SPANDIDOS PUBLICATIONS

Germline mutations of *BRCA1* in two Korean hereditary breast / ovarian cancer families

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Received September 19, 2005; Accepted November 11, 2005

Abstract. Testing for cancer susceptibility gene, in particular mutations in the *BRCA1* gene in association with hereditary breast/ovarian cancer has been extensively studied. We investigated germline mutations in the *BRCA1* gene from two Korean hereditary breast/ovarian cancer families using direct DNA sequencing. Blood samples of the thirteen family members were studied. We found three missense mutations; 3232 A→G, 2731 C→T, 3667 A→G. These mutations were involved in the altered coding of amino acids. According to the BIC database, clinical significance of these mutations is regarded as favor polymorphisms. Therefore, these genetic variations are not believed to be involved in the development of the disease, but may be associated with breast/ovarian cancers in another yet undefined way. For further clinical significance of these variations, additional study such as a case-controlled haplotyping study is needed.

Introduction

Breast cancer is the most common cancer in Korean women and the incidence of ovarian cancer is the ninth in order with increasing tendency (1). Although most cases of breast and ovarian cancer occur sporadically in women with no previous personal or family history of cancer, it is well known that 5-7% of breast cancers and 10% of ovarian cancers are hereditary with a pattern of autosomal-dominant inheritance (2).

Testing for cancer susceptibility genes support a variety of clinical decisions by providing results that indicate risk for future disease, confirmation of diagnoses and more recently, therapeutic selection and prognosis. The *BRCA1* associated with hereditary breast/ovarian cancer is the most extensively studied cancer susceptible gene to date. *BRCA1* was originally isolated using positional cloning techniques (3). Germline alterations in the gene result in a predisposition for developing early-onset breast and ovarian cancer with a penetrance as high as 85% and 65%, respectively (4). The protein products of the *BRCA1* gene regulate, at least in part, transcriptional activation, DNA repair, cell-cycle checkpoint control and chromosomal re-modeling (5).

Although about 1500 genetic variants of *BRCA1* have been described in the Breast Cancer Information Core (BIC) (http://research.nhgri.nih.gov/bic/), there is little data on the contribution of germline *BRCA1* mutations to breast and/or ovarian cancer in Korea (6,7). To detect mutations, a variety of mutation screening methods, including dHPLC, CSGE, DGGE, SSCP and direct DNA sequencing have been adopted in Korea (8). Direct DNA sequencing is regarded as the 'gold standard' for sensitivity (9).

To further understand the implication of genetic variants, we performed mutational analysis on the *BRCA1* gene in two families having two or more affected first- or second-degree relatives with breast and/or ovarian cancer using direct DNA sequencing.

Materials and methods

Subjects. We evaluated germline mutations of *BRCA1* from 13 blood samples in two families including two or more affected first- or second-degree relatives with breast and/or ovarian cancer. One proband is a patient with ovarian cancer and the other one is a patient with breast cancer (Fig. 1).

Genomic DNA isolation. Using an iNtRon blood genomic DNA purification kit (iNtRON Biotechnology, Seoul, Korea) and in accordance with the manufacturer's protocols, genomic DNA was extracted from peripheral blood lymphocytes.

PCR program. DNA was amplified by PCR for the 24 exons of the *BRCA1* gene using previously published primer sets (10) that yielded 169- to 400-base pair products. Because of its size, exon 11 was screened using 19 overlapping primer sets (Table I). PCRs were performed with genomic DNA containing 50 ng of genomic DNA, 1 μl of each primer at 5 pmol/μl, 2 μl of a mixture of dNTPs (each at 2.5 mM), 2.5 μl of 10X PCR buffer, 1 μl of Taq polymerase, and distilled water was added to a final volume of 25 μl. For amplification,

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Key words: ovarian neoplasms, breast neoplasms, genes, *BRCA1*, Koreans

Table I. *BRCA1* primers used for amplification and SSCP analysis of exon 11.

