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JNCI J Natl Cancer Inst (2022) 114(5): djac024

https://doi.org/10.1093/jnci/djac024
First published online January 25, 2022
Article

ATM Germline-Mutated Gastroesophageal Junction Adenocarcinomas: Clinical Descriptors, Molecular Characteristics, and Potential Therapeutic Implications

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Abstract

Background: Gastroesophageal junction (GEJ) adenocarcinoma is a rare cancer associated with poor prognosis. The genetic factors conferring predisposition to GEJ adenocarcinoma have yet to be identified. **Methods:** We analyzed germline testing results from 23 381 cancer patients undergoing tumor-normal sequencing, of which 312 individuals had GEJ adenocarcinoma. Genomic profiles and clinico-pathologic features were analyzed for the GEJ adenocarcinomas. Silencing of ATM and ATR was performed using validated short-interfering RNA species in GEJ, esophageal, and gastric adenocarcinoma cell lines. All statistical tests were 2-sided. **Results:** Pathogenic or likely pathogenic *ATM* variants were identified in 18 of 312 patients (5.8%), and bi-allelic inactivation of *ATM* through loss of heterozygosity of the wild-type allele was detected in all (16 of 16) samples with sufficient tumor content. Germline *ATM*-mutated GEJ adenocarcinomas largely lacked somatic mutations in *TP53*, were more likely to harbor *MDM2* amplification, and harbored statistically significantly fewer somatic single nucleotide variants (2.0 mutations/Mb vs 7.9 mutations/Mb; $P < .001$). A statistically significantly higher proportion of germline *ATM*-mutated than *ATM*–wild-type GEJ adenocarcinoma patients underwent a curative resection (10 [100%] vs 92 [86.8%], $P = .04$; Fisher's exact test.), A synthetic lethal interaction between short-interfering RNA silencing of *ATM* and *ATR* was observed in the models analyzed. **Conclusions:** Our results indicate that germline pathogenic variants in *ATM* drive oncogenesis in GEJ adenocarcinoma and might result in a distinct clinical phenotype. Given the high prevalence of germline *ATM*-mutated GEJ adenocarcinomas, genetic testing for individuals with GEJ adenocarcinomas may be considered to better inform prognostication, treatment decisions, and future cancer risk.

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The ataxia telangiectasia-mutated (*ATM*) gene encodes a protein kinase responsible for the phosphorylation of targets in the DNA damage response (DDR) to double-strand breaks (1,2). Pathogenic loss-of-function variants affecting *ATM* were first recognized to cause autosomal recessive ataxia telangiectasia (3). Heterozygous pathogenic or likely pathogenic (P/LP) variants

Received: August 9, 2021; **Revised:** November 22, 2021; **Accepted:** January 19, 2022

in *ATM* have more recently been associated with an increased risk of breast and pancreatic cancers (4-7). Although germline *ATM* P/LP variants have been reported in individuals with cancers of gastroesophageal origin (8), the association between germline *ATM* alterations and gastroesophageal junction (GEJ) adenocarcinomas has not been established.

In addition to its role in inherited cancer susceptibility, *ATM* is also somatically mutated in a subset of sporadic cancers, including pulmonary, gynecologic, genitourinary, and hematopoietic malignancies (9). *ATM* deficiency increases genomic instability in a form of chromosomal alterations driving oncogenesis, and cancer cells lacking *ATM* activity are sensitive to radiation (10,11). As a result, *ATM* deficiency may render cancer cells sensitive to DNA-damaging agents and be a promising therapeutic target for DDR inhibitors (12). In preclinical studies, the combination of *ATM*-related (*ATR*) and poly (ADP-ribose) polymerase (PARP) inhibition resulted in rapid cancer cell death without prolonged drug exposure (13,14). Early clinical trial data for *ATR* inhibitors suggest that ATM-deficient tumors may be sensitive to *ATR* inhibition (15-18). Furthermore, *ATM* P/LP germline or somatic variants have been associated with improved tumor control following radiation therapy in a large pan-cancer cohort, qualifying *ATM* mutations as a potential biomarker of radiation therapy response (11). Thus, *ATM* status is not only important for cancer predisposition but also for therapeutic decision making.

Whether there is a link between germline *ATM* P/LP variants and the development of GEJ adenocarcinoma, an aggressive tumor that arises at the interface of the esophagus and stomach, remains to be defined. The incidence of GEJ adenocarcinomas in the United States has been rising over the last 40 years (19) and has an overall poor prognosis, with 5-year survival averaging approximately 30%. Early detection and advances in therapeutic approaches are needed to improve outcomes and lessen the burden of this disease.

To investigate the potential association between GEJ adenocarcinoma and germline alterations in *ATM*, sequencing results from 23 381 patients who had genetic testing at Memorial Sloan Kettering (MSK) were analyzed. In this study, we characterize the clinico-pathologic and genomic features of GEJ adenocarcinomas in patients with P/LP germline *ATM* alterations and explore potential therapeutic implications for *ATM*-deficient GEJ adenocarcinomas.

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Methods

Patients and Samples

All patients in this study had a cancer diagnosis, consented to a protocol approved by the MSK Institutional Review Board, and received cancer agnostic germline testing in the context of tumor-normal sequencing using the Federal Drug Administration (FDA)-cleared MSK Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay (May 2015 to September 2021). Patients with diagnoses of upper gastrointestinal malignancies were consented to MSK-IMPACT as a standard of practice during this time. A total 140 of 312 GEJ cases, including 6 of 18 *ATM*-positive cases, were previously reported (8). Patients were classified as having GEJ either from the pathological report for those who had resection or by combination of fluorodeoxyglucose positron emission tomography imaging with endoscopy for those without surgical resection. Cases were not classified as GEJ if there was discordance between the endoscopy and fluorodeoxyglucose positron emission tomography imaging report. The Siewart classification was retrieved from patient charts. For patients consenting to this testing with a diagnosis of GEJ adenocarcinoma, archived formalin-fixed paraffin-embedded (FFPE) tumor samples were used for ATM immunohistochemistry.

