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**Brief Communication**

**The landscape of somatic genetic alterations in breast cancers from ATM germline mutation carriers**

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**ABSTRACT**

Pathogenic germline variants in ataxia-telangiectasia mutated (ATM), a gene that plays a role in DNA damage-response and cell cycle checkpoints, confer an increased breast cancer (BC) risk. Here, we investigated the phenotypic characteristics and landscape of somatic genetic alterations in 24 BCs from ATM germline mutation carriers by whole-exome and targeted sequencing. ATM-associated BCs were consistently hormone receptor-positive and largely displayed minimal immune infiltrate. Although 79.2% of these tumors exhibited loss-of-heterozygosity of the ATM wild-type allele, none displayed high activity of mutational signature 3 associated with defective homologous recombination DNA (HRD) repair. No TP53 mutations were found in the ATM-associated BCs. Analysis of an independent dataset confirmed that germline ATM variants and TP53 somatic mutations are mutually exclusive. Our findings indicate that ATM-associated BCs often harbor bi-allelic inactivation of ATM, are phenotypically distinct from BRCA1/2-associated BCs, lack HRD-related mutational signatures, and that TP53 and ATM genetic alterations are likely epistatic.

The protein kinase ataxia-telangiectasia, ATM, plays a central role in the activation of DNA damage response following DNA double-strand breaks and in cell cycle checkpoint control (1). Heterozygous germline mutations in ATM occur in ~1% of the population and are associated with increased breast cancer (BC) risk (2,3). In addition to germline nonsense and frameshift mutations, one rare ATM missense variant, c.7271T>G (p.V2424G), confers a particularly strong BC risk (2) and may act in a dominant-negative manner (4).

Here, we assessed the phenotype and repertoire of somatic genetic alterations in BCs from 24 patients with germline pathogenic ATM variants, and whether somatic loss of heterozygosity (LOH) of the ATM wild-type allele occurs in these tumors. As a secondary aim, we set out to establish whether ATM-associated breast cancers harboring the c.7271T>G variant had genomic evidence supportive of its dominant-negative mode of action. Approval by the local ethics committees of the contributing authors’ institutions and patient written informed consents were obtained. Methods are detailed in the [Supplementary Methods](https://oup.silverchair-cdn.com/oup/backfile/Content_public/Journal/jnci/PAP/10.1093_jnci_djx213/3/djx213_supp.zip?Expires=1517353232&Signature=TIvwzEogH4t4oI5~1jo1UV0QLluD4Mv6CRPXj1UuQVsZ9jXFrVhAr0Eb2FaNLp42GJxsJRNyLRBkPpJV0np0--XEMrBxOmiA2LqSihtFUbCRMpjJLukxvl5o23HrEm3c-Eq7rFmuh1Arhmu06MYDc4plKZ8Dif8iiPnMDtm71nHJ59R4FZxlPRQ3W2SKON5zzBL765ceHFMaZSczYqRFN5GRAukbwstjUS3aoqDwhDfyvp6xQtdAxESxEG4abD4ldSlDrQVMoDAMnrvgDOLxNDf3FLX2mi-IRi4KUNLGZCxvw5AWMElKBnml8bCfJWpnZ1tLiF1RelfzXW1e6m6mwA__&Key-Pair-Id=APKAIUCZBIA4LVPAVW3Q) (available online). Two-sided P values <0.05 were considered statistically significant. For CoMEt, default settings and significance levels were adopted (5).

