

Mutation in Brief**Mutational Analysis of BRCA1 and BRCA2 Genes in Chinese Ovarian Cancer Identifies 6 Novel Germline Mutations**

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Germline mutations in the BRCA1 and BRCA2 genes predispose women to breast and ovarian cancer. An incidence of 5% and 3.3% respectively has been reported of BRCA1 and BRCA2 mutations in women with ovarian cancer unselected for family history. The contribution of BRCA1 and BRCA2 mutations to ovarian cancer in Chinese women is unknown. A total of 60 samples of ovarian cancer diagnosed in Chinese unselected for age or family history were analyzed for BRCA mutations using the protein truncation test. The entire coding exon of BRCA1 of 53 cases and that of exon 11 of BRCA2 of 43 cases were successfully screened. Six germline (11.3%) mutations (633C>T, 1080delT, 1129delA, 2371-2372delTG, 3976-3979delGTGA, and IVS 22+7 A>G) were detected in BRCA1. One germline mutation (3337C>T) (2.1%) was detected in BRCA2. None of these seven cases were associated with strong family history of breast and/or ovarian cancer. Five out of our six BRCA1 mutations and the one BRCA2 mutation identified are novel. Our 11.3% incidence of BRCA1 mutations in ovarian cancer found amongst Chinese with insignificant family history is apparently higher than that previously reported in other populations. It suggests that BRCA1 mutation may play a significant role in the development of sporadic ovarian cancer in Chinese women. © 2000 Wiley-Liss, Inc.

KEY WORDS: BRCA1; BRCA2; Chinese, ovarian cancer

INTRODUCTION

A wide variation in the incidence and characteristics of inherited BRCA1 and BRCA2 gene (MIM#s 113705 and 600185) mutations has been observed amongst the various populations studied, with some specific mutations being apparently characteristic to some particular ethnic origin (Szabo & King 1997). Molecular studies of BRCA mutations to date, however have largely been focused on patients from Caucasian background. The Japanese are one of the few Asian populations that have studied BRCA mutations extensively, demonstrating mutations unique to that ethnic group. The only information regarding BRCA gene mutations in

2 Khoo et al.

the Chinese population, have been in relation to breast cancer (Li et al 1999; Tang et al 1999) and our own recent publication reporting somatic BRCA1 mutations in both breast and ovarian cancers. (Khoo et al 1999)

The incidence of BRCA1 germline mutations previously reported in ovarian cancers unselected for age and family history ranges between 2-6% (Matsushima et al 1995; Takahashi et al 1995; Stratton et al 1997; Janezic et al 1999). In these studies most of the mutation carriers were shown to have positive family history of breast and/or ovarian cancer. BRCA2 germline mutations have also been reported at an incidence of 3 – 4 % in sporadic ovarian cancers (Foster et al 1996; Takahashi et al 1996). The contribution of BRCA1 and BRCA2 mutations to ovarian cancer in Chinese women is unknown.

To investigate the contribution of BRCA1 and BRCA2 germline mutations to ovarian cancer in the Chinese population of Hong Kong, we have studied a series of ovarian tumor samples from Chinese women diagnosed with ovarian carcinoma. Sixty rapidly frozen fresh tumor specimens were collected, all cases being unselected for age or family history. The entire BRCA1 coding sequence and exon 11 of BRCA2 were analyzed using the protein truncation test.

MATERIALS AND METHODS

Samples

Tumor samples were obtained from excision specimens of ovarian cancer patients treated in Queen Mary Hospital, a regional hospital and a teaching hospital of the University of Hong Kong. Non-tumor tissue was also collected whenever available. Informed consent in accord with ethical guidelines was obtained from individuals donating blood specimens for analysis. Genomic DNA as well as total cellular RNA was extracted from the tumor samples by standard procedures. Prior to extraction, a histological section was cut from all blocks to confirm the identity of tissues analyzed. In cases in which mutations were identified, genomic DNA was further extracted from the patient's non-tumor tissue and sequenced together with tumor DNA.

Protein truncation test (PTT)

The entire coding exon of the BRCA1 gene (Genbank accession number, U14680) was analyzed by the PTT reaction as described previously (Ozcelik et al. 1996; Ozcelik et al. 1997). Genomic DNA or cDNA synthesized from tumor RNA was used in the PTT analysis of exon 11 of BRCA1 and BRCA2, and of exons 2-10, 12-24 of BRCA1 respectively. A total of six overlapping primer pairs for BRCA1, and three overlapping primer pairs of exon 11 of BRCA2 were used. The PCR reaction was carried out in a total volume of 30 μ l containing 10mM Tris-HCl (pH 8.3), 50 mM KCl, 1.0 mM MgCl₂, 0.10% gelatin, 200 μ M of each dNTP, 200 nmol of each forward and reverse primers, 2.5 units of AmpliTaq DNA polymerase and 100-200ng of nucleic acids. Amplification consisted of 35 cycles each of 30 sec at 94°C, 30 sec at 55°C and 120 sec at 72°C. PCR products from tumor and normal control samples were used directly for in vitro protein synthesis by the TnT/T7 coupled rabbit reticulocyte lysate system (Promega) in the presence of [³⁵S]methionine (Amersham). The synthesized protein products were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were fixed, dried and exposed to autoradiography.

