CASE REPORT

Challenges in Interpreting Germline Mutations in BARD1 and ATM in Breast and Ovarian Cancer Patients

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Abstract: Next-generation sequencing promotes identification of mutations in non-BRCA1/2 genes in hereditary cancer families. The contribution of mutations in moderate penetrance genes to hereditary cancer risk is not well established. Here, we report a family with early onset breast and fallopian tube cancer that was identified as carrying germline mutations in BARD1 and ATM genes. Loss of heterozygosity studies suggest a causative role of the BARD1 mutation in the development of primary peritoneal cancer, but fail to confirm an association between germline ATM mutations and breast cancer development in this family. Complexities in interpreting implications of mutations in moderate-risk cancer susceptibility genes are discussed.

Key Words: ATM, BARD1, breast cancer, ovarian cancer, primary peritoneal cancer

Genetic testing with Next Generation Sequencing (NGS) has dramatically increased testing for mutations in non-BRCA1/2, moderate-penetrance cancer susceptibility genes with an associated two- to fourfold increase in cancer risk (1,2). Currently, available multi-gene panels routinely incorporate genes in the Fanconi Anemia/BRCA pathway, including ATM and BARD1 that are involved in DNA repair or cell cycle regulation.

Here, we report on a family with early onset breast cancer (BC) and primary peritoneal cancer (PPC) that was found to carry two deleterious germline mutations in the ATM and BARD1 genes. Analysis of tumor tissue for loss of heterozygosity (LOH) for the corresponding mutations in BARD1 and ATM was performed to provide insight into the role of these mutations in the development of hereditary breast and ovarian cancer.

CASE PRESENTATION

Patient F is a 41 year old female diagnosed with T2N3MX ER+, PR+, HER2+ invasive ductal carcinoma. Her mother (Patient J) was diagnosed with stage 3 serous PPC at age 70 years. A maternal aunt (patient R) had BC diagnosed at 62 years old (Fig. 1). Patient F had negative germline BRCA1/2 testing, but was found to carry the familial germline mutations in BARD1 and ATM. Prior to her death, Patient R had participated in a research study that provided access to her breast tumor and normal tissue. Postmortem analysis of Patient R’s normal breast tissue identified the presence of the familial ATM mutation only.

To investigate the potential contribution of these mutations to the development of the breast and peritoneal cancers, we analyzed all available cancer tissue...
for LOH at the familial mutation sites in BARD1 and ATM in individuals with a corresponding germline mutation.

Formalin fixed paraffin embedded sections were dissected to obtain tumor and adjacent normal tissue after review by a breast pathologist. Extracted genomic DNA was PCR amplified with primers for BARD1 or ATM (Fig. 2), and the purified products were then reamplified with nested primers and gel purified. The final products were analyzed by Sanger sequencing.

Genotyping of the BC from patient F demonstrated presence of the wild-type (wt) allele in both BARD1 (Fig. 2) and ATM (data not shown). Genotyping of the PPC tissue from Patient J demonstrated loss of the wt allele for the BARD1 gene, although the wt allele for ATM was present (Fig. 2). Genotyping of normal breast tissue from Patient R identified the familial germline ATM mutation only. LOH analysis of breast tumor identified the presence of the wt ATM allele.

**DISCUSSION**

**Bard1 and Ovarian Cancer**

The BARD1 gene is the main binding partner of BRCA1 and is critical for the tumor suppressor functions of BRCA1 (3). LOH analysis of Patient J’s PPC demonstrated absence of wt BARD1 thus suggesting the germline BARD1 mutation had a causative role in her PPC development consistent with the tumor suppressor model. Previously, Thai et al. identified a germline BARD1 missense mutation (Gln564His) in a 73 year old patient diagnosed with clear cell ovarian carcinoma, an infiltrating lobular carcinoma of the breast, and a clear cell endometrial cancer (4). They also demonstrated absence of the BARD1 wt allele in the ovarian tumor. Additional data addressing BARD1 and ovarian cancer risk is limited (5). Walsh et al. did not find LOH for BARD1 in a fallopian tube cancer from a woman with a germline BARD1 mutation (c. 2148delCA), the only case with an inherited BARD1 mutation identified among 360 women with unselected ovarian or fallopian tube cancer (6). In a recent large case–control series, an increased prevalence of BARD1 mutations were found among women with ovarian cancer, but only four women with mutations were identified, and two also had BRCA1 mutations (7). While reports have identified germline BARD1 mutations in families with suspected hereditary breast and ovarian cancer, these series are small (8–10). Additional case–control studies or larger family studies assessing the segregation of germline BARD1 mutations with ovarian cancer will be necessary to clarify the magnitude of any associated ovarian cancer risk with inherited BARD1 mutations.