Next-Generation Sequencing

Representative FFPE tumor sections were sequenced using the FDA-authorized New York State–approved MSK-IMPACT, a capture-based Next-Generation Sequencing assay capable of identifying mutations, copy number alterations, and select gene fusions. Additionally, blood from the same patient was also sequenced using the same Next-Generation Sequencing platform and analyzed for germline alterations in up to 88 genes implicated in cancer predisposition syndromes. The specifics of the MSK-IMPACT somatic and germline assays were reported previously (20,21).

Histopathology and Immunohistochemistry

FFPE tissue sections were stained with hematoxylin-and-eosin. The response of tumor to previous chemotherapy and radiation therapy was reported using the modified Ryan scheme (22-25), which associates a tumor regression score to a specific set of histologic criteria seen in the posttreatment microscopic examination. For details, please see Supplementary Methods (available online).

Immunohistochemical analysis of ATM expression was performed using a monoclonal antibody (*ATM* clone Y170; catalogue no. ab32420; Abcam Plc, Cambridge, UK). Lymphocytes in the mantle and paracortical tonsil areas were used as positive controls. Cancer cells with a complete absence of nuclear expression were labeled as complete loss of ATM. Any other degree of nuclear expression in cancer cells was considered positive for ATM expression. All slides were reviewed and scored by 2 independent pathologists (T.E.-J. and J.S.R.-F.) without prior knowledge of *ATM* mutational status.

Short-Interfering RNA (siRNA) Silencing of ATM and ATR

Cell lines of gastrointestinal junction (ESO26), esophageal (OACM51C), and gastric (SNU601) origin were transfected with siRNA targeting *ATM* and *ATR*. Cell viability was evaluated using luminescent cell viability assay, and immunoblotting was performed to determine the effect of siRNA on ATM and ATR protein expression. For detailed description of cell culture, siRNA transfection, cell viability assay, and immunoblotting methods, see Supplementary Methods (available online).

Burden Analysis

The carrier frequency of loss-of-function and ClinVar-classified P/LP *ATM* variants in the Genome Aggregation Database (26) was compared with the carrier frequency in the GEJ cancer patient cohort using the Fisher exact test (2-sided; $P < .05$ was considered statistically significant).

Statistical Analysis

Comparisons of categorical and continuous variables were performed by the Fisher's exact and Wilcoxon-Mann-Whitney *U* tests, respectively. Multiple testing correction using the

Benjamini-Hochberg method was applied to control for the false discovery rate as needed. *P* less than .05 was considered statistically significant. All tests used were 2-tailed. Statistical analyses were conducted using R (v.3.1.2). Visualization of the mutational landscape was generated through the *oncoplot* function of the R package maftools (27). Comparison of the number of nonsynonymous single nucleotide variants between groups (germline *ATM*–wild-type and germline *ATM*-mutated GEJ tumors) was assessed by the Wilcoxon-Mann-Whitney *U* test. Comparisons of frequencies of genes altered by somatic genetic alterations (nonsynonymous mutations, gene amplifications, and/or homozygous deletions) between germline *ATM*–wild-type and germline *ATM*-mutated GEJ tumors were performed using the Fisher's exact test. Analysis of mutually exclusive gene pairs was performed using CoMEt (28) and DISCOVER (29), as previously described.

Results

Molecular Features of Germline ATM-Mutated GEJ Adenocarcinoma

P/LP *ATM* variants were detected in 247 of cases from the total pan-cancer cohort of 23 381. GEJ adenocarcinoma was the cancer diagnosis with the highest rate of *ATM* P/LP germline variants (g*ATM*-mut) at 5.8% (18 of 312 cases; Figure 1, A, Table 1; and Supplementary Table 1, available online). The prevalence of *ATM* P/LP germline variants was statistically significantly higher in GEJ adenocarcinoma compared with a large pan-ethnic cohort in the Genome Aggregation Database (Supplementary Table 2, available online) as well as other disease sites in the MSK-IMPACT cohort (Figure 1, A). Allele-specific copy number analysis revealed bi-allelic inactivation of *ATM* through clonal loss of heterozygosity (LOH) of the wild-type allele in all g*ATM*-mut cases with sufficient tumor content to assess (n = 16 of 16; Figure 1, B). Two of the 18 patients with *ATM* germline P/LP variants also had additional germline pathogenic variants detected by the 88 gene hereditary cancer susceptibility panel. One patient carried a pathogenic variant in *BARD1*, whereas the other carried a pathogenic variant in *BRCA1*. In these cases, LOH was detected by fraction and allele-specific copy number estimates from tumor sequencing (27) for *ATM* but not for *BARD1* or *BRCA1*, respectively, indicating that *ATM* may be driving tumorigenesis in these tumors (28) (Table 1). Among 294 germline *ATM*–wild-type cases (g*ATM*-wt), 28 cases contained other P/LP germline variants in low penetrance cancer susceptibility genes or genes associated with an autosomal recessive disease as well as 3 cases with germline *TP53*, *MSH2*, and *PALB2* pathogenic variants, respectively (29).

g*ATM*-mut and g*ATM*-wt GEJ cancers differed in their repertoires of somatic genetic alterations. Whereas somatic *TP53* mutations were highly recurrent in g*ATM*-wt GEJ adenocarcinomas (231 of 294, 78.6%), these were observed in only 2 of the gATM-mut tumors (2 of 18; 11.1%; $P < .001$; Figure 1, C). *MDM2* was amplified in 33.3% (n = 6 of 18) of g*ATM*-mut cases and in only 5.4% (n = 16 of 294; $P = .001$) of g*ATM*-wt tumors (Figure 1, C). g*ATM*-wt GEJ cancers harbored a statistically significantly higher number of somatic single nucleotide variants than g*ATM*-mut cases (2.0 mutations/Mb vs 7.9 mutations/Mb, $P < .001$; Figure 1, D). No notable differences were observed in copy number profiles (Supplementary Figure 1, available online).