Exon	Sense	Antisense	Product length (bp)
11.0	5'-GTGAATTTTCTGAGACGGATGTA	5'-GAGCTGGCATGAGTATTTGTG	169
11.1	5'-AGAGGCATCCAGAAAAGTATCAGG	5'-GGGAGTCCGCCTATCATTACA	239
11.2	5'-ACAGCCTGGCTTAGCAAGGAG	5'-CCCCATCATGTGAGTCATCAGA	278
11.3	5'-AGAAACTGCCATGCTCAGAGAATC	5'-ATGAGGATCACTGGCCAGTAAGTC	245
11.4	5'-TGTATTGGACGTTCTAAATGAGGT	5'-TTGTGAGGGGACGCTCTTGTA	266
11.5	5'-GCATTTGTTACTGAGCCACAGATA	5'-TCTATTGGGTTAGGATTTTTCTCA	263
11.6	5'-CAAACGGAGCAGAATGGTCA	5'-GCCTGGTAGAAGACTTCCTCCTC	244
11.7	5'-TCCACAATTCAAAAGCACCTAAAA	5'-CTCTGGGAAAGTATCGCTGTCAT	299
11.8	5'-GCAACTGGAGCCAAGAAGAGTAAC	5'-TTTGCAAAACCCTTTCTCCACTTA	256
11.9	5'-TTGTCAATCCTAGCCTTCCAAGAG	5'-TTTTGCCTTCCCTAGAGTGCTAAC	224
11.10	5'-TATGGCACTCAGGAAAGTATCTCG	5'-GCGCTTTGAAACCTTGAATGTAT	270
11.11	5'-ACAGTCGGGAAACAAGCATAGAA	5'-TTTGGCATTATCAACTGGCTTATC	314
11.12	5'-AGGCTTTCCTGTGGTTGGT	5'-TTACGGCTAATTGTGCTCACTG	306
11.13	5'-AACATTCCAAGTACAGTGAGCACA	5'-AGATGCATGACTACTTCCCATAGG	378
11.14	5'-TCCTGGAAGTAATTGTAAGCATCC	5'-GGCCCCTCTTCGGTAACC	325
11.15	5'-TCCTAGCCCTTTCACCCATACA	5'-AGATGCCTTTGCCAATATTACCTG	274
11.16	5'-TGCTACCGAGTGTCTGTCTAAGAA	5'-AGAAAGGATCCTGGGTGTTTGTAT	209
11.17	5'-GCTAGCTTGTTTTCTTCACAGTGC	5'-AAGTTTGAATCCATGCTTTGCTCT	218
11.18	5'-CAGGGAGTTGGTCTGAGTGAC	5'-GCTCCCCAAAAGCATAAAC	181

each sample was denatured at 94˚C for 2 min and subjected to 35 cycles of PCR (94˚C for 30 sec, 55˚C for 15 sec, 60˚C for 15 sec, and extension at 72˚C for 1 min on Applied Biosystems DNA thermal cycler); this was followed by incubation at 72˚C for 10 min.

SSCP analysis. For SSCP analysis, the PCR products were mixed with an equal volume of formamide loading dye. The mixture was heated at 95˚C for 5 min to denature the DNA. After denaturation, the sample was chilled on ice for a few minutes. The samples were then run on an acrylamide gel under non-denaturing conditions in an electrophoresis apparatus. Electrophoresis was carried out in 0.5X TBE for 3-5 h at 4˚C.

Direct sequencing. All sequence variants were confirmed by using the PCR products of each sequence variant and Big Dye on an ABI3100 DNA sequencer (Applied Biosystems).

Results

In two breast and/or ovarian cancer families, 13 blood samples were evaluated for germline mutations in *BRCA1*. SSCP analysis using PCR products of the whole *BRCA1* gene revealed that there were band shifts in only exon 11 compared to the wild-type *BRCA1* gene (Fig. 2). All members of Park family showed a band shift in the location of the exon 11.13, except for a son of the proband who did not have the genetic mutation (Fig. 1). Also, all members of the Moon family had two genetic mutations in SSCP. The locations identified were exon 11.11 and exon 11.14.

Direct DNA sequencing of exon 11, which is known as a 'hot spot', found three missense mutations as follows: 3232 A→G, 2731 C→T, 3667 A→G (Fig. 3). These mutations altered coding of amino acids in the study family.

To understand the clinical significance of these missense mutations, we referred to the BIC database, which has about 1500 genetic variations registered. All of the genetic mutations that we found in two Korean families have already been entered into the BIC database. They are identified as favor polymorphisms. In addition, they have reference SNP numbers (Table II).

