Atypical Medullary Breast Carcinoma in a Family Carrying the 5382insC *BRCA-1* Mutation

To the Editor:

The tumor suppressor genes *BRCA-1* and *BRCA-2* identified in mid-1990s were thought initially to be responsible for the majority of hereditary breast and/or ovarian cancer. However, it is now generally believed that germline mutations in *BRCA-1* and *BRCA-2* account for only 15–20% of familial breast cancers and less than 5% of breast cancers overall (1). The possibilities for the remaining 80% of family clusters are a combination of a small number of moderately strong genes and a larger number (possibly 100 or more) of weaker genes (2). Nevertheless, the two genes *BRCA-1* and *BRCA-2* are of high penetrance and confer a quite increased lifetime risk of breast and ovarian cancer. According to a recent large epidemiologic study, breast cancer risk exceeds 80% by age 80 years for both *BRCA-1* and *BRCA-2* mutation carriers, while the ovarian cancer risk reaches 55% and 28%, respectively, for the two genes by the same age (3). It seems imperative therefore to be able to identify these high-risk alleles for proper genetic counseling and therapeutic management of the proband and progeny.

Mutation analysis in *BRCA-1* and *BRCA-2* is cumbersome and expensive since both genes are extremely large, with a total cDNA of 16.7 kb, and mutations are scattered throughout the entire coding region. To reduce the cost of such screening and to increase its effectiveness, certain features like histologic and biologic characteristics of the tumors of the probands have to be taken into account as well. It is evident now that the phenotype of a tumor with a *BRCA-1* germline mutation is different from the phenotype of a *BRCA-2*-associated tumor and that both cases differ from the phenotype of a sporadic case (4,5).

BRCA-1 tumors tend to be of a higher grade and higher mitotic index than the sporadic cases. Most of them are negative for both estrogen and progesterone receptors and are associated with aneuploidy and high S-phase. The presence of p53 somatic mutations confers a far more aggressive pattern in the *BRCA-1* tumor compared to a sporadic tumor. *BRCA-2* tumors are more heterogeneous and lie somewhere in between. Recently these observations have been verified at the molecular level with the use of a cDNA microarray through the identification of 176 genes that are differentially expressed in *BRCA-1* or *BRCA-2* tumors (6). This allows for the possibility that, in the future, transcriptional profiling might be able to correctly classify a tumor and therefore bypass the need for a complete genetic analysis.

However, at the moment these techniques are not widely available and the ability of techniques routinely employed in pathology laboratories to correctly identify the gene involved could be exploited. This could promptly direct the patient for genetic counseling even in the absence of family history. A very sensitive portion of patients for this is the group of women less than 40 years of age that develop breast and/or ovarian cancer. It is surprising that in a study of such a group, 27% of those identified with a deleterious *BRCA-1* mutation had no family history at all (7). More recently, in a larger study selecting for women diagnosed with breast cancer before 36 years of age but who had no family history of the disease, a prevalence of 3.6% for *BRCA-1* mutations was found (8). These data indicate that other criteria for inclusion of a patient in a genetic study have to be used besides a pedigree pointing to a hereditary pattern (9). There is always a chance that family history might be hidden due to either low penetrance caused by the presence of modifying alleles of other genes or to male transmission of the gene or to a relatively low number of family members without excluding the possibility of a de novo mutation.

We present here a case report of a multiparous, 39-year-old woman with breast cancer where multiple evidence suggested a *BRCA-1*-associated carcinoma besides her family pedigree, which included a mother with bilateral metachronous breast cancer at age 51 and 55 years. At the end of our investigation and after a *BRCA-1* mutation was detected, a grandmother with cancer at an unidentified

The Breast Journal, Volume 9, Number 3, 2003 260–262

age (eventually breast cancer in her 80s) and an aunt (mother's sister) with colon cancer at age 56 years were also mentioned. She presented with a palpable lump at the outer quadrant of the right breast 6 months after giving birth to her third child and soon after she quit breast-feeding. She had an early menarche (at 12 years old), has used contraceptives for 6 months, her first pregnancy was at age 30 years, and she breast fed all three of her children.

Mammographic evidence and clinical examination suggested possible malignancy. Fine-needle aspiration cytology confirmed that the mass was malignant. The patient underwent a quadrantectomy with ipsilateral axillary lymphectomy and received six cycles of cyclophasphamide, adriamycin, and fluouracil (CAF) chemotherapy followed by postoperative regional and ipsilateral axillary radiotherapy.