Clinico-Pathologic Characteristics

Epidemiologic characteristics of g*ATM*-mut and g*ATM*-wt GEJ adenocarcinomas were compared with identify potential differences between the 2 groups (Supplementary Table 3, available online). No differences in sex or median age at diagnosis were observed between the g*ATM*-mut and g*ATM*-wt GEJ cancers. g*ATM*-mut GEJ cancer patients did have a statistically significantly higher proportion of first-degree family relative with a cancer diagnosis (77.8% vs 43.2%, $P = .006$, Fisher's exact test).

g*ATM*-mut GEJ cancer patients had a numerically higher (n = 10, 55.6%) frequency of localized disease at presentation than patients with g*ATM*-wt GEJ cancers (n = 106, 36.1%; $P = .13$, Fisher's exact test). Of the patients with resectable disease at presentation, more patients in the g*ATM*-mut underwent curative intent surgery compared with the g*ATM*-wt patients (10 [100%] vs 92 [86.8%], $P = .04$, Fisher's exact test). Six of 10 (60.0%) of the g*ATM*-mut GEJ cancer patients underwent preoperative chemoradiation and 4 (40.0%) underwent preoperative chemotherapy or chemotherapy plus immunotherapy. In the g*ATM*-wt GEJ cancer group, 84 patients who underwent resection had preoperative treatment (chemoradiation: 70, 83.3%; chemotherapy: 14, 16.7%). Pathologic complete response was observed in 3 (30.0%) g*ATM*-mut patients, all of whom had preoperative chemoradiation, compared with 12 (14.3%) in the g*ATM*-wt cohort. Recurrence of disease in those who had primary curative intent surgery was observed in only 1 of 10 (10.0%) g*ATM*-mut patients and 52 of 92 (56.5%) in g*ATM*-wt ($P = .006$, Fisher's exact test).

Histologic Findings and ATM Protein Expression

Microscopically, the 9 g*ATM*-mut adenocarcinomas were moderately differentiated with glandular formation and cribriform structures within a dense desmoplastic stroma, ofttimes displaying a brisk inflammatory infiltrate (Supplementary Figure 2, available online). On central review of the cases included in this study, no statistically significant differences in the histologic features of g*ATM*-wt vs g*ATM*-mut GEJ cancers were identified (data not shown).

To determine whether immunohistochemical screening can be used to identify GEJ cases with P/LP *ATM*-germline variants, we used a discovery cohort of 49 and a validation cohort of 59 GEJ adenocarcinomas for which tissue was available. The discovery cohort contained 7 gATM-mut and 42 g*ATM*-wt cases, whereas the validation cohort was composed of 3 g*ATM*-mut and 56 gATM-wt cases. All GEJ adenocarcinomas with P/LP *ATM* variants from both cohorts (discovery [n = 7] and validation [n = 3]) displayed complete loss of ATM expression by immunohistochemistry (Figure 2). In addition, 3 g*ATM*-wt adenocarcinomas that lacked ATM immunohistochemical expression but did not harbor *ATM* P/LP germline variants were identified: 2 cases from the discovery cohort harbored *ATM* somatic truncating mutations with LOH of the WT allele, and 1 case from the validation cohort lacking ATM expression by immunohistochemistry that did not harbor *ATM* P/LP germline or somatic variants but displayed high microsatellite instability. Among the remaining 95 gATM-wt cases that had intact ATM immunohistochemical expression, a single case was found to harbor a mono-allelic somatic missense *ATM* mutation, which was classified as a variant of uncertain significance and not expected to result in absent or truncated protein. Based on the combined cohort of 108 GEJ adenocarcinomas for which tissue was available for further analysis, immunohistochemistry displayed 100% (73.5%-100%) sensitivity and 99.0% (94.3%-100%) specificity to