Discussion

The *BRCA1* gene is located on chromosome 17q21 and has a total length of about 100 kb. This gene consists of 24 exons and the coding region starts at the middle of exon 2. The size of the mRNA is 7.8 kb and *BRCA1* product, localized in the nucleus (11), is a phosphorylated protein that consists of 1863 amino acids and has a molecular weight of 220 kDa. The phosphorylation is dependent on the cell cycle (12). *BRCA1* is known to have several functions. One is repair of DNA damage by interacting with Rad51 and participation in homologous recombination (13). Another is regulation of gene expression by performing as a cofactor of many transcription factors. For instance, *BRCA1* functions as a coactivator in p53-

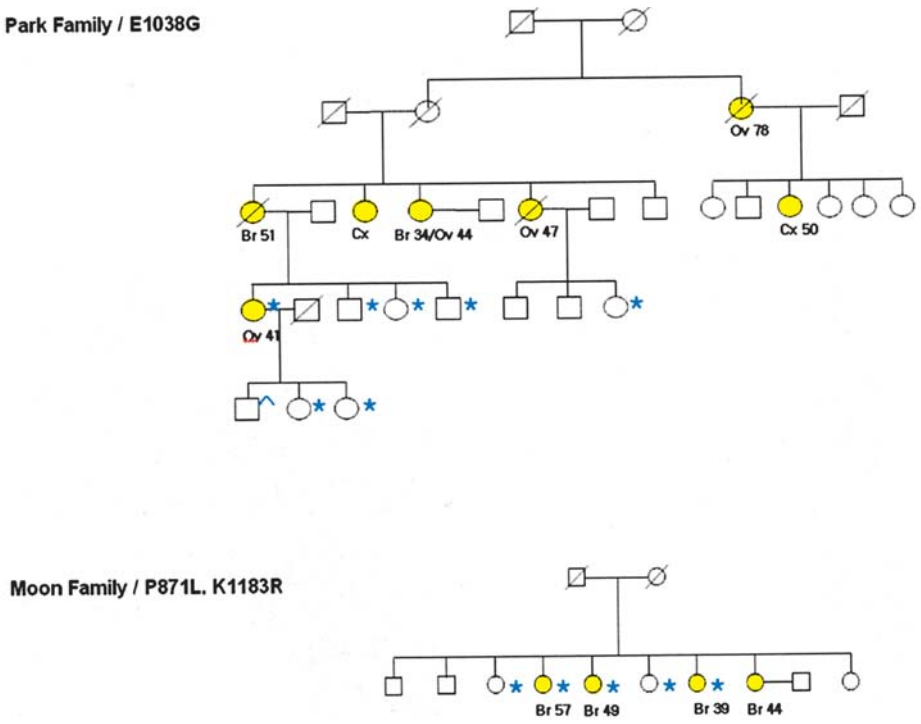

Figure 1. Korean breast and breast-ovarian cancer families with germline *BRCA1* missense mutations. Cancer types, age at diagnosis and mutation status are shown. *Confirmed from blood sample to carry mutation; ^Confirmed from blood sample not to carry mutation; Circles, patients with cancer; Br, breast cancer; Ov, ovarian cancer; Cx, cervical cancer.

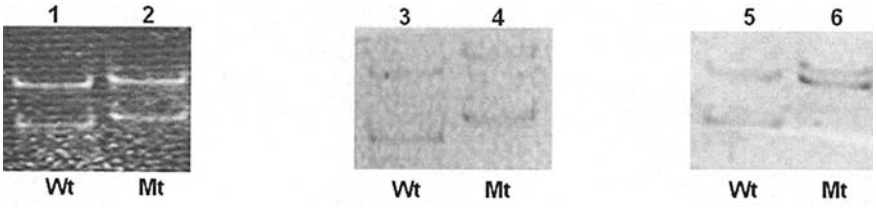

Figure 2. Single-strand conformational polymorphism (SSCP) assay for germline mutation in *BRCA1* exon 11. Lanes 1, 2, exon 11.13 PCR products; Lanes 3, 4, exon 11.11 PCR products; Lanes 5, 6, exon 11.14 PCR products; Wt, wild-type; Mt, mutant type.

dependent transcription (14) and as a corepressor in estrogen response transcription (15). Therefore, *BRCA1* is believed to regulate cell growth, apoptosis and cell function by regulating gene expression. The other function is to maintain chromosomal stability. *BRCA1*-deficient cultured cells show decreased cell growth and hypersensitivity to ionizing radiation, resulting in DNA damage associated with chromosomal abnormalities (16-18). Overall, *BRCA1* in its non-mutated form function as a tumor suppressor gene. The process of cancer development in individuals with germline mutations in the *BRCA1* gene requires somatic inactivation of the remaining wild-type allele.

