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Editorial note:

Family history has long been known to be an important risk factor of breast cancer. In this issue of Topical Update, Dr. Ui Soon Khoo gives us an update on the two major susceptibility genes associated with this disease, and discusses on the practical aspects. We welcome any feedback or suggestions. Please direct them to Dr. Polly Lam (e-mail: lamwy@ha.org.hk) of Education Committee, the Hong Kong College of Pathologists. Opinions expressed are those of the authors or named individuals, and are not necessarily those of the Hong Kong College of Pathologists.

Hereditary Breast and Ovarian Cancer – the BRCA1 and BRCA2 genes

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Background

Breast cancer is the leading female cancer in Hong Kong. Now at 52.1 per 100,000 (Hong Kong Cancer Registry, 2008) its incidence has been steadily rising over the last few decades, and is the highest reported in Asian regions. There are two major breast and ovarian susceptibility genes, BRCA1 and BRCA2. About 30-70% of patients with hereditary breast/ovarian cancer and about 5-10% of all breast and/or ovarian cancer cases harbor a germline mutation in these genes[1]. The defective gene is inherited in autosomal dominance pattern. Individuals carrying a mutation in the BRCA1 or BRCA2 genes have a 85% lifetime risk of breast cancer, and a lifetime risk for ovarian, fallopian tube or primary peritoneal cancer that ranges from 35-60% for BRCA1 and 10-27% for BRCA2[2].

BRCA mutation carriers tend to develop breast cancer at a young age, may have bilateral breast cancer or have a personal history of both breast and ovarian cancer. There is also an increased risk for prostate and pancreatic cancer as well as male breast cancer in BRCA2 mutation carriers. Other features of increased likelihood of hereditary susceptibility include the presence of two or more individuals in the family with breast cancer, the presence of both breast and ovarian cancer in the family, breast cancer in one or more male family members, and one of more members with two primary cancers. To estimate the probability of heritable genetic mutation in a family, one has to

take into account the age of onset of breast cancer, the number of affected relatives, biological relationships of affected relatives, the ratio of affected to unaffected relatives as well as the presence/absence of associated malignancies and ethnic background.

Clinical and pathologic features

Gene expression microarray profiling of breast cancer has identified a distinct subtype called basal-like cancer which is characterized by an expression signature that is similar to basal/myoepithelial cells of the breast[3]. Basal-like cancer is the subtype observed in BRCA1-related breast cancers, representing 80-90% of breast cancers arising in BRCA1 mutation carriers and about 15% of sporadic breast cancers associated with reduced BRCA1 mRNA expression[4].

Although there is as yet no internationally accepted definition for basal-like cancers, basal cytokeratin markers, singly or in combination, such as CK5/6, CK14, and CK17 by immunohistochemistry have been used to identify basal phenotype[5]. These cancers are usually of high histological grade, with features of medullary-like cancers. Most metaplastic cancers also display basal-like phenotype. Basal-like cancers typically do not express hormone receptors or HER-2 (triple negative phenotype). Ductal carcinoma in-situ (DCIS) with basal-like phenotype has been reported, suggested to be the precursor lesion to invasive basal-like cancer.
TP53 mutations have been found at high frequency in breast cancers with germ-line BRCA1 mutations (97%) as well as in sporadic basal-like breast carcinomas (92%) independent of BRCA1 status[6]. DCIS with basal-like phenotype was also found strongly associated with p53 accumulation.

Patients with basal-like cancer are usually younger and associated with poorer clinical outcome with development of metastases within the first 5 years, shorter survival and relatively high mortality rate. They are more strongly associated with family history, more frequently “interval cancers” (i.e. cancers arising between annual mammograms), and with specific mammographic features demonstrating rapid progression. They also show a specific pattern of distant metastases to brain and lung.

BRCA2 related breast cancers contain a significant proportion of tubular and lobular carcinoma not commonly found in BRCA1 mutation carriers. These cancers tend to be of medium to high grade, more often estrogen receptor positive and more commonly associated with ductal carcinoma in-situ. BRCA1 and BRCA2 related ovarian carriers tend to be advanced stage high-grade serous carcinomas.

Genetic testing

Genetic testing aims at identifying the mutation that predisposes the individual or the family to cancer. In families where germ-line mutations in BRCA1 and BRCA2 have been identified, estimates for breast cancer risk can be made with greater accuracy. Both BRCA genes are very large genes. Several hundred different mutations have been identified but only a few of these mutations have been found repeatedly in unrelated families.

Identification of a specific mutation in a family, therefore, is a complex process and must usually begin by testing a blood sample from a family member who has had breast or ovarian cancer, called “index" testing. If a specific mutation is identified through index testing, then "carrier" testing is possible for family members who wish to learn whether or not they have inherited that mutation and the associated cancer risks.

A negative result from families where no mutation has been identified cannot exclude the possibility that other genes, as yet unknown, may be involved in that family.

Although basal-like breast cancer appears associated with BRCA1 mutations, there is as yet no recommendation that genetic test be carried out on these cases. The recommendation is against routine referral for genetic counseling and BRCA testing for women without specific family history patterns.