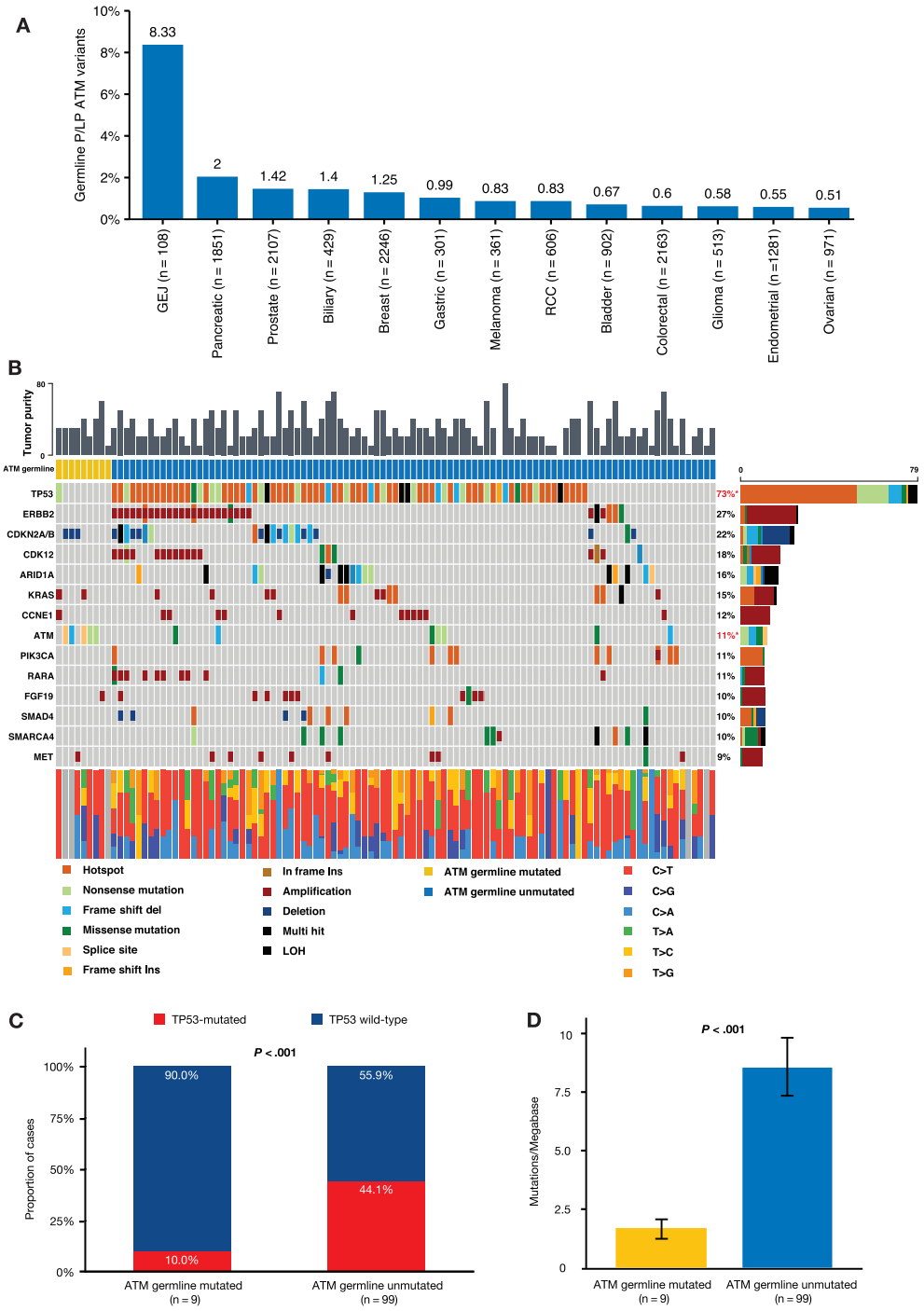

Figure 1. Prevalence and molecular characteristics of germline ATM-mutated gastroesophageal junction (GEJ) adenocarcinoma. **A)** Prevalence of pathogenic or likely pathogenic (P/LP) germline variants in ATM identified in a pan-cancer cohort: *P* values for multiple pairwise comparison were calculated using Pearson's χ^2 test with Benjamini-Hochberg correction yielding the following for each cancer type: pancreatic ($P < .001$), gastric ($P = .01$), biliary ($P = .004$), prostate ($P < .001$), breast ($P < .001$), bladder ($P < .001$), melanoma ($P < .001$), renal cell carcinoma ($P < .001$), colorectal ($P < .001$), esophageal adenocarcinoma ($P = .02$), ovarian ($P < .001$), glioma ($P < .001$), endometrial ($P < .001$), and esophageal squamous cell ($P = .23$). **B)** Landscape of recurrent ($n \geq 10$) nonsynonymous somatic mutations in *ATM* P/LP germline variants (gATM-mut; n = 18) and germline *ATM*–wild-type cases (gATM-wt; n = 294) GEJ tumors detected by targeted Memorial Sloan Kettering Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) sequencing. The effects of the somatic alterations are **color-coded** according to the legend. Loss-of-heterozygosity (LOH) of the wild-type allele is displayed by **diagonal bars**. **Bar charts** (top) indicate the tumor purity for each sample. **Phenobar** (top) provides information about *ATM* germline status. **Stacked barplots** (bottom) show the fraction of different transitions or transversions in each sample colored according to the legend. The **right bar** refers to total counts of pathogenic alternation per gene. **C)** *TP53* is more frequently mutated in gATM-wt than in gATM-mut GEJ adenocarcinomas. Only genes with more than 4 mutations in the samples from each group were included in the analysis. The statistical difference of the 2 groups was compared by 2-sided Fisher's exact tests, corrected for multiple testing through the Benjamini-Hochberg method ($P < .001$). **D)** Comparison of mean number of single nucleotide variants (SNVs) between gATM-wt and gATM-mut GEJ adenocarcinomas. Plotted are the mean number of SNVs, with **error bars** indicating SDs. Statistical comparison was performed using 2-sided Wilcoxon-Mann–Whitney *U* test.

