

## Letter to the Editor

# Genetic counseling of medullary breast cancer patients

### To the Editor:

Germline mutations in the *BRCA1* and *BRCA2* genes account for approximately 40% of families with evidence of inherited susceptibility to breast cancer but for only 2–3% of all breast cancer cases. However, these genes are highly penetrant, and mutations in either of these genes confer a 60–85% lifetime risk of breast cancer and a 15–40% lifetime risk of ovarian cancer (1). Mutation analysis in the *BRCA1* and *BRCA2* genes is cumbersome and expensive, because both genes are extremely large, with a total cDNA of 16.7 kb, and mutations are scattered throughout the entire coding region. It is therefore imperative for genetic counselors to be able to efficiently identify those patients in real need of the complete genetic analysis of both genes.

An overrepresentation of the rare subtype of medullary breast carcinoma among patients with mutations in the *BRCA1* gene has recently been reported, while this is not occurring for the *BRCA2* gene (2–4). The inclusion of the histology of medullary breast cancer as an independent selection criterion for referral to *BRCA1* mutation analysis, besides family history and age, has been proposed as a possibility for genetic counseling (2–4). However, in a recent investigation of 42 cases of typical and atypical medullary breast cancer, only three *BRCA1* mutations (7%) were detected, and these were among young patients with a significant family history (5).

It has also been proposed that besides family history, other morphological and biological features of breast cancer could improve the efficacy of *BRCA1* mutation screening (6, 7). It is now realized that the tumor phenotype of *BRCA1* mutation carriers differs significantly from the phenotype of sporadic breast cancer, while the phenotype of *BRCA2* tumors lies somewhere in between. *BRCA1* tumors tend to be of a higher grade and higher mitotic index than the sporadic cases (8). They are often negative for both estrogen and progesterone receptors (ER and PgR) and are associated with aneuploidy, high S-phase, and presence of p53 somatic mutations that confer a far more aggressive pattern in the *BRCA1* tumor compared with a sporadic one (8).

In the most recent and largest of all cohorts of cases with a *BRCA1* germline mutation ( $n = 165$ ), these observations were substantiated and three immunohistochemical markers emerged as the ones showing significant difference compared to sporadic breast cancer: 90% and 79% of the *BRCA1* tumors were negative for the presence of the hormone receptors ER and PgR, respectively, and 97% of them were negative for the overexpression of the c-erbB-2 oncogene (9). Our data agree with the above remarks and we also suggest to include a fourth parameter: an estimate of the tumor proliferation with either the Ki-67 proliferation marker assessed immunohistochemically or the proliferation profile obtained from cytometry which in our *BRCA1* tumors is always extensive (10, 11).

The goal of our study was to test for the above two proposals and examine their validity alone or in combination in a group of medullary carcinoma patients. Medullary carcinoma of the breast is rather scarce (<3% of breast cancer cases in most populations), but we managed to collect phenotypic and genetic data from 17 Greek patients suffering from this rare subtype. Patients were asked about their family history in counseling sessions. After informed consent, mutation analysis was performed on genomic DNA isolated from blood leukocytes employing a combination of the Protein Truncation Test (PTT) for the large exon 11 of the *BRCA1* gene and DNA sequencing on an ABI 310 Genetic Analyzer (Perkin-Elmer, Applied Biosystems, Foster City, CA, USA) of the other small exons and all exon/intron boundaries of the *BRCA1* gene (including exon 11) (10). Complex rearrangements constituting about 10% of *BRCA1* mutations were not covered by our mutation detection methods. Histological diagnosis and classification in the corresponding tumor specimens on hematoxylin and eosin slides were based on criteria previously described (12, 13). We performed immunohistochemistry (IHC) with the LSAB methodology by using the following monoclonal antibodies for the four phenotypic parameters mentioned above: 1D5 (ER), 10A9 (PgR), MIB-1 (Ki-67), and 3B5 (c-erbB-2), all purchased from

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Immunotech-Coulter (Marseille Cedex, France). Most of the IHC work was performed on paraffin sections; however, in a few occasions, frozen tissue was available and image cytometry with the CAS 200 (Becton Dickinson, San Jose, CA, USA) was also performed on Feulgen-stained imprints for DNA-ploidy, S-phase, and proliferative fraction estimation. Also, because an image analysis cytometer was available, we quantitated the fraction of cells being positive for the Ki-67 marker in the IHC. According to our reference values, proliferation was considered as 'high' when the Ki-67 antibody stained more than 20% of the cells (11).

The mean age of our group of patients who underwent medullary breast cancer diagnosis was 46 years (range 31–65 years). Four of the carcinomas were classified as atypical. Only five patients had a positive family history (29%). Seven out of the 17 tumor specimens (41%) were showing the phenotype of a BRCA1 carcinoma: negative for estrogen and progesterone hormone receptors and the *c-erbB-2* gene and also highly proliferative as seen by the intense Ki-67 immunostaining and/or high S-phase/proliferative fraction. BRCA1 mutations were detected in two of the patients: the 5382insC mutation in exon 20 by DNA sequencing and the rare R1203X mutation in exon 11 with PTT screening methodology. The 5382insC mutation is recurrent in the Greek population (10) and was detected in a familial case with atypical medullary cancer previously described (11). The R1203X mutation seems rare (being reported six times to the BIC database) and was found in a 41-year-old woman with typical medullary carcinoma who did not possess known family history due to the fact that she was adopted. If only the family history was used as referral criterion, this mutation would have been missed and the patient would have lost important medical information about her clinical follow-up and genetic counseling. On the other hand, the mutation detection efficiency is rather low when only the histology criterion of medullary carcinoma was considered (12% or two out of 17 cases). According to our data in the Greek population, the BRCA1 mutation screening efficiency could be increased to 22%, if the genetic analysis would be reduced to a group of patients possessing either positive family history or the appropriate BRCA1 phenotypic characteristics in their tumors (two out of nine cases). Genetic counseling might therefore benefit from taking into account phenotypic tumor criteria besides family history alone, when dealing with patients with medullary breast cancer. Although the present sample size was small, it might eventually become part of a series

of patient cohorts in the literature that could add to our ability to accurately identify individuals most likely to have BRCA1 mutations.

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