Pathogenic germline variants in ATM comprised 15 (62.5%) missense mutations (all but one c.7271T>G), 2 (8.3%) nonsense mutations, 5 (20.8%) frameshift deletions, 1 (4.2%) in-frame deletion and 1 (4.2%) large deletion (**Supplementary Table 1**). The median age of breast cancer diagnosis of the patients was 46 years (range 32-79 years). All ATM-associated BCs included in this study were estrogen receptor (ER)-positive, 4 (16.7%) were HER2-positive and all but one displayed low levels of stromal tumor-infiltrating lymphocytes (TILs**; Figure 1**, **Supplementary Figure 1**, **Supplementary Table 1**). Whole-exome (WES, n=12) and high-depth targeted-capture massively parallel sequencing using the MSK-IMPACT assay [**Supplementary Methods** and (6)] (n=12) of tumor and matched normal tissue/peripheral blood revealed that the repertoire of somatic genetic alterations of ATM-associated BCs included alterations affecting genes recurrently altered in ER-positive BCs (7), such as PIK3CA (29.2%) and GATA3 mutations (16.7%), and CCND1 (20.8%) and HER2 amplification (16.7%) (**Figures 1A** and **2A**; **Supplementary Figures 2** and **3**, **Supplementary Table 2**). Interestingly, somatic mutations affecting TP53 were not detected in any of the tumors. Allele-specific copy number analysis revealed bi-allelic inactivation of ATM through clonal LOH of the wild-type allele in 19 of 24 cases (79.2%, **Figure 1A**). In addition, 78.6% (11/14) of the BCs from c.7271T>G germline mutation carriers harbored somatic LOH of the ATM wild-type allele (**Figure 1A**), suggesting that this missense mutation may act through bi-allelic inactivation of ATM. Thus the hypothesis that bi-allelic inactivation of c.7271T>G is not required for pathogenicity was not confirmed. WES of both the ductal carcinoma in situ (DCIS) and synchronous invasive BC from ATM2 revealed that the two lesions were clonally related and that LOH of the ATM wild-type allele occurred in the DCIS, suggesting that bi-allelic inactivation of ATM is an early event in the development of ATM-associated BCs (**Figure 1B**).

A comparison of ATM-associated BCs sequenced in this study with those of BRCA1-associated and BRCA2-associated BCs from The Cancer Genome Atlas (TCGA) (7) and International Cancer Genome Consortium (ICGC) (8) demonstrated that ATM-associated BCs statistically significantly less frequently displayed an ER-negative/HER2-negative phenotype than BRCA1- or BRCA2-associated BCs (**Figure 2B**). ATM-associated BCs were also found to have low levels of stromal TILs (median 5.0%, range 1.0%-20.0%; **Figure 1A**, **Supplementary Table 1**, **Supplementary Figure 1**), whereas BRCA1-associated BCs commonly display prominent stromal TILs (median 35%, up to 70%-85%) (9,10). This was further confirmed by differences in the cytolytic activity of the immune infiltrate [CYT score (11)] of BCs from TCGA, which revealed statistically significantly higher CYT scores in BRCA1-associated BCs than in ATM-associated BCs (**Figure 2C**). A comparison of the mutational profiles of the ATM-associated BCs sequenced in this study with those of BRCA1- and BRCA2-associated BCs from TCGA/ICGC revealed that TP53 (0.0% vs 68.6%, P<0.001, and 0.0% vs 27.3%, P=0.004, respectively, Fisher’s exact test) was statistically significantly less frequently mutated in ATM-associated BCs (**Supplementary Figure 4**). In addition, a re-analysis of an independent set of BCs from ATM germline mutation carriers from TCGA (n=8; **Supplementary Figure 5**) confirmed that pathogenic ATM germline mutations, but not BRCA1 or BRCA2 germline mutations, are mutually exclusive with somatic TP53 mutations [CoMEt (5), p=0.04; **Figure 2D**), suggesting that ATM and TP53 genetic alterations may be epistatic. Our findings expand on previous observations in other malignancies, where somatic ATM and somatic TP53 mutations were found to be mutually exclusive (12,13).

Many cancer predisposition genes associated with an elevated risk for BC are DNA repair-related (e.g. BRCA1, BRCA2 and PALB2) and the BCs these patients develop have been shown to often display genomics features consistent with HR deficiency (HRD) (14), such as large-scale state transitions (LSTs) (15,16), mutational signature 3 (8,17,18) and rearrangements signatures RS1, RS3, or RS5 (8). We and others have previously shown that BRCA1- and BRCA2-associated BCs display high LST scores in >85% of cases (16) and mutational signature 3 in >50% of cases (7,8,18). Five (41.7%) of the 12 ATM-associated BCs subjected to WES here and four (50.0%) of the 8 ATM-associated BCs from TCGA displayed high LST scores, all of which displayed bi-allelic ATM inactivation (**Figure 1A**, **Supplementary Figure 6**). Further, consistent with recent results (18), none of the 12 ATM-associated BCs subjected to WES here and only 1/8 ATM-associated BCs from TCGA, which displayed ATM bi-allellic inactivation, harbored mutational signature 3 (**Figure 1A**). None of the three ATM-associated BCs analyzed by ICGC harbored mutational signature 3 or rearrangement signatures RS1, RS3, or RS5 (8). These findings suggest that the risk and evolution of BC in patients with ATM germline mutations may be driven by mechanisms independent of HR-DNA repair defects, or that the DNA repair defects caused by ATM mutations result in mutational signatures distinct from those of bona fide HR-related genes.

