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BRAF polymorphisms and the risk of ovarian cancer of low malignant potential

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Abstract

Objective. The object of this study was to test the hypothesis that *BRAF* is a low-risk susceptibility gene for low malignant potential (LMP) ovarian cancer. A recent study of the relationship between *BRAF* polymorphisms and malignant melanoma identified strong linkage disequilibrium across the *BRAF* gene with one of the three most common haplotypes (haplotype *C*) having a population attributable risk of approximately 1.6%. We therefore hypothesized that the same *BRAF* haplotype may confer an increased risk of serous ovarian tumors of low malignant potential.

Methods. We genotyped 383 cases of LMP ovarian cancer, including 234 of serous histology, and 987 controls for seven SNPs, representative of the most common *BRAF* gene haplotypes, using MALDI-TOF mass spectrometry.

Results. Haplotype information was obtained for 369 LMP ovarian cancer cases and 983 healthy controls. None of the haplotypes were found to be associated with risk of LMP ovarian cancer (OR for haplotype *C* 0.81, 95% CI = 0.54–1.22), or with the risk of serous LMP ovarian cancer (OR for haplotype *C* 0.90, 95% CI = 0.56–1.45).

Conclusion. We found no evidence to suggest that *BRAF* is a low-risk LMP ovarian cancer susceptibility gene.

Keywords: BRAF; Susceptibility; Polymorphism; Ovarian cancer; Low malignant potential; LMP

Introduction

The pathogenesis of ovarian cancer is poorly understood, as is the relationship between borderline (low malignant potential) and invasive ovarian adenocarcinoma. There is evidence to suggest that serous ovarian cancers of low malignant potential (LMP) will not progress to high-grade ovarian cancer but, in contrast, mucinous LMP tumors share specific somatic mutations with their benign and invasive counterparts and may be part of a continuum [1–4].

The *RAF* family of genes (including *BRAF*) encode cytoplasmic serine–threonine kinases that bind to Ras, mediating a cellular response to growth signals. Somatic missense mutations in the kinase domain of *BRAF* have been identified in common moles [5] and malignant melanomas [6,7], as well as in other types of cancer [8–11], including serous ovarian tumors of low malignant potential [12,13]. However, *BRAF* gene mutations are rare in invasive and in non-serous tumors [12,13]. Therefore,

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further knowledge of *BRAF* gene involvement in ovarian tumorigenesis may help to gain a better understanding of the etiology of LMP tumors and the nature of their relationship with their malignant counterparts.

The majority of ovarian cancer patients present with no remarkable family history [14,15], making it unlikely that high penetrance germline mutations, in *BRAF* or any other gene, play an important role in disease susceptibility. Instead, heritable genetic factors that may be involved in susceptibility to ovarian cancer are likely to be associated with small increases in risk, and could be conferred by relatively common variants. If they occur at a high frequency within the population, they may be important risk factors at the population level. Meyer et al. [16] found a suggestion for a possible relationship between *BRAF* polymorphisms and malignant melanoma. More recently, a study by James et al. [17] identified strong linkage disequilibrium across the *BRAF* gene in Caucasians from Australia, and found one of the three most common haplotypes (haplotype *C*) to have a population attributable risk of malignant melanoma of approximately 1.6%. No studies to date have examined the association between *BRAF* variants and the risk of LMP ovarian cancer.

We hypothesized that the *BRAF* haplotype *C*, identified by James et al. [17], may confer an increased risk for serous ovarian cancer of low malignant potential. We set out to test this hypothesis in a case-control study, comprising 383 cases, including 234 of serous histology (the largest collection of LMP ovarian cancer cases to genotyped date), and 987 healthy controls.

Materials and methods

Subjects

A case-control sample, drawn from six case-control studies conducted in three different countries (Table 1), comprised 383 ovarian cancer cases of low malignant potential, with no selection for family history, and 987 healthy controls. Of the 383 tumors, 234 were serous, and the remainder mucinous (128), endometrioid (7), clear cell (2), and undetermined or mixed histologies (12). Information on potential or known ovarian cancer risk factors was available for most cases. Age was known for all but one of the cases (99.7%), tubal ligation and parity for 85%, and hysterectomy, oral contraceptive pill (OCP) use, and smoking for 82% of cases. The age range was 19–95 years. Questionnaire information regarding ethnicity was available for 48% of cases. 32% reported Caucasian ethnicity, while the remaining 16% were of mixed ethnicity.

Limited information on potential or known ovarian cancer risk factors was available for controls, including age (for 91%) tubal ligation (for 37%), hysterectomy, OCP use, parity, and smoking (for 35%). Ages ranged from 20 to 80 years. Ethnicity information was available for 78% of control subjects. 71% were of Caucasian ethnicity, while the remaining 9% were of mixed ethnicity.

Details of the six studies are as follows:

1. Familial Registry of Ovarian Cancer (FROC). Patients with epithelial ovarian cancer diagnosed between March 1, 1997 and July 31, 2001 were identified through the Greater Bay Area Cancer Registry operated by the Northern California Cancer Centre as part of the Surveillance, Epidemiology and End Results (SEER) Program of the National Cancer Institute. We used rapid case ascertainment to identify cases within 1 month of diagnosis. Eligible patients were those diagnosed with invasive or LMP epithelial ovarian cancer at ages 20 years to 64 years who resided in six Bay Area counties. Of the 579 women who provided epidemiologic data and a blood or mouthwash sample, 115 patients were diagnosed with LMP epithelial ovarian cancer. Control women were identified through random-digit dial and were frequency-matched to cases on race/ethnicity and 5-year age group. Full description of the study design and methods are available in McGuire et al. [18]. DNA was purified from peripheral blood leucocytes ($n = 218$) using the Puregene Kit (Gentra Systems, Minneapolis, MN) and from exfoliated cells in buccal mouthwash rinses ($n = 12$) as previously described [19]. DNA was quantified by spectrophotometry.

Table 1
Sources of ovarian cancer cases and controls

Source	Cases	(% Genotyped)	Case source location	Controls	(% Genotyped)	Control source location
FROC	115	(99)	San Francisco Bay Area	115	(100)	San Francisco Bay Area
QIMR	94	(88)	SWH and RBH, Australila	594	(100[a])	ATR
DUMC	76	(100)	North Carolina, USA	141	(100)	North Carolina, USA
IRV	43	(100)	Irvine, USA	53	(98)	Irvine, USA
DKFZ	29	(93)	Heidelberg and Freiburg, Germany	55	(96)	Heidelberg and Freiburg, Germany
PAH	26	(100)	Southampton, UK	29	(97)	Southampton, UK
Total	383			987		

ATR = Australian Twin Registry; DKFZ = Deutsches Krebsforschungszentrum for German Cancer Research Center; DUMC = Duke University Medical Center; FROC = Familial Registry of Ovarian Cancer; IRV = Population-based cancer registry of Orange County; PAH = Princess Anne Hospital; QIMR = Queensland Institute of Medical Research; RBH = Royal Brisbane Hospital; SWH = Survey of Women's Health.
[a] Genotype drawn from Melanoma Study by James et al. [29].