Testing for mutations of inherited cancer susceptibility genes raises many issues for the individual and family, with medical, psychological, and social implications. Hence the benefits of routine screening for mutations have to be balanced with adverse ethical, legal and social consequences that could result from this. Individuals are strongly recommended to receive genetic counseling prior to testing. Blood samples for genetic testing are accepted only after informed consent has been given.

Local findings

The frequency of BRCA mutations and the magnitude of cancer risks vary across different populations[7]. For familial breast/ovarian cancer families, the prevalence of BRCA1 and BRCA2 mutations in Caucasians and African Americans (42.2%, 27.9% respectively) is much higher compared with Asians (5-20%)[8].

On the other hand, For sporadic ovarian cancer, the 11.3% incidence of BRCA1 mutations in Chinese is one of the highest reported worldwide[9]. Founder mutations, presumed to have arisen in a single ancestor of a specific ethnic group many generations ago, have been identified in many ethnic groups including the Chinese population[10].

The majority of germline mutations in the BRCA genes lead to truncated protein which disrupts the function of the encoded proteins. Somatic mutations in BRCA1 and BRCA2 are rare[11]. Reduced expression of BRCA1 protein and promoter hypermethylation has been demonstrated in both breast and ovarian cancer. On the other hand, increased BRCA2 protein expression with promoter hypomethylation has been found in sporadic ovarian cancer[12].

Interventions offered

The interventions that can be offered to women with BRCA1 or BRCA2 mutation carriers include intensive screening, chemoprevention, prophylactic mastectomy and/or oophorectomy. There remains insufficient evidence on the effectiveness of intensive surveillance with mammography or the benefits of chemoprevention with selective estrogen receptor modulators in improving health outcomes for women with BRCA1 or BRCA2 mutations[13]. Although the use of MRI, ultrasonography, and mammography in combination has a high sensitivity of 95%, the effect of this increased detection on morbidity and mortality remains unclear[14]. There is however fair evidence that prophylactic surgery for these women significantly decreases the incidence of breast and ovarian cancer[15]. Oophorectomy reduced ovarian cancer risk by 85-100% and reduced breast cancer risk by 53-68%.

Origin of High-grade Serous Malignancies.

The acceptance of prophylactic oophorectomy as the treatment strategy for women with BRCA mutations and at high risk for the development of ovarian carcinoma, led to the recognition of clinically occult tubal carcinomas and serous tubal intraepithelial carcinoma (STIC) originating in the distal fallopian tube, particularly the fimbriae, making an important contribution to determining the ultimate site of origin pelvic high-grade serous malignancy[16].

Detailed examination of prophylactic salpingo-oophorectomies has revealed the presence of serous tubal intraepithelial carcinoma (STIC) in approximately 5% cases, with about 80% of these early carcinomas originating in the distal fallopian tube, particularly the fimbrae[17]. Tumors arising from this region are extremely small and previously often went unrecognized, emphasizing the importance of complete histologic sampling of fallopian tubes and ovaries in all salpingo-oophorectomy specimens. Detailed routine pathological examination of the fimbria following the protocol “Sectioning and Extensively Examining the FIMbrial end” (SEE-FIM)[17] is now the recommended method of handling prophylactic salpingo-oophorectomy specimens for BRCA mutation carriers (Table 1)[18] as the outcome and management of these individuals would depend on the status of her fallopian tubes.

The American College of Obstetrician and Gynecologists now recommend that women with BRCA1 or BRCA2 mutations, aged above 40 years or when childbearing is complete, should be

offered risk-reducing bilateral salpingo-oophorectomy with microscopic examination of ovaries and fallopian tubes for occult cancer and thorough visualization of the peritoneal surfaces with pelvic washings.

Tubal carcinomas originating in the distal fallopian tube has since been identified irrespective of BRCA status and has also been shown to be the source of one half of primary peritoneal serous carcinomas. STICs have significant cytological atypia, absence of cilia, are highly proliferative, and in 80% of cases highlighted by nuclear accumulation of mutated p53 protein, with TP53 mutations found in almost all cases.

p53 immunostaining has also revealed the presence of small linear p53 positive foci in non-neoplastic mucosa of the distal fallopian tube, called “p53 signatures”. Evidence suggests that these “p53 signatures” are a precursor of pelvic serous carcinoma, and probably the earliest lesion in a continuum of tubal serous carcinogenic sequence. These are now shown to be a relatively common finding in the fallopian tube, its prevalence in BRCA mutation carriers similar to that in women with unknown BRCA status. Such findings have important clinical implications which include recommending salpingetcomy at the time of simple hysterectomy.

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Table 1. The SSS-FIM Protocol for examining the fallopian tubes of prophylactic salpingo-oophorectomy specimens.

1. Fix tubes and ovaries in formalin for 1-2 hours to reduce risk of exfoliation during sectioning. Submit the entire tube and ovary for histology in the following manner:
2. Amputate the distal 2 cm of tube, which is to be sectioned sagitally into 4 sections.
3. Serially section the remaining portion of tubes at 2 to 3 mm intervals.
4. Serially section the ovaries at 2 to 3 mm intervals.
5. Perform p53 and MIB-1 immunostaining for areas showing cytological atypia and loss of cilia