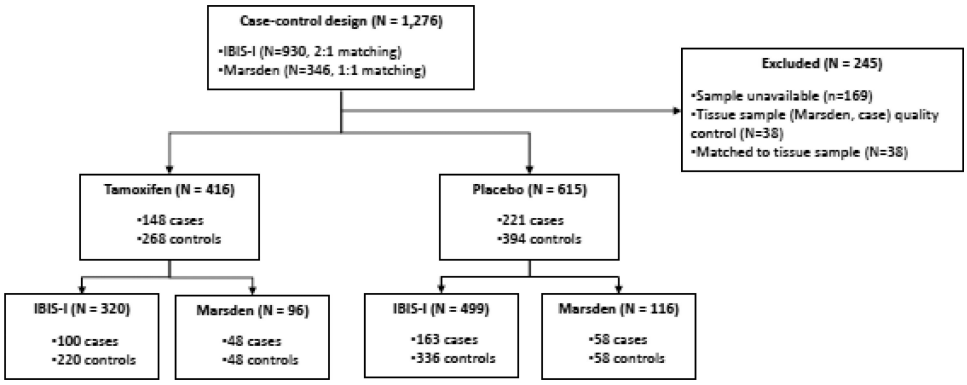

Figure 1.
Consort diagram

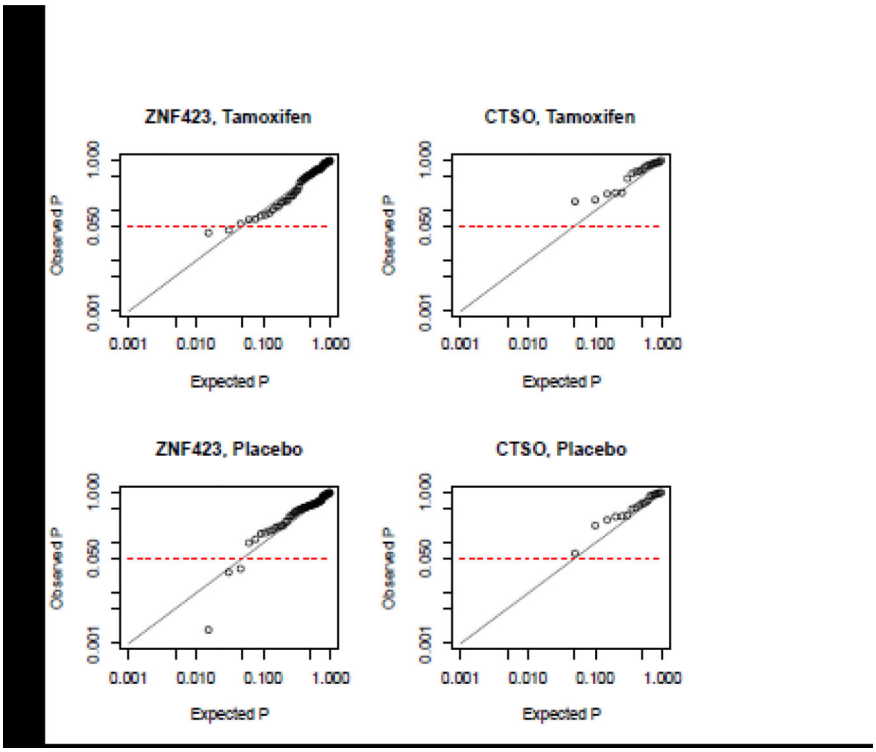

Figure 2.

P-values from conditional logistic regression likelihood ratio tests for the Oncoarray SNPs in *ZNF423* and in/near *CTSO*. Each point is a SNP with minor allele frequency greater than 1%. The expected distribution of P under the null is the diagonal.

Table 1

Baseline characteristics according to case control status.

	Cases	Controls	P
Total	369	662	
Placebo	221	394	
Tamoxifen	148	268	
ER-Positive	273		
ER-Negative	65		
Unknown	31		
Age (y), median (IQR)	50 (45-54)	49 (46-54)	0.9
Premenopausal	191 (52%)	316 (48%)	0.06
Perimenopausal	19 (5%)	28 (4%)	
Postmenopausal	159 (43%)	318 (48%)	
BMI (kg/m^2), median (IQR)	25.7 (22.7-28.7)	25.4 (22.9-29.4)	0.3
TC, median RR (IQR)	2.4 (1.9-3.0)	2.1 (1.7-2.7)	<0.001

BMI: body mass index; ER: estrogen-receptor; IQR: inter-quartile range; RR: 10y risk relative to general population; TC: Tyrer-Cuzick model.

Table 2

Genotype results for minor (m) and major (M) allele combinations, by trial arm and case status.

SNP	Status	Arm	n	mm	mM	MM	OR (95% CI)	P	Ingle OR (95% CI)
rs8060157	Case	Placebo	220	49 (22%)	100 (45%)	71 (32%)	1.10 (0.87 - 1.40)	0.409	
	Control		394	72 (18%)	190 (48%)	132 (34%)			
	Case	Tamoxifen	147	26 (18%)	76 (52%)	45 (31%)	0.99 (0.73 - 1.34)	0.939	0.70 (0.60 - 0.81)
	Control		267	54 (20%)	121 (45%)	92 (34%)			
rs10030044	Case	Placebo	218	40 (18%)	103 (47%)	75 (34%)	1.01 (0.79 - 1.29)	0.929	
	Control		392	70 (18%)	190 (48%)	132 (34%)			
	Case	Tamoxifen	144	24 (17%)	66 (46%)	54 (38%)	1.00 (0.76 - 1.33)	0.991	1.42 (1.23 - 1.65)
	Control		265	41 (15%)	128 (48%)	96 (36%)			

OR, odds ratio; CI, confidence interval