The *BRCA1* gene is very large and hundreds of specific different mutations of *BRCA1* have been described. Most of the mutations are unique to the family in which they occur (19). Myriad Genetic Laboratories, Inc., where most variants in *BRCA1* were discovered and classified, classify genetic variants as deleterious, suspected deleterious, uncertain clinical significance, favor polymorphism and polymorphism/neutral (20). Mutations that result in a truncated protein can be assumed to be deleterious. The most common deleterious mutations for *BRCA1* are non-sense or frame-shift mutations that begin prior to, or at, codon 1853. In addition, some specific missense mutations and splice mutations are recognized as deleterious. Although many variations that are not deleterious mutations have been found, many remain to be identified.

Ethnicity may affect genetic mutations. Founder mutations have been identified in various ethnic groups including Ashkenazi Jews, Icelanders, Russians, and Israelis (21-23). However, it is still necessary to screen the entire sequences of *BRCA1* genes in Asian women. Most studies of *BRCA1* mutations in Japan reported many frameshift or non-sense mutations as well as polymorphisms and unclassified variants in *BRCA1* gene, but there appears not to be specific Japanese 'hot spots' for *BRCA1* mutations (24). A previous study in 21 Korean hereditary breast/ovarian cancer families identified only 5 deleterious mutations in *BRCA1* gene; 2 frameshift and 3 non-sense mutations, without polymorphisms or unclassified variants. They used the protein truncation test for exon 11 of *BRCA1* as a screening method (7). The present study found three genetic variations in *BRCA1* in two Korean families with hereditary breast and/or ovarian cancer (Table II). All mutations identified were also reported in the Japanese

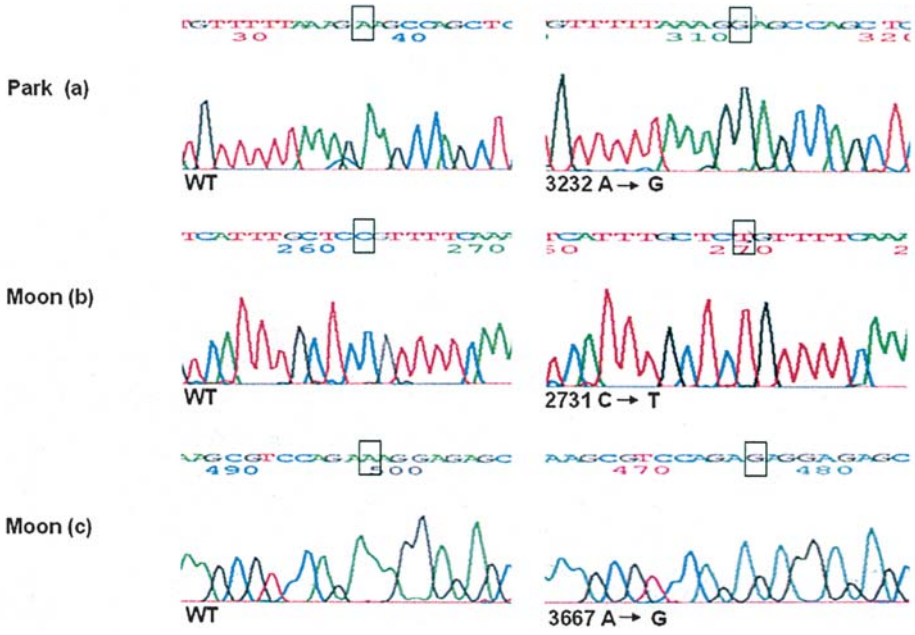

Figure 3. Missense mutations identified by direct sequencing. Direct PCR based sequencing was performed on all samples in which a shift band pattern was observed in SSCP analysis. (a) Park family: Left, wild-type sequence of a portion of exon 11; Right, missense mutation sequence of a portion of exon 11 (3232 A→G). (b) and (c) Moon family: Left, wild-type sequence of a portion of exon 11; Right, missense mutation sequences of a portion of exon 11 (2731 C→T), (3667 A→G).

Table II. Polymorphisms in *BRCA1* gene.

