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Do *BRCA1* modifiers also affect the risk of breast cancer in non-carriers?

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ABSTRACT

We studied whether or not single nucleotide polymorphisms (SNPs), which have been shown to modify the risk of breast cancer in women with a *BRCA1* mutation, are associated with cancer risk in unselected (non-hereditary) breast cancer patients. We genotyped seven SNPs in six distinct genes (*PHB*, *RAD51*, *ITGB3*, *TGFB1*, *VEGF*, *MTHFR*) in 1100 unselected Polish breast cancer patients and 1100 controls. The frequencies of genotypes were similar in cases and controls. In a subgroup analysis, we found a positive association between the homozygous genotype *PHB* 1630C/T and medullary breast cancer (odds ratio (OR) = 4.0, 95% confidence interval (CI) 1.1–14.0). *PHB* 1630C/T was also associated with tumours negative for oestrogen receptor (OR = 2.2, 95% CI 1.13–4.4) or progesterone receptor (OR = 2.8, 95% CI 1.4–5.8). Our results show that, in general, the single nucleotide polymorphisms which modify the risk of hereditary breast cancer in Poland do not modify the risk of sporadic breast cancer. The *PHB* 1630 C/T single nucleotide polymorphism was associated with breast cancers with clinical features typical for *BRCA1*-positive tumours and is deserving of further study.

1. Introduction

Breast cancer is the most common malignancy among Polish women with a lifetime risk of approximately 6%. This malignancy is diagnosed in 12,000 new cases and causes 5000 deaths every year. It is estimated that in Poland 50–60,000 women are currently affected by breast cancer. The three common founder mutations in *BRCA1* gene (4153delA, 5328insC, and C61G) account for the majority (~90%) of *BRCA1* mutations in Polish breast–ovarian cancer families. However, mutations in the *BRCA1* gene are responsible for only about 3% of all breast cancers and for the majority of patients, the genetic contribution remains unknown.[1–3]

Numerous studies have examined low-penetrance susceptibility single nucleotide polymorphisms (SNPs) in candidate genes for breast cancer. There are at least two categories of SNPs of interest: (1) SNPs which are believed to modify the risk of cancer in women from the general population and (2) SNPs which are believed to modify the risk of cancer in women who are already at an elevated risk (e.g. because of a mutation in *BRCA1*, *BRCA2* or *CHEK2*). It is of interest to know whether or not these two groups of SNPs are over-lapping, i.e.

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whether or not the SNPs which are relevant in the high-risk group are also relevant to women at average risk.

Recently, we have identified several SNPs which were associated with breast or ovarian cancer risk in Polish *BRCA1* mutation carriers.[4–8] The SNPs were localised within genes which are important in cancer development and progression, including: regulation of cell growth (*PHB*, *ITGB3*, *TGFB1*, *VEGF*), DNA repair (*RAD51*) or folate metabolism, which is essential in DNA biosynthesis and methylation (*MTHFR*). Only SNPs known to have functional activity were included. We conducted univariate and multivariate analyses; in the latter, the odds ratios (OR) were adjusted for other risk factors, including age at first live birth, parity, breastfeeding, age at menarche, oral contraceptive use, smoking, and body mass index.

In the current study, we sought to determine whether or not the SNPs which modified the risk of breast cancer in women with a *BRCA1* mutation are also associated with cancer risk in unselected breast cancer patients, or if the observed associations are restricted to mutation carriers. We analysed seven single nucleotide polymorphisms in six genes in a group of 1100 unselected Polish breast cancer cases and 1100 controls.

2. Materials and methods

2.1. Study participants

The study was performed on a series of 1100 prospectively-ascertained cases of invasive breast cancer patients (participation rates above 95%) who were diagnosed at the Regional Oncology Hospital (Szczecin) in the years 2002, 2003, 2006 and 2007 or the University Hospital from 2002 to 2007 in Szczecin, West-Pomerania, Poland. Patients with pure intraductal or intralobular cancer were excluded (DCIS or LCIS) but patients with DCIS with micro-invasion were included. Twenty-nine women carried one of the three Polish founder *BRCA1* mutations (4153delA, 5328insC, and C61G) and were excluded from the present analyses.

The control group was comprised of 1100 healthy adult females with a negative family history of cancer residing the region of Szczecin. These controls were part of a population-based study of the 1.3 million inhabitants of West Pomerania performed in 2003 and 2004 which was designed to identify familial aggregations of cancer by our centre. Cancer-free control women with a negative cancer family history were identified by a review of the records of the population based study and invited for an interview. The participation rate was 55%. These individuals were chosen for this study to be sex-, age- and geographically matched with the breast cancer cases (year of birth was matched within 2 years). Women affected with any malignancy or with any cancers diagnosed in a first- and second-degree relative were excluded from this control group.

All patients and controls were invited for an interview. During the interview the goals of the study were explained, informed consent was obtained, genetic counselling was given and a blood sample was taken for DNA analysis. A central pathology review was conducted in Szczecin by two pathologists associated with the study. Each case was reviewed with regard to histology (medullary, ductal, lobular, tubulo-lobular, DCIS with microinvasion, other). Information was recorded on age at diagnosis, stage, grade and lymph node status, oestrogen receptor, progesterone receptor status and bilaterality.

Table 1 – Primers, probes and annealing temperatures for SimpleProbe analysis.

PHB 1630 C/T (rs 6917)		
Forward	5′ CGTGAGAAGGGCAGTCTCTGA 3′	53 °C
Reverse	5′ TGCATCCTGCTGGGGCTGAA 3′	
Probe	FAM 5′ CTGCCAAAGACGTGTCCGACC 3′Phos	
RAD51 135 G/C (rs1801320)		
Forward	CTGGGGCAAGCGAGTAGAGA	56 °C
Reverse	TCCGACTTCACCCCGCGG	
Probe	FAM 5′ CCCAACGCCCCTGGCTTACGCTCCA 3′ Phos	
ITGB3 L59P (rs5918)		
Forward	5′ TGGGCTCCTGTCTTACAGG 3′	52 °C
Reverse	5′ GGCACAGTTATCCTTCAGCAG 3′	
Probe	FAM 5′CTGCCTCCGGGCTCACCTCGCTG 3′ Phos	
TGFB1 –509 C/T (rs1800469)		
Forward	5′ GGAGGGTGTCAGTGGGAGGA 3′	58 °C
Reverse	5′ TTCTTACAGGTGTCTGCCTCCTGA 3′	
Probe	FAM 5′ CTTCCATCCCTCAGGTGTCCTGTT 3′ Phos	
VEGF 936 C/T (rs3025039)		
Forward	5′ ACTCCGGCGGAAGCATTCCC 3′	56 °C
Reverse	5′ GGGCTCGGTGATTTAGCAGCAAGA 3′	
Probe	5′ CCAAGAGGGACCGTGCTGGGTCAC 3′ FAM	
MTHFR A222V (rs1801133)		
Forward	5′ GAAGAATGTGTCAGCCTCAAAG 3′	52 °C
Reverse	5′ CCTGAAGCACTTGAAGGAG 3′	
Probe	5′ TGAAATCGACTCCCGCAGACAC 3′ FAM	
MTHFR E429A (rs1801131)		
Forward	5′ GGTTCTCCCGAGAGGTAAAG 3′	53 °C
Reverse	5′ AGCTGCTGAAGATGTGGG 3′	
Probe	FAM 5′ CAGTGAAGCAAGTGTCTTTGAAG 3′Phos	