This study has important limitations. This series was deliberately enriched for patients with the relatively high risk ATM c.7271T>G missense variant; hence, albeit not representative of a consecutive series of patients with ATM-associated BC, it allowed us to investigate whether LOH of the ATM wild-type allele would be present in these cancers. Given the limited mutational burden of ATM-associated BCs, signature 3 could only be defined in the cases subjected to WES.

Despite these limitations, our study has revealed that ATM-associated BCs are phenotypically distinct from BRCA1-associated BCs, have somatic genetic alterations similar to those of non-BRCA1/non-BRCA2-associated ER-positive BCs (7,8), and lack TP53 somatic mutations and largely mutational signature 3. Interestingly, germline mutations affecting ATM are reported to be associated with response to Poly(ADP)Ribose Polymerase (PARP)inhibitors in prostate cancer (19) and this association has been presumed to be mediated via defective HR-DNA repair. Although bi-allelic genetic alterations affecting ATM and other HR-DNA repair-related genes are mutually exclusive (16), the majority of ATM-associated BCs lack genomic features of HRD and consistently lack TP53 somatic mutations; hence, it is plausible that ATM functions other than those related to HR DNA repair may mediate this association and the increased BC risk. Our results also warrant further studies to define the role ATM mutations play in oncogenesis and the molecular basis of the mutual exclusivity of ATM germline variants and TP53 somatic mutations in BC.

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REFERENCES

1. Shiloh Y, Ziv Y. The ATM protein kinase: regulating the cellular response to genotoxic stress, and more. Nat Rev Mol Cell Biol. 2013;14:197-210.

2. Goldgar DE, Healey S, Dowty JG, et al. Rare variants in the ATM gene and risk of breast cancer. Breast Cancer Res. 2011;13:R73.

3. Swift M, Morrell D, Massey RB, et al. Incidence of cancer in 161 families affected by ataxia-telangiectasia. N Engl J Med. 1991;325:1831-1836.

4. Chenevix-Trench G, Spurdle AB, Gatei M, et al. Dominant negative ATM mutations in breast cancer families. J Natl Cancer Inst. 2002;94:205-215.

5. Leiserson MD, Wu HT, Vandin F, et al. CoMEt: a statistical approach to identify combinations of mutually exclusive alterations in cancer. Genome Biol. 2015;16:160.

6. Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): A Hybridization Capture-Based Next-Generation Sequencing Clinical Assay for Solid Tumor Molecular Oncology. J Mol Diagn. 2015;17:251-264.

7. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012;490:61-70.

8. Nik-Zainal S, Davies H, Staaf J, et al. Landscape of somatic mutations in 560 breast cancer whole-genome sequences. Nature. 2016;534:47-54.

9. Nolan E, Savas P, Policheni AN, et al. Combined immune checkpoint blockade as a therapeutic strategy for BRCA1-mutated breast cancer. Sci Transl Med. 2017;9.

10. Massink MP, Kooi IE, van Mil SE, et al. Proper genomic profiling of (BRCA1-mutated) basal-like breast carcinomas requires prior removal of tumor infiltrating lymphocytes. Mol Oncol. 2015;9:877-888.

11. Rooney MS, Shukla SA, Wu CJ, et al. Molecular and genetic properties of tumors associated with local immune cytolytic activity. Cell. 2015;160:48-61.

12. Negrini S, Gorgoulis VG, Halazonetis TD. Genomic instability--an evolving hallmark of cancer. Nat Rev Mol Cell Biol. 2010;11:220-228.

13. Pettitt AR, Sherrington PD, Stewart G, et al. p53 dysfunction in B-cell chronic lymphocytic leukemia: inactivation of ATM as an alternative to TP53 mutation. Blood. 2001;98:814-822.

14. Maxwell KN, Wubbenhorst B, Wenz BM, et al. BRCA locus-specific loss of heterozygosity in germline BRCA1 and BRCA2 carriers. Nat Commun. 2017;8:319.

15. Foo TK, Tischkowitz M, Simhadri S, et al. Compromised BRCA1-PALB2 interaction is associated with breast cancer risk. Oncogene. 2017;36:4161-4170.

16. Riaz N, Blecua P, Lim RS, et al. Pan-cancer analysis of bi-allelic alterations in homologous recombination DNA repair genes. Nat Commun. 2017;8:857.

17. Alexandrov LB, Nik-Zainal S, Wedge DC, et al. Signatures of mutational processes in human cancer. Nature. 2013;500:415-421.