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Table 1. Summary of GEJ adenocarcinoma cases with germline P/LP ATM variants

Case ID	Sex	Ethnicity	Age, y	ATM variant (NM_000051.4)	ATM variant type	1st or 2nd degree relative with ATM-associated cancer	ACMG variant classification	Additional notes
MSK_GEJ_07	Male	White	61	c.8418+5_8418+8delGTGA	Intronic deletion	No	Pathogenic	NA
MSK_GEJ_08	Male	White	62	c.8977C>T (p.Arg2993*)	Truncating SNV	Yes (pancreato-biliary, prostate and breast)	Pathogenic	NA
MSK_GEJ_09	Male	White	66	c.3802delG (p.Val1268*)	Truncating SNV	Yes (pancreato-biliary and breast)	Pathogenic	NA
MSK_GEJ_10	Male	Ashkenazi Jewish	69	c.1027_1030delGAAA (p.Glu343Ilefs*2)	Truncating frameshift	Yes (pancreato-biliary and breast)	Pathogenic	NA
MSK_GEJ_11	Male	South Asian	43	c.217_218delGA (p.Glu73Metfs*26)	Truncating frameshift	No	Pathogenic	NA
MSK_GEJ_13	Male	White	50	c.3894dupT (p.Ala1299Cysfs*3)	Truncating frameshift	Yes (prostate)	Pathogenic	NA
MSK_GEJ_15	Female	White	58	c.8147T>C (p.Val2716Ala)	Missense	No	Pathogenic	carrier of BARD1 (NM_000465) c.2300_2301delTG (p.Val767Aspfs*4) with no LOH detected by FACETS
MSK_GEJ_59	Male	White	69	c.7327C>T (p.Arg2443*)	Truncating SNV	Yes (pancreato-biliary and breast)	Pathogenic	carrier of BRCA1 exon 12 duplication with no LOH detected by FACETS
MSK_GEJ_108	Male	White	62	c.3154-2A>G	Essential splice site SNV	Yes (pancreato-biliary)	Pathogenic	NA
MSK_GEJ_109	Male	Ashkenazi Jewish	65	c.9022C>T (p.Arg3008Cys)	Missense	Yes (breast)	Pathogenic	NA
MSK_GEJ_110	Male	White	48	c.5932G>T (p.Glu1978*)	Truncating SNV	No	Pathogenic	NA
MSK_GEJ_111	Male	White	44	c.5228C>T (p.Thr1743Ile)	Missense	Yes (breast)	Likely pathogenic	NA
MSK_GEJ_112	Male	White	74	c.5712dupA (p.Ser1905Ilefs*25)	Truncating frameshift	No	Pathogenic	NA
MSK_GEJ_113	Male	White	59	c.1A>C (p.Met1?)	Start loss	Yes (prostate)	Likely pathogenic	NA
MSK_GEJ_114	Female	White	35	exons 17-63 deletion; c.?_2467_9171_? del (p.Ala823_Val3056delins28)	Multi-exon deletion	Yes (breast, pancreato-biliary, and prostate)	Pathogenic	NA

(continued)

Table 1. (continued)

Case ID	Sex	Ethnicity	Age, y	ATM variant (NM_000051.4)	ATM variant type	1st or 2nd degree relative with ATM-associated cancer	ACMG variant classification	Additional notes
MSK_GEJ_115	Female	White	67	c.331 + 1G>A	Essential splice site SNV	No	Likely pathogenic	Personal history of cervical and parathyroid cancer
MSK_GEJ_116	Female	White	23	c.6573–9G>A	Intronic SNV	Yes (pancreatic and prostate)	Likely pathogenic	NA
MSK_GEJ_117	Female	White	56	7638_7646delTAGAATTTC (p.Arg2547_Ser2549del)	In-frame deletion	Yes (breast)	Pathogenic	Personal history of breast cancer at 51 y

[a]FACETS = fraction and allele-specific copy number estimates from tumor sequencing; GEJ = gastroesophageal junction; LOH = loss of heterozygosity; P/LP = pathogenic or likely pathogenic; SNV = single nucleotide variant.

identify bi-allelic *ATM* somatic or germline alterations (Supplementary Table 4, available online) and 100% (69.2%-100%) sensitivity and 96.9% (91.3%-99.4%) specificity in identifying germline cases harboring bi-allelic *ATM* germline alterations (Supplementary Table 5, available online).

ATM Loss and Sensitization to ATR Inhibition

Synthetic lethality between loss of function in *ATM* and ATR inhibition has been reported in preclinical systems, though not specifically in GEJ carcinomas, suggesting that ATM deficiency in GEJ adenocarcinomas may present a targetable vulnerability for ATR inhibitors (30-32). To define whether an ATM and ATR are in a synthetic lethal interaction in a GEJ model, we silenced ATM, ATR, or both in cell lines of GEJ, esophageal, and gastric cancer origin using validated siRNAs as well as confirmed the loss of ATM and ATR by immunoblotting (Figure 3, A-C). Cell viability analysis revealed that although ATM and ATR silencing was synthetically lethal in the 3 cells lines, the most conspicuous effect was observed in the GEJ-derived cell line model, (ESO26 cells, $P = .002$; Figure 3, D-F). Consistent with these observations, increased H2A histone family member X (H2AX) phosphorylation on serine 139 (g-H2AX), a well-defined marker of DNA double strand breaks (33), on treatment with a potent and selective ATR inhibitor RP-3500 was observed in *ATM*-depleted ESO26 cells compared with cells transfected with a control siRNA (Supplementary Figure 3, available online).These results suggest that ATM and ATR are synthetic lethal in a GEJ tissue background and encourage further investigation of the utility of ATR inhibitors in ATM-mutated GEJ tumors.

Discussion

Germline P/LP variants in *ATM* are known to increase the risk of breast (4,34), pancreatic (35,36), and prostate (37,38), cancers. Here we expand the repertoire of human cancers whose development may be underpinned by *ATM* P/LP germline variants to include GEJ adenocarcinoma. In our series, the rate of *ATM* P/P germline variants in GEJ adenocarcinoma was 5.8%. Somatic LOH of the *ATM* wild-type allele as well as loss of ATM protein expression were observed in all GEJ adenocarcinomas from the patients with *ATM* P/LP germline variants tested, indicating a likely disfunction of ATM in these cancers.

