

Null Results in Brief

Prohibitin 3' Untranslated Region Polymorphism and Breast Cancer Risk

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Introduction

Prohibitin is an antiproliferative protein (1) and may function as a tumor suppressor through interaction with the retinoblastoma tumor suppressor protein and its family members (2). In addition, somatic mutations have been identified in human cancers, including breast cancer (3, 4). Interestingly, the prohibitin 3' UTR³ exhibits the characteristics of a *trans*-acting regulatory RNA that is able to arrest cell proliferation (5). A polymorphic variant allele (*T* allele) in the 3'UTR of prohibitin (C to T at nucleotide 1703) lacks the tumor suppressor function (6) and has been associated with an increased risk of breast cancer, particularly in women who have been diagnosed before the age of 50 years and have at least one first-degree relative with the disease (OR = 4.8). However, in a subsequent study, Spurdle *et al.* (7) did not observe any increased risk associated with the 3' UTR *T* allele in a cohort of Australian women diagnosed with breast cancer before the age of 50 years. Given the important antiproliferative function that prohibitin possesses, it is plausible that the *T* variant could represent a cancer-predisposing allele. The frequency of the *T* allele is high in Caucasian populations; therefore, it could potentially contribute significantly to the population risk of breast cancer. Given the conflicting conclusions of the only two studies that have investigated this polymorphism in cancer predisposition, we conducted a case-control study among British women who had bilateral breast cancer or had a family history of breast cancer or had been diagnosed with breast cancer before the age of 40 years.

Materials and Methods

Two hundred ninety-one breast cancer cases were selected on the basis of an age of onset <40 years, a family history of breast cancer (defined as two or more cases of breast cancer in a first- or second-degree female relative) irrespective of age at onset, or bilateral breast cancer irrespective of family history or age at onset. All breast cancer cases were systematically ascertained through breast clinics in the Wessex region of southern England

as described previously (8, 9). Briefly, women were invited to take part in a research study, the primary goal of which was to ascertain and verify family histories for segregation analysis. The breast cancer cases diagnosed before 40 years of age were consecutively ascertained without regard to family history. The group of women with bilateral breast cancer were ascertained in the same clinics, but the selection criterion was the presence of bilateral breast cancer diagnosed after 39 years of age. The familial breast cancer cases consisted of women presenting to the same clinics with a strong family history of breast or ovarian cancer or both. Family histories were verified to the greatest possible extent from medical records and death certificates. Blood was taken from all recruits who consented to molecular analysis for breast cancer-predisposition genes. The age range of the breast cancer cases was 19–79 years, with a mean age of 38 years. The controls represented the population from which the cases arose and consisted of 233 Caucasian female volunteers who were either staff at the Princess Anne Hospital, Southampton, United Kingdom, or patients attending for nonneoplastic disease conditions. The age of the controls ranged from 18 to 84 years, with a mean age of 39 years. For all groups, normal genomic DNA was prepared from blood lymphocytes. Epidemiological data, such as reproductive factors, oral contraceptive use, smoking, and obesity, were not available for either the cases or controls. However, both control and cancer groups were drawn from the same geographical area, which was a predominantly Anglo-Saxon population.

Genomic DNA was extracted and analyzed for the prohibitin 3' UTR using an allele-specific PCR reaction. The PCR involved two fluorescently labeled reverse primers (5'-6-carboxyfluorescein-AGGAACGTAGGTCGGACACG-3' and 5'-hexachloro-6-carboxyfluorescein-AGGAACGTAGGTCGGACACA-3') specific for the *C* and *T* alleles, respectively, and a common forward primer (5'-CCCCAGGGTCTCTAAAAC-TTG-3'). The alleles were separated on sequencing gels and analyzed using a scanning laser fluorescence imager (Bio-Rad FX Molecular Imager). Control samples with a known genotype (confirmed by direct sequencing) were included in each PCR batch. In addition, 20% of cases and controls were repeated to assess the consistency of the PCR assay.

Comparison of frequencies were analyzed using Fisher's exact test. ORs and 95% CIs were calculated using the relevant 2 × 2 contingency tables. All statistical calculations were two-sided and performed using InStat Version 3.01 (GraphPad Software Inc., San Diego, CA).

Results and Discussion

The study had 80% power to detect an OR of ≥ 1.7 for carriers heterozygous for the prohibitin 3' UTR *T* allele and an OR ≥ 3.1 for carriers homozygous for the *T* allele. There was no deviation from Hardy-Weinberg equilibrium among the cases ($P = 0.9$) or controls ($P = 0.9$), and the frequency of the *T* allele was similar to that reported in the two previous studies (Table 1). We did not detect a difference in the genotype

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³The abbreviations used are: UTR, untranslated region; OR, odds ratio; CI, confidence interval.

Table 1 Prohibitin 3' UTR C/T polymorphism genotype distribution among cases and controls

Group	CC		CT		TT		TT or CT	
	N (%)	P value ^a	N (%)	P value ^a (OR, 95% CI)	N (%)	P value ^a (OR, 95% CI)	N (%)	P value ^a (OR, 95% CI)
Controls	170 (71.4)		61 (25.6)		7 (3.0)		68 (28.6)	
All breast cancer	188 (64.6)	1.00	93 (32.0)	0.1 (1.3, 0.9–2.0)	10 (3.4)	0.6 (1.3, 0.5–3.5)	103 (35.4)	0.1 (1.3, 0.9–2.0)
Under 40 BrCa ^b	142 (68.3)	1.00	59 (28.4)	0.5 (1.2, 0.7–1.7)	7 (3.3)	0.8 (1.2, 0.4–3.5)	66 (31.7)	0.5 (1.2, 0.7–1.7)
Familial ^c BrCa	123 (64.1)	1.00	65 (33.8)	0.1 (1.5, 0.9–2.2)	4 (2.1)	0.7 (0.7, 0.2–2.8)	69 (35.9)	0.1 (1.4, 0.9–2.1)

^a Fisher's exact test (two-sided) for the relevant genotype using the CC genotype as reference. The ORs and 95% CIs are shown in brackets.

^b BrCa, breast cancer.

^c Family history is defined as two or more cases of breast cancer reported in first or second degree female relatives or bilateral breast cancer.

frequency of the C/T polymorphism under either a codominant or dominant model. There was no significant association of the T allele with breast cancer risk among the women diagnosed with breast cancer before the age of 40 years, among those with bilateral breast cancer, or among those who reported a family history of breast cancer (defined as at least two cases of breast cancer in first or second degree female relatives). Consequently, our study does not support the findings of the North American case control study that has suggested a large increase in risk of breast cancer (OR = 4.8) associated with the T allele among younger women with a first-degree relative with the disease. We concur with Spurdle *et al.* (7) that the T allele of the prohibitin 3' UTR polymorphism is not associated with an increased risk of breast cancer among women diagnosed with breast cancer before age 40 years, irrespective of whether they have a family history of the disease.

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