DNA sequence analysis

Fresh frozen tumor DNA samples showing truncated protein products on PTT, as well as the DNA extracted from additional tumor and non-tumor sections of these cases were analyzed by direct sequencing in both sense and anti-sense directions using Thermo Sequenase radiolabeled terminator cycle sequencing kit (Amersham Life Sciences) as described by the manufacturer.

RESULTS AND DISCUSSION

The ovarian cancer cases ranged from ages 25 to 84 years, with an average age of 53 years (median 51.5 years). All cases were unselected for family history. We successfully analyzed the entire coding region of the BRCA1 gene for 53 of the 60 frozen ovarian tumors, for which six mutations were found (Figure 1, Table 1). The entire exon 11 of the BRCA2 gene was successfully analyzed in 48 cases, which resulted in the identification of one BRCA2 mutation (2.1 %) (Figure 1, Table 1).

Table 1. BRCA1 and BRCA2 mutations detected and clinical characteristics of mutation carriers

Case	Age	Gene	Exon	Mutation	Family history of cancer	Cancer type	Personal history of other cancers
G106	43	BRCA1	8	633C>T	Ovary (M) Liver (F)	Serous cystadenocarcinoma, grade 3	None
G4	62	BRCA1	11	1080delT	Nil	Serous cystadenocarcinoma, grade 2	None
G50	46	BRCA1	11	1129delA	Breast (PA), Endometrium (PA)	Serous cystadenocarcinoma, grade 3	None
G116	44	BRCA1	11	2371-2372delTG	Liver (F), Oesophagus (MGM)	Serous cystadenocarcinoma, grade 3 Bilateral	Endometrial carcinoma
G105	46	BRCA1	11	3976-3979delGTGA	Ovary (MC)	Endometriod adenocarcinoma	None
52	52	BRCA1	Int 22	IVS 22+7 A>G	Breast (PA) NPC (PGF)	Serous cystadenocarcinoma, grade 3 Bilateral & omental	None
50	64	BRCA2	11	3337C>T	Nil	Mucinous adenocarcinoma, grade 3	None

F, father; M, mother; MGM, maternal grandmother; MA, maternal aunt; MC, maternal cousin; PA, paternal aunt; PGF, paternal grandfather