Family	Exon	Codon	Nucleotide change[a]	Amino acid change	Reference SNP (rs) number	BIC[b]
Park	11	1038	3232 A>G	Glu to Gly	16941	-
	11	871	2731 C>T	Pro to Leu	799917	-
Moon	11	1183	3667 A>G	Lys to Arg	16942	-

[a]Nucleotide number are based on U14680; [b]Change entered in the BIC database are marked as -.

studies (24). According to the BIC database, three variations are all regarded as favor polymorphisms. They are given reference SNP (rs) numbers (http://www.ncbi.nlm.nih.gov). Therefore, these genetic variations are not believed to be causative mutations for cancer, but may be associated with the disease in another yet undefined manner. For a more detailed understanding of the clinical significance, further study such as a case-controlled haplotyping is needed. Here we may not have found any deleterious mutations reported in the Korean cancer families, because of the small number of samples studied. Instead, we identified polymorphisms, not found in a previous study for Korean women, this difference could have resulted from different screening methods.

At first, we used a single-strand conformational polymorphism (SSCP), which showed a band-shift in exon 11. Then direct DNA sequencing was performed. Many studies have used SSCP or denaturing high-performance liquid chromatography (DHPLC), but direct DNA sequencing is considered optimal and as the 'gold standard'.

BRCA1-related breast cancers can be associated with a poor prognosis, but *BRCA1*-related ovarian cancers are associated with a high frequency of serous adenocarcinoma and a good outcome (24). Among patients with FIGO stage III ovarian cancer, the 5-year survival rate of *BRCA1*-mutation positive patients is reported as 78.6%, as compared with only 30.3% in control patients, with other etiologies for their ovarian cancer (25). The clinicopathologic features of one proband with ovarian cancer were consistent with the familial ovarian cancer with a *BRCA1* mutation. The proband had primary surgery with adjuvant chemotherapy 14 years ago. Pathologic diagnosis was stage IIIc serous adenocarcinoma. Recurrence developed six years after the primary treatment and she had cytoreductive surgery and palliative chemotherapy, total of 50 cycles to date. She has been very sensitive to platinum-based chemotherapy. One possible mechanism supporting the favorable outcome in BRCA carriers with ovarian cancer is the increased sensitivity to platinum induced DNA damage in BRCA heterozygotes (26).

Although this study investigated a small number of samples of the Korean *BRCA1* families, it identified genetic variations of *BRCA1* genes through direct DNA sequencing methods screening the entire sequences. All identified

were favor polymorphisms and are therefore not to be causative mutations in the development of the breast/ovarian cancer. Because only a limited number of cases have been analyzed in Korea, for further understanding of the genetic variations in the *BRCA1* gene in the Korean population, additional study is required.