The molecular features of g*ATM*-mut GEJ adenocarcinomas were found to differ from its g*ATM*-wt counterpart. All tested g*ATM*-mut GEJ cancers were found to harbor LOH of the wild-type (functional) allele, a near-complete absence of somatic alterations in *TP53*, increased rate of *MDM2* amplification, and a lower number of somatic single nucleotide variants. Mutual exclusivity of *ATM* and somatic *TP53* mutations were previously reported between the 2 genes (11,39,40), and *MDM2* amplification is a known alternative mechanism of *TP53* inactivation (41,42). The results of our study suggest that germline P/LP variants in *ATM* play a role in GEJ adenocarcinoma development and may increase the risk of this disease. The molecular data presented in this study provide support for the association between P/LP *ATM* variants and GEJ adenocarcinoma, given that the rate of g*ATM*-mut samples for GEJ adenocarcinoma was statistically significantly higher compared with any other cancer, including breast, prostate, and pancreatic cancer, in which *ATM* is known to play a role as a genetic risk factor. In addition, g*ATM*-mut GEJ adenocarcinomas appear to be primarily driven by ATM deficiency. Future molecular and epidemiological studies of P/LP *ATM* carriers are

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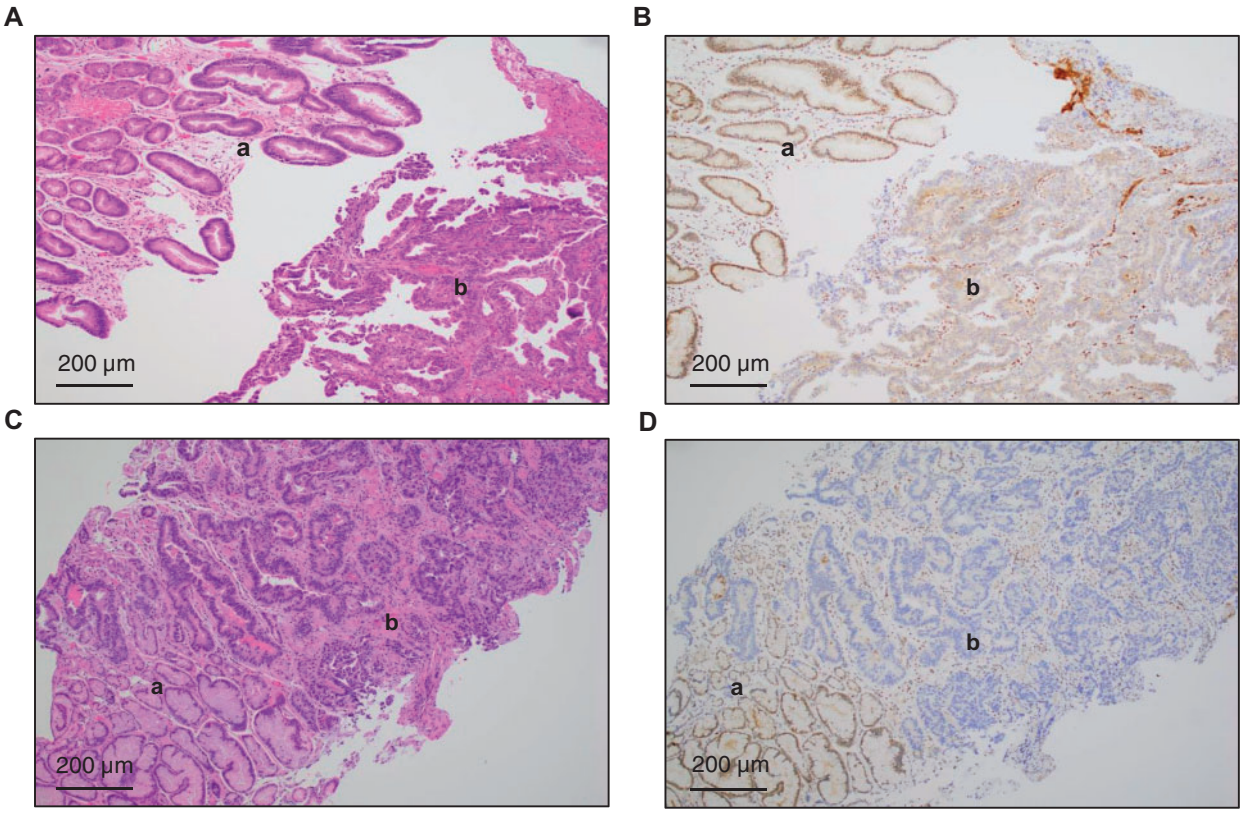

Figure 2. Immunohistochemical analysis of ATM expression in gastroesophageal junction (GEJ) adenocarcinomas. **A–D**) Representative micrographs of the hematoxylin-and-eosin (H&E) and ATM immunohistochemical assessment of 2 cases of ATM-mutated GEJ adenocarcinomas. Note the retention of the nuclear expression in the normal glands (marked by **a**) and the complete loss of nuclear expression in the malignant proliferating glands (marked by **b**). **A, C**) H&E, X40. **B, D**) ATM immunohistochemistry.

needed to confirm our findings and determine the relative risk of developing GEJ adenocarcinoma.