Our first line of evidence for a *BRCA-1* carcinoma was the presence of medullary carcinoma in her histology report, since an overrepresentation of this type of carcinoma in *BRCA-1* mutation carriers has been reported (10). The tumor consisted of a well-circumscribed, 2.5 cm diameter solid mass diagnosed as atypical medullary carcinoma with squamous metaplasia. The architecture was predominantly syncytial (more than 75% of the tumor) growing as a solid sheet of tumor cells with indistinct cell borders and containing pushing margins. Extensive necrosis and a high number of mitotic cells were present. Areas of tumor margins showed focal lymphocyte infiltration, however, the stromal infiltration of mononuclear cells was sparse. An intraductal component was present as well. Atypical pleomorphic nuclei and bizarre-looking giant cells were abundant. One of 24 axillary lymph nodes excised was infiltrated with cancer cells.

The first lesion, excised from the mother's right breast when she was premenopausal at age 51 years, showed a complete analogous architecture and image in the archival slides according to the two experienced pathologists in our group, and this is, to the best of our knowledge, the first report of such a *BRCA-1* family. Four years later the mother developed a second lesion in the other breast diagnosed as infiltrating ductal carcinoma of grade III, lymph node positive (two of seven), and she eventually died from metastatic disease. So far there is no established guideline for medullary carcinoma to be an absolute indication for *BRCA-1* testing, however, it has been proposed as a cost-effective *BRCA-1* mutation analysis approach to screen all medullary carcinomas (11). The fact that the mother suffered from bilateral breast cancer and the early onset of cancer in the daughter (less than 40 years old) further reinforced the *BRCA-1* hypothesis.

The second line of evidence came from the immunohistochemical and biologic characteristics of the tumor. Estrogen receptors (ERs) and progesterone receptors (PRs) were both negative as assessed by the immunohistochemical LSAB method performed on cryostat frozen sections (clones 1D5 and 10A9 from Immunotech-Coulter). Neither p53 nuclear accumulation in more than 10% of the cells by the DO-1 monoclonal antibody nor a membrane overexpression of the product of c-*erb*B-2 gene was observed. Proliferation as reflected by the MIB-1 count was extensive: the Ki67 marker stained 26% of the cells as measured in the CAS 200 image cytometer. No *BRCA-1* nuclear staining with the MS-110 antibody was observed in the corresponding paraffin section. Imprints of the frozen tissue were Feulgen stained and 200 nuclei were measured in a CAS 200 image cytometer. The tumor was found to be aneuploid (DNA Index 1.51) and the total proliferative fraction was estimated to be at least 33% while the objective over 5C cells percentage was found quite elevated (37%). Eight giant cells with DNA mass over 44 pg were recorded (the largest one contained 49 times the DNA amount of a regular cell). Except for p53, all other data fitted the pattern of a BRCA1 related tumor.

Our genetic analysis approach includes first the protein truncation test (PTT) for exon 11 of *BRCA-1* and exons 10 and 11 of *BRCA-2*, representing 60% of the coding region of both genes; in case of a negative result, we proceed with DNA sequencing (12). In our patient, PTT analysis was performed only for exon 11 of *BRCA-1* and was found to be negative. Based on the aforementioned evidence, instead of performing PTT analysis for *BRCA-2*, we proceeded directly with DNA sequencing for the rest of the *BRCA-1*. A 5382insC truncating mutation was revealed at exon 20 of *BRCA-1*. Since the mother was not alive, we tested the mother's sister and found her to be positive for the 5382insC mutation, and therefore we assumed that both the grandmother and her mother were obligate carriers of the same mutation.

Family pedigree is not always easy to obtain or ascertain. Most of the times a familial pattern is recognized, such as in our case (her mother's breast cancer was immediately reported), however, the hereditary pattern is established from additional data on older generations (usually not available or obscure) or other cancers in the family (first regarded as not relevant to mention, like the aunt's colon cancer). Therefore it is imperative for the laboratories involved in cancer evaluation to cooperate in order to achieve an efficient and cost-effective screening for the two genes *BRCA-1* and *BRCA-2*.

We present here such a case where we efficiently detected a *BRCA-1* mutation without the financial burden of *BRCA-2* screening and where features like family history, age of onset, and histologic, immunohistochemical, and cytometric data intercalated in order to achieve the best result. This is useful not only for the course of the patient's disease (bilateral cancer is usually a problem to be addressed) and the choice of therapy, but also for her progeny. An analogous effort to identify the *BRCA-1* signature in a tumor might also prove to be rewarding in the group of young women less than 40 years old with breast and/or ovarian cancer irrespective of family history.

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