References

1. Ministry of Health and Welfare RoK: Annual report of cancer registry programme in the Republic of Korea (2000.1-2000.12), 2003.
2. Claus EB, Schildkraut JM, Thompson WD and Risch NJ: The genetic attributable risk of breast and ovarian cancer. Cancer 77: 2318-2324, 1996.
3. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, *et al*: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66-71, 1994.
4. Easton DF, Ford D and Bishop DT: Breast and ovarian cancer incidence in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. Am J Hum Genet 56: 265-271, 1995.
5. Kerr P and Ashworth A: New complexities for BRCA1 and BRCA2. Curr Biol 11: R668-676, 2001.
6. Ahn SH, Hwang UK, Kwak BS, Yoon HS, Ku BK, Kang HJ, Kim JS, Ko BK, Ko CD, Yoon KS, Cho DY, Kim JS and Son BH: Prevalence of BRCA1 and BRCA2 mutations in Korean breast cancer patients. J Korean Med Sci 19: 269-274, 2004.
7. Kang HC, Kim IJ, Park JH, Kwon HJ, Won YJ, Heo SC, Lee SY, Kim KH, Shin Y, Noh DY, Yang DH, Choe KJ, Lee BH, King SB and Park JG: Germline mutations of BRCA1 and BRCA2 in Korean breast and/or ovarian cancer families. Hum Mutat 20: 235, 2002.
8. Eng C, Brody LC, Wagner TM, Devilee P, Vijg J, Szabo C, Tavtigian SV, Nathanson KL, Ostrander E and Frank TS: Interpreting epidemiological research: blinded comparison of methods used to estimate the prevalence of inherited mutations in BRCA1. J Med Genet 38: 824-833, 2001.
9. Orban TI, Csokay B and Olah E: Sequence alterations can mask each other's presence during screening with SSCP or heteroduplex analysis: BRCA genes as examples. Biotechniques 29: 94-98, 2000.
10. Friedman LS, Ostermeyer EA, Szabo CI, Dowd P, Lynch ED, Rowell SE and King MC: Confirmation of BRCA1 by analysis of germline mutations linked to breast and ovarian cancer in ten families. Nat Genet 8: 399-404, 1994.
11. Wilson CA, Ramos L, Villasenor MR, Anders KH, Press MF, Clarke K, Karlan B, Chen JJ, Scully R, Livingston D, Zuch RH, Kanter MH, Cohen S, Calzone FJ and Slamon DJ: Localization of human BRCA1 and its loss in high-grade, non-inherited breast carcinomas. Nat Genet 21: 236-240, 1999.
12. Ruffner H and Verma IM: BRCA1 is a cell cycle-regulated nuclear phosphoprotein. Proc Natl Acad Sci USA 94: 7138-7143, 1997.
13. Moynahan ME, Chiu JW, Koller BH and Jasin M: Brca1 controls homology-directed DNA repair. Mol Cell 4: 511-518, 1999.
14. Ouchi T, Monteiro AN, August A, Aaronson SA and Hanafusa H: BRCA1 regulates p53-dependent gene expression. Proc Natl Acad Sci USA 95: 2302-2306, 1998.
15. Fan S, Wang J, Yuan R, Ma Y, Meng Q, Erdos MR, Pestell RG, Yuan F, Auborn KJ, Goldberg ID and Rosen EM: BRCA1 inhibition of estrogen receptor signaling in transfected cells. Science 284: 1354-1356, 1999.
16. Powell SN and Kachnic LA: Roles of BRCA1 and BRCA2 in homologous recombination, DNA replication fidelity and the cellular response to ionizing radiation. Oncogene 22: 5784-5791, 2003.
17. Venkitaraman AR: Functions of BRCA1 and BRCA2 in the biological response to DNA damage. J Cell Sci 114: 3591-3598, 2001.
18. Welcsh PL and King MC: BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. Hum Mol Genet 10: 705-713, 2001.
19. Welcsh PL, Owens KN and King MC: Insights into the functions of BRCA1 and BRCA2. Trends Genet 16: 69-74, 2000.
20. Frank TS, Deffenbaugh AM, Reid JE, Hulick M, Ward BE, Lingenfelter B, Gumpper KL, Scholl T, Tavtigian SV, Pruss DR and Critchfield GC: Clinical characteristics of individuals with germline mutations in BRCA1 and BRCA2: analysis of 10000 individuals. J Clin Oncol 20: 1480-1490, 2002.
21. Johannesdottir G, Gudmundsson J, Bergthorsson JT, Arason A, Agnarsson BA, Eiriksdottir G, Johannsson OT, Borg A, Ingvarsson S, Easton DF, Egilsson V and Barkardottir RB: High prevalence of the 999del5 mutation in icelandic breast and ovarian cancer patients. Cancer Res 56: 3663-3665, 1996.
22. Robles-Diaz L, Goldfrank DJ, Kauff ND, Robson M and Offit K: Hereditary ovarian cancer in Ashkenazi Jews. Fam Cancer 3: 259-264, 2004.
23. Struewing JP, Hartge P, Wacholder S, Baker SM, Berlin M, McAdams M, Timmerman MM, Brody LC and Tucker MA: The risk of cancer associated with specific mutations of BRCA1 and BRCA2 among Ashkenazi Jews. N Engl J Med 336: 1401-1408, 1997.
24. Arai M, Utsunomiya J and Miki Y: Familial breast and ovarian cancers. Int J Clin Oncol 9: 270-282, 2004.
25. Aida H, Takakuwa K, Nagata H, Tsuneki I, Takano M, Tsuji S, Takahashi T, Sonoda T, Hatae M, Takahashi K, Hasegawa K, Mizunuma H, Toyoda N, Kamata H, Torii Y, Saito N, Tanaka K, Yakushiji M, Araki T and Tanaka K: Clinical features of ovarian cancer in Japanese women with germ-line mutations of BRCA1. Clin Cancer Res 4: 235-240, 1998.
26. Cass I, Baldwin RL, Varkey T, Moslehi R, Narod SA and Karlan BY: Improved survival in women with BRCA-associated ovarian carcinoma. Cancer 97: 2187-2195, 2003.