Our findings also suggest that g*ATM*-mut GEJ adenocarcinomas tended to be more indolent and presented at an earlier stage compared with g*ATM*-wt GEJ adenocarcinomas. Though these observations need to be tested in additional cohorts, the findings here support the contention that g*ATM*-mut GEJ cancers may be sensitive to radiation (11), because only 10% of patients with resections post treatment had recurrent disease. Consistent with previous observations demonstrating that ATM and ATR loss of function are synthetically lethal (32,43), silencing of ATM and ATR in GEJ, esophageal, and gastric adenocarcinoma cell lines resulted in statistically significant loss of cell viability as demonstrated here and in Roulston et al. (32) Alterations in DDR and homologous recombination (HR) DNA repair-related genes have received attention as potential therapeutic targets through synthetic lethality, with PARP inhibitors being FDA approved for the treatment breast, ovarian, and pancreatic cancers in patients with *BRCA1/2* germline P/LP variants as well as prostate cancer patients with mutations affecting DDR- or HR-related genes (43). Sensitivity to PARP inhibitors, however, varies according to the DDR or HR genes affected in cancers. For example, the benefit of PARP inhibition appears to be less conspicuous in prostate cancer patients with loss of *ATM* than in those with loss of *BRCA2*; conversely, loss of *ATM* may result in increased sensitivity to ATR inhibition (15,44). Our in vitro findings in the GEJ model demonstrate that in a GEJ adenocarcinoma background, concurrent silencing of ATM and ATR resulted in a statistically significant reduction in cell viability, supporting the notion that g*ATM*-mut GEJ cancers may be potential candidates for treatment with ATR inhibitors. Further studies are warranted to test this hypothesis.

Smoking, obesity, and gastroesophageal reflux disease are the well-recognized risk factors for GEJ adenocarcinoma (45). Screening programs for early detection of GEJ adenocarcinomas are not in use or practical because of low incidence of the disease and are limited for patients with gastroesophageal reflux disease and Barrett's esophagus as risk factors (46). Given the high prevalence of germline P/LP *ATM* variants in GEJ adenocarcinoma compared with the general population and other cancer types in our cohort, screening GEJ adenocarcinoma patients for germline P/LP variants in *ATM* should be considered. Based on our findings, immunohistochemistry appears to be rather sensitive and to display a high negative predictive value for the presence of a germline P/LP *ATM* variant (ie, none of the GEJ adenocarcinomas with *ATM* germline or somatic bi-allelic *ATM* variants expressed ATM by immunohistochemistry). Thus, this approach may be used to identify cases, and by extension families, that may be at risk of developing *ATM*-associated cancers. A similar approach has been used for Lynch syndrome, in which immunohistochemistry for mismatch repair proteins is used to determine which mismatch repair genes should undergo further analysis using molecular methods (47). Alternatively, given the high rate of P/LP *ATM* variants in GEJ adenocarcinoma, a recommendation for patients with GEJ adenocarcinomas to undergo genetic testing for *ATM* may also be considered.

This study has several limitations. Despite the large cohort of cancers subjected to MSK-IMPACT sequencing, the relative sample