18. Polak P, Kim J, Braunstein LZ, et al. A mutational signature reveals alterations underlying deficient homologous recombination repair in breast cancer. Nat Genet. 2017;49:1476-1486.

19. Mateo J, Carreira S, Sandhu S, et al. DNA-Repair Defects and Olaparib in Metastatic Prostate Cancer. N Engl J Med. 2015;373:1697-1708.

20. Gao J, Aksoy BA, Dogrusoz U, et al. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal. 2013;6:pl1.

FIGURE LEGENDS

Figure 1. Genomic characterization of ATM-associated BCs.

A, Recurrent (n≥3) non-synonymous somatic mutations in ATM-associated BCs detected by whole-exome sequencing (left) and targeted MSK-IMPACT sequencing (right). ATM germline and somatic mutation types are color-coded according to the legend. Loss of heterozygosity (LOH) of the wild-type allele is displayed by a diagonal bar. Clonal LOH for ATM and clonal mutations are displayed by yellow and blue boxes, respectively. The phenobar (top) provides information about estrogen receptor (ER) and HER2 status, histologic grade, stromal tumor-infiltrating lymphocytes (TILs), age at diagnosis, large-scale state-transitions (LSTs) and mutational (Mut) signatures. The percentage of the genome altered (copy number, see Methods) is shown for each case below. Indel, small insertion and deletion; SNV, single nucleotide variant. B, Results of whole-exome sequencing analysis of the ductal carcinoma in situ (DCIS) and invasive ductal carcinoma of no special type (IDC) of case ATM2. Top, micrographs of representative hematoxylin and eosin-stained sections of the DCIS (left) and IDC (right). Scale bars, 500µm. Middle, circos plot of the DCIS (left) and IDC (right) depicting the somatic mutations and copy-number alterations across the genome. Mutations are shown along the outside, including annotations of mutation type; copy-number alterations are depicted along the center ring color-coded according to the legend, and the chromosomal position is shown in the inside ring. Bottom, cancer cell fractions (CCFs) of mutations identified in the DCIS (top) and IDC (bottom), color-coded according to the legend. LOH of the wild-type allele is displayed by a diagonal bar. Clonal LOH for ATM and clonal mutations are displayed by yellow and red boxes, respectively. Right, phylogenetic tree depicting the inferred evolution of the DCIS and IDC (see Methods). The length of the branches is representative of the number of somatic mutations that distinguishes the DCIS from the IDC, and selected somatic mutations are illustrated along the branches.

Figure 2. Comparison of ATM-associated BCs with BRCA1- and BRCA2-associated BCs, and assessment of mutual exclusivity of germline ATM mutations and somatic TP53 mutations.

A, Non-synonymous somatic mutation burden in ATM-associated BCs subjected to whole-exome sequencing in this study (n=12; median 38, range 15-114), ATM-associated BCs from TCGA (n=8; median 40, range 14-78), BRCA1- (TCGA/ICGC; n=51; median 1, range 1-698) and BRCA2-associated BCs (TCGA/ICGC; n=55; median 58, range 0-255). Statistical significance was evaluated by the two-sided Mann-Whitney U test. B, Estrogen receptor (ER) and HER2 status of ATM-associated BCs sequenced here (n=24) and BRCA1- (TCGA/ICGC; n=48) and BRCA2-associated BCs (TCGA/ICGC; n=51) from TCGA/ICGC with ER and HER2 information available. ATM-associated BCs statistically significantly less frequently displayed an ER-negative/HER2-negative phenotype than BRCA1- or BRCA2-associated BCs as evaluated by the two-sided Fisher’s exact test. C, Cytolytic activity of the immune infiltrate (CYT) score as defined by RNA-sequencing analysis following methods described by Rooney et al. (11) of ATM-associated BCs (TCGA; n=8) and BRCA1- (TCGA; n=20) and BRCA2-associated BCs (TCGA; n=24) from TCGA. Statistical significance was evaluated by the two-sided Mann-Whitney U test. D, Mutual exclusivity analysis of germline ATM (TCGA; n=8), germline BRCA1 (TCGA; n=20) and germline BRCA2 variants (TCGA; n=24) (red bars) with somatic TP53 mutations (TCGA, n=244) (blue bars) using CoMEt (5) in 929 breast cancer from TCGA (see **Supplementary Methods**). For CoMEt, default settings and significance levels were adopted. OncoPrints were generated using OncoPrinter from cBioPortal (20).