**Bard1 and Breast Cancer**

The role of germline BARD1 mutations in the development of BC is also unclear (4,8–14). Germline BARD1 mutations in those with suspected hereditary BC have been identified but an associated increased relative risk for BC has not been demonstrated (15,16). Patient F was identified with germline mutations in BARD1 and ATM, and a BC diagnosis at age 41. Genotyping of the cancer demonstrated retention of the BARD1 and ATM wt alleles and it is therefore uncertain whether these mutations contributed to the development of BC in this patient. We cannot exclude that a somatic mutation elsewhere in the BARD1 gene, an epigenetic event, or BARD1 protein modification occurred. Insufficient DNA precluded further analysis.

**Atm and Breast Cancer**

ATM’s role as a moderate penetrance BC susceptibility gene is well established, as heterozygous female carriers are estimated to have a 2–4 fold increase in BC risk as compared to the general population (1,17,18). However, despite identifying
germline mutations in ATM in three affected relatives in the current report, none of the tumors analyzed (two breast and one peritoneal cancer) demonstrated LOH for ATM. Therefore, we did not identify a causative role for the germline ATM mutation in the cancers in this family.

Complexities in Counseling Moderate Penetrance Genes

This case highlights the complexities of understanding the contribution of inherited moderate-penetrance gene mutations to familial cancer and the resulting challenges for genetic counseling. This family includes two women with BC, and one with PPC all of whom inherited a germline mutation in ATM, yet, we were unable to demonstrate LOH for ATM in any of the three tumors. Patient F with BC and Patient J with PPC also inherited a germline BARD1 mutation, but only the PPC demonstrated LOH for BARD1. Thus, inherited mutations in moderate-penetrance genes may not necessarily explain every cancer in a family. As mentioned, one limitation of this analysis includes the possibility of undetected genomic alterations in

|| Gene | Forward primer (5'-3') | Reverse primer (5'-3') | PCR product size (bp) |
|------|------------------------|------------------------|-----------------------|
| BARD1| R1: ATTTCTGAGGCACCCTTT | R1: ACAGATCTGAATGTTTGGAGT | 270                   |
|      | R2: GAGGGCCAGGTGGTTGTAAC | R2: TGAGTCTCTTTACCATTGGCTGA |                       |
| ATM  | R1: AGAAGGTGGCCAAGTGACTCA | R1: TGGGGAAACAGGAGCAAAAT | 197                   |
|      | R2: AGTTGCCAAGGTAGTCGAGT | R2: ACCCTTATTGAGACAATGCAA | 159                   |

Figure 2. (a) Sanger sequencing with forward primer for p.L316* in BARD1 (c.947T>G) in tumor tissue of Patient J (demonstrating LOH for BARD1 mutation) (b) Sanger sequencing with forward primer for p.L316* in BARD1 (c.947T>G) in tumor tissue of Patient F (confirming presence of germline BARD1 mutation but lack of LOH) (c) Sanger sequencing with forward primer for 5 bp deletion in ATM at chr 11:108181013 in tumor tissue of Patient J (confirming presence of germline ATM mutation but lack of LOH) (d) Sanger sequencing with forward primers for 5pb deletion in ATM at chr 11:108181013 in normal tissue of Patient J (also confirming germline mutation) (e) PCR primers for the amplification of BARD1 and ATM sequences from genomic DNA, R1-Round 1, R2-Round 2.
BARD1 or ATM, however, the potential for phenocopies or genetic heterogeneity in these families should also be considered.

In contrast, in families with mutations in high-penetrance genes such as BRCA1/2, cancer development can be attributed almost entirely to the familial mutation and noncarriers have cancer risks similar to the general population. With moderate-penetrance genes, one cannot assume that cancer diagnoses in a family are caused solely by the mutation identified. Rather, a polygenic model has been proposed in which the moderate-penetrance gene mutation works in concert with other modifiers to increase risk of cancer (19–21). Counseling and risk management for these families can therefore be complex. Individuals who test negative for known familial mutations in moderate-risk genes may receive false reassurance if health care providers do not recognize cancer risks unaccounted for by the familial mutation.

CONCLUSION

As NGS panel testing becomes widespread, more families like this will be identified. Evaluation of LOH may help further our knowledge regarding the relationship between non-BRCA1/2 germline mutations and associated cancer risk. In the meantime, clinicians should use caution when interpreting the implications of mutations in moderate-penetrance genes.

CONFLICT OF INTEREST

The authors do not report any conflicts of interest.

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