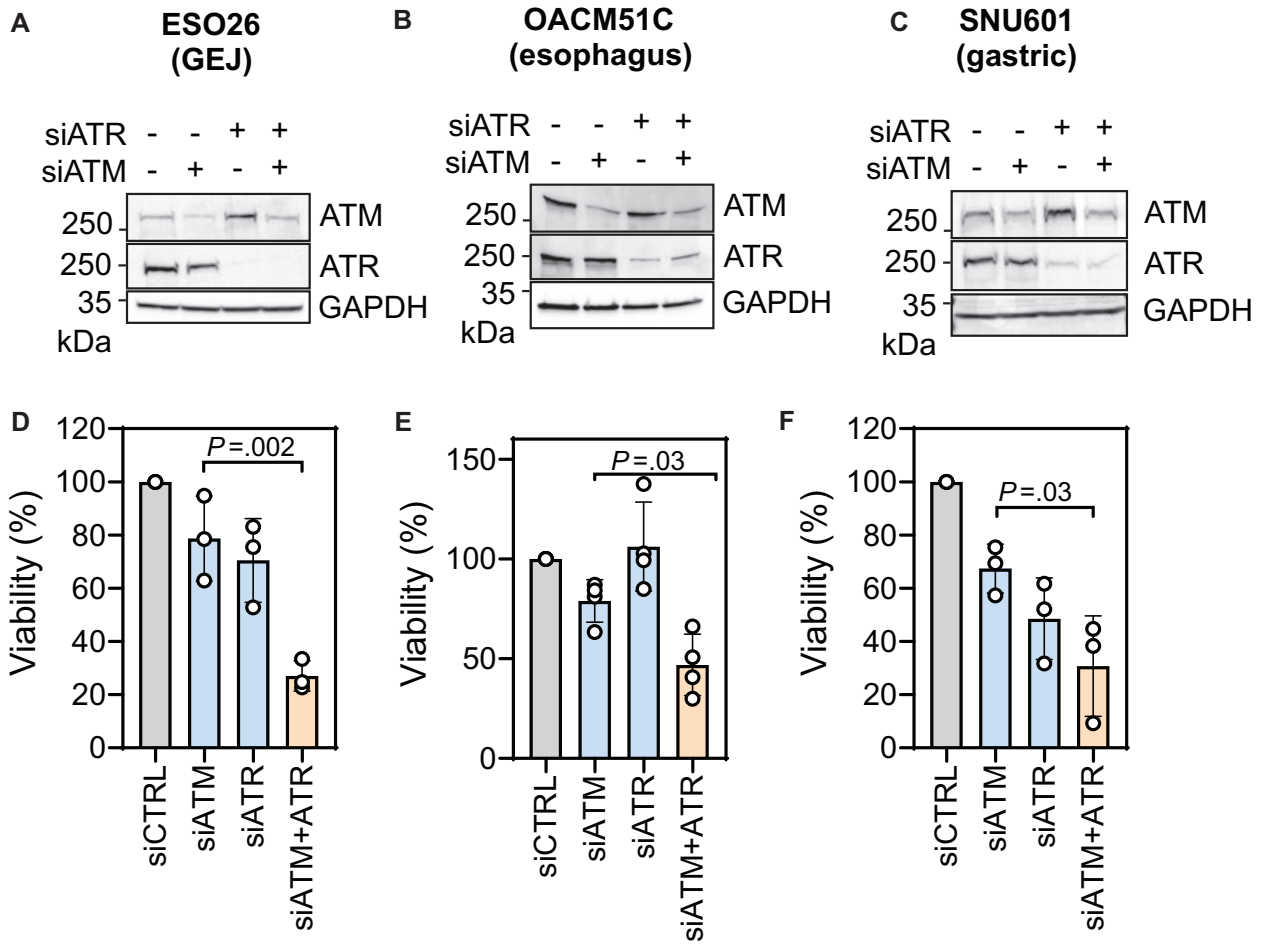

Figure 3. ATM and ATR short-interfering RNA (siRNA) silencing in gastroesophageal junction (GEJ), esophageal, and gastric adenocarcinoma cell lines. **A-C**) Representative immunoblots showing downregulation of ATM and ATR expression by their respective siRNAs in the indicated cell lines. Whole cell extracts were probed with the indicated antibodies, glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as a loading control. **D-F**) Quantification of viability of the indicated cell lines on downregulation of ATM, ATR, or both, suggesting synthetic lethality between ATM and ATR in the respective tissue backgrounds. Cells were transiently transfected with the indicated siRNAs, and viability was measured with a CellTiter Glo assay. Data represented as individual values from 3 and more independent biological replicates normalized to a non-targeting siRNA control (**circles**) with mean (**bars**) and the **error bars** represent the SD. *P* values were calculated with 1-way analysis of variance (ANOVA). All statistical tests were 2-sided.

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size of GEJ adenocarcinomas included in this study was small, reflecting the rarity of these cancers. Additionally, we were unable to directly test the synthetic lethal interaction–caused ATR silencing in a GEJ adenocarcinoma cell line harboring a germline or somatic *ATM* P/LP variant, given that cell lines or patient-derived xenografts with these characteristics are not commercially available. Despite these limitations, here we demonstrate germline *ATM* P/LP variants are frequent in GEJ adenocarcinomas, and these tumors likely constitute a distinct subset of GEJ cancers with associated clinico-pathologic and molecular features. Although additional studies are needed to ascertain the penetrance and risk of GEJ adenocarcinoma in germline P/LP ATM carriers, our findings suggest that *ATM* testing of patients with GEJ adenocarcinomas may be justified. Finally, given the potential therapeutic implication of ATM loss of function in a substantial subset of GEJ adenocarcinomas, further studies testing novel potential therapeutic approaches for ATM-deficient GEJ cancers are warranted.

Funding

JSR-F is funded in part by the Breast Cancer Research Foundation. This work was partly supported by the National Institutes of Health/National Cancer Institute grant (P50 CA247749 01), the Marie-Josée and Henry R. Kravis Center for Molecular Oncology, the Precision, Interception and Prevention Program; the Robert and Kate Niehaus Center for Inherited Cancer Genomics; and the National Institutes of Health/National Cancer Institute Cancer Center Support Grant (P30 CA008748).

Notes

Role of the funders: The funding sources had no role in the study design; collection, analysis, and interpretation of the data; writing of the manuscript; or decision to submit the manuscript for publication.

Disclosures: JSR-F reports receiving personal/consultancy fees from Goldman Sachs, REPARE Therapeutics, Paige.AI and Eli Lilly, membership of the scientific advisory boards of VolitionRx, REPARE Therapeutics, Paige.AI and Personalis, membership of the Board of Directors of Grupo Oncoclinicas, and ad hoc membership of the scientific advisory boards of Roche Tissue Diagnostics, Ventana Medical Systems, Novartis,

Genentech and InVicro. JSR-F owns REPARE Therapeutic stocks. MZ, MZ, VR and MK are full-time employees of REPARE Therapeutics and receive salary and equity compensation. All remaining authors have no conflicts of interest to declare.

Jorge S. Reis-Filho, MD, PhD, who is a *JNCI* Associate Editor and a coauthor on this article, was not involved in the editorial review or decision to publish this article.

Author contributions: TEJ: Data curation, Writing—original draft, Investigation, Writing—review and editing, Formal Analysis; MM: Data curation, Writing—original draft, Investigation, Writing—review and editing, Formal Analysis; DC: Data curation, Writing—original draft, Investigation, Writing—review and editing, Formal Analysis; MZim: Data curation, Writing—original draft, Investigation, Methodology, Writing—review and editing, Formal Analysis; VR: Data curation, Investigation, Writing—review and editing; AM: Data curation, Investigation, Software, Visualization, Writing—review and editing; FD: Data curation, Investigation, Writing—review and editing; PS: Data curation, Investigation, Software, Writing—review and editing; MP: Data curation, Writing—review and editing; JSS: Data curation, Writing—review and editing; OCB: Data curation, Writing—review and editing; YK: Data curation, Writing—review and editing; AC: Data curation, Writing—review and editing; MR: Data curation, Writing—review and editing; GYK: Data curation, Writing—review and editing; YYJ: Data curation, Writing—review and editing; MZim: Data curation, Methodology, Resources, Writing—review and editing; MK: Data curation, Resources, Writing—review and editing, Formal Analysis; ZS: Data curation, Writing—review and editing; JS: Data curation, Writing—review and editing; JSRF: Data curation, Writing—original draft, Investigation, Methodology, Project administration, Resources, Supervision, Writing—review and editing, Formal Analysis; DM: Data curation, Writing—original draft, Methodology, Project administration, Resources, Supervision, Writing—review and editing, Formal Analysis.

Data Availability

Genomic data are available on the MSK cBioPortal at https://www.cbioportal.org/study/summary?id=egc_msk_2022.

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