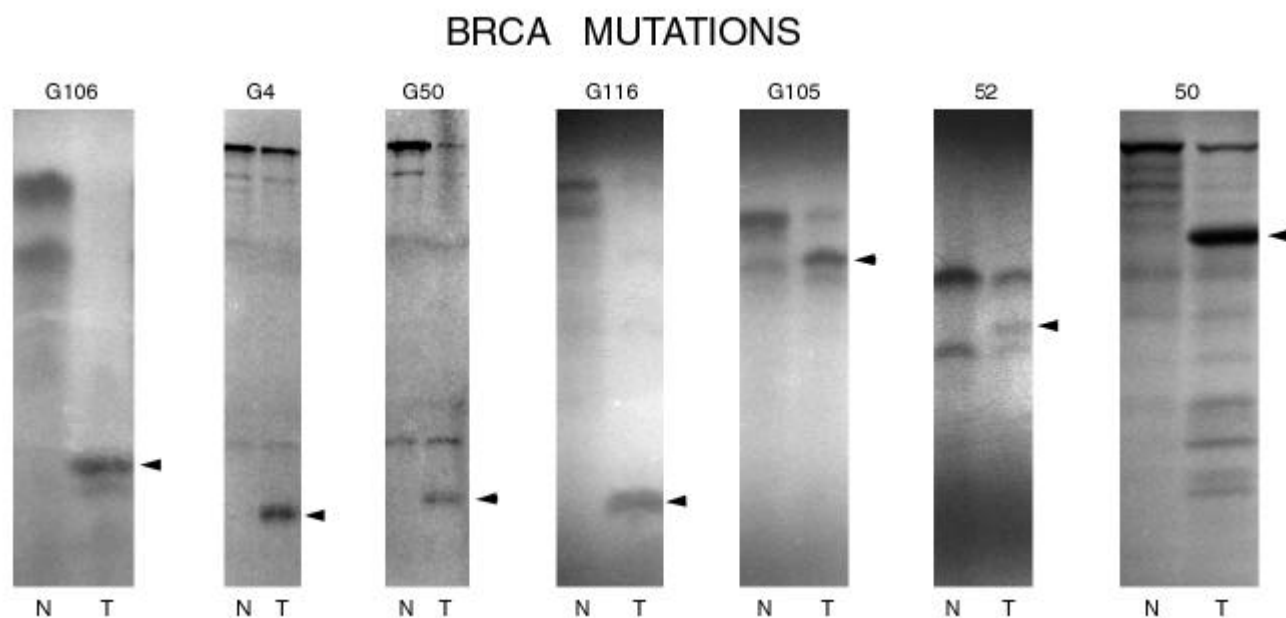


Figure 1. Protein truncation test showing truncated protein products of cases with BRCA mutations. T = tumor, N = normal sample BRCA1 non-sense mutation was found in case G106. Frame-shift BRCA1 mutations in exon 11 were involved in cases G4, G50, G116 and G105. Case 52 was found to harbor a BRCA1 aberrant splicing mutation in IVS 22+7 A>G which results in a stop codon after splicing. A non-sense BRCA2 mutation was found in case 50.

All seven mutations were confirmed to be germline by direct sequencing of non-tumor DNA. Four of the BRCA1 mutations, 1080delT, 1129delA, 2371-2372delTG and 3976-3979delGTGA, were frame-shift mutations. One case was found to harbor an aberrant splicing mutation (BRCA1 IVS 22+7 A>G) which resulted in a truncated protein product. Two nonsense mutations were detected, a 633C>T in BRCA1 and a 3337C>T in BRCA2. The finding of BRCA1 mutations in six of 53 cases, represents a prevalence of 11.3% (95% CI = 4.27% - 23.03%)

4 Khoo et al.

Five out of the reported six BRCA1 mutations and the one BRCA2 mutation identified in this study are novel. The 1129delA BRCA1 mutation has been previously reported in a Dutch patient with ovarian cancer (Breast Cancer Information Core 1999). Although the BRCA1 mutations we have identified were found scattered throughout the entire length of the gene, mutations 1080delT and 1129delA, are noted to be clustered within 50bp of each other. The location of these two mutations, together with the mutations 633C>T and 2371-2372delTG, make up 4 out of the 6 BRCA1 mutations that we found to be located at the 5' region of the gene. This observation is concurrent with the reported connection between the risk of ovarian cancer and mutations in the 5' two thirds of the gene (Gayther et al 1995).

The age at the time of diagnosis of the 7 cases with BRCA mutations ranged between 43 to 64 years, the average age being 48.8 years. Carriers of BRCA1 mutations were mainly serous cystadenocarcinoma (5 cases), with one case of endometrioid adenocarcinoma. The patient with the BRCA2 mutation had a poorly differentiated mucinous adenocarcinoma, and was aged 64 at the time of diagnosis. These clinical features are comparable with that found in the literature (Rubin et al 1996; Aida et al 1998). Unlike in breast cancer where BRCA1 mutations can be found in younger patients, BRCA1 mutations are notably uncommon in young ovarian cancer patients.

Analysis for family history of cancer of all seven patients having BRCA mutations, showed that four had one other member of the family with either a breast or an ovarian cancer (Table 1). Patient G50, had a paternal aunt who developed breast cancer aged 45 years, and another paternal aunt diagnosed with carcinoma of the uterine corpus at the age of 60. No other cases had a family history of breast and/or ovarian cancer of several affected family members. A family history of carcinoma of liver and esophagus, as well as nasopharyngeal carcinoma (which are relatively more common cancers in this region) were found in the families of three other patients. The patient with the BRCA2 germline mutation was noted to have no family history of cancer.

In contrast to the previously reported incidence of 2-5%, our prevalence of 11.3% of BRCA1 germline mutations in Chinese ovarian cancers unselected for age and family history is relatively high, and the association with family history is weak. These findings are consistent with a more recent report by Rubin et al 1998 which noted an 8.6% incidence of BRCA1 mutations with a weak or non-existent association of mutation carriers with family history. Our finding of one BRCA2 mutation (2.1%) is at an incidence comparable to that found in the literature.

The incidence of ovarian carcinoma in the Chinese population in Hong Kong of 7.7 per 100,000 (Hospital Authority 1999) is about half of that found in Caucasians (Bjorge et al 1997; Tortolero et al 1995). Our apparently higher incidence of BRCA1 mutations in Chinese ovarian cancer in individuals with rather unremarkable family history suggests that BRCA1 mutation may play a significant role in the development of sporadic ovarian cancer in Chinese women. The possibility of differences in penetrance of some BRCA1 mutations in the general population compared to that estimated in high-risk families should also be considered. Further study with a larger sample size would be important in order to better define the frequency of the BRCA1 and BRCA2 gene mutations in Chinese breast and ovarian families. Such information will be useful in properly appraising the usefulness of genetic screening in our population.

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