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ATM Variants and Breast Cancer Risk in North Macedonia: Focus on the Regionally Enriched *p.(Leu2492Arg)* Variant

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Background: Germline pathogenic variants (PVs) in the ataxia-telangiectasia mutated (*ATM*) gene are established moderate-risk factors for breast cancer (BC), however, population-specific variant spectra and the clinical significance of many missense variants remain incompletely characterized.

Aims: To evaluate the prevalence of *ATM* variants in a large cohort of patients with BC from North Macedonia and compare it with that in the general population, with a particular focus on the frequency of the p.(Leu2492Arg) variant and its distribution relative to global genomic datasets.

Study Design: This study was conducted as a retrospective case–control analysis.

Methods: *ATM* variants were analyzed in 1,211 patients with BC from North Macedonia using a targeted hereditary cancer gene panel. These findings were compared with those from 1,303 population-based controls analyzed by clinical exome or whole-exome sequencing.

Results: Pathogenic *ATM* variants were identified in 1.9% of BC cases and 0.4% of controls, indicating a significantly increased risk of BC [odds ratio (OR) = 5.02, $p = 0.0006$]. Most PVs were protein-truncating, with six recurrent variants accounting for over 70% of detections, suggesting regional enrichment. Carriers showed a significantly higher prevalence of human epidermal growth factor receptor 2-positive tumors (OR = 2.92, $p = 0.0189$). Variants of uncertain significance were observed at comparable frequencies in cases and controls. The p.(Leu2492Arg) missense variant was more frequently detected in cases than in controls (1.9% vs. 1.1%; OR = 1.78, $p = 0.086$) and exhibited a markedly higher allele frequency in this population than in global databases.

Conclusion: These findings confirm *ATM* as a clinically relevant BC susceptibility gene in North Macedonia and highlight the population-specific enrichment of both PVs and the p.(Leu2492Arg) missense variant. The results emphasize the importance of using population-matched controls and regional genomic data for accurate risk assessment and variant interpretation.

INTRODUCTION

The ataxia-telangiectasia mutated (*ATM*) gene, located on chromosome 11q22.3, encodes a serine/threonine protein kinase that is essential for maintaining genomic stability.[1] Comprising 63 exons, ATM acts as a central regulator of cell cycle checkpoint signaling pathways. It is primarily activated in response to DNA double-strand breaks, which represent one of the most cytotoxic forms of DNA damage.[2] ATM activation is mediated by the MRE11–RAD50–NBS1 (MRN) complex, after which it phosphorylates multiple downstream targets, including p53, BRCA1, CHEK2, and H2AX. This signaling cascade coordinates cell cycle arrest, DNA repair, or apoptosis, thereby preventing the accumulation and propagation of genomic damage.[3] Pathogenic variants (PVs) in these genes impair DNA repair mechanisms and increase cancer susceptibility.[4] Beyond its role in genome maintenance, ATM also influences several physiological processes, including neurological function, immune response, and aging. Biallelic PVs in *ATM* cause *ataxia-telangiectasia* (A–T), a rare autosomal recessive disorder characterized by progressive neurodegeneration, immunodeficiency, radiosensitivity, premature

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aging, and a markedly increased risk of cancer, particularly lymphoid malignancies.[5] Although A–T is inherited in a recessive manner, heterozygous carriers of pathogenic *ATM* variants also have an elevated risk of cancer, particularly breast, prostate, and pancreatic cancers.[6] Importantly, cancer risk varies considerably depending on the specific *ATM* variant. Protein-truncating variants (PTVs) are generally associated with a two- to four-fold increased risk of breast cancer (BC),[7] whereas the clinical significance of many missense variants remains uncertain.[8] BCs associated with *ATM* PVs often exhibit distinct clinical features, including hormone receptor positivity, poor differentiation, and increased lymph node involvement.[7] *ATM* variants may also influence treatment response, particularly in the context of radiotherapy, where variant-specific effects on contralateral BC risk have been reported.[9] Despite growing evidence linking *ATM* variants to BC, the clinical interpretation of many *ATM* missense variants remains challenging. Furthermore, although *ATM*-associated BC risk has been extensively studied, populations from Southeastern Europe remain underrepresented in global genomic datasets. In this study, we analyzed *ATM* germline variants in a large cohort of patients with BC from North Macedonia and compared them with population controls. We estimated the prevalence of PVs and evaluated the frequency of the c.7475T > G, p.(Leu2492Arg) variant relative to global datasets. These findings provide insight into regional variant distribution and contribute to the improved interpretation of *ATM* variants in an underrepresented population.

MATERIALS AND METHODS

The case cohort included 1,211 patients diagnosed with invasive BC between 2009 and 2025. Clinical and demographic data were obtained for all patients. To estimate the carrier frequency of *ATM* PVs in the general population, we analyzed sequencing data from 1,303 individuals referred for genetic testing for pediatric and non-cancer conditions. The control cohort had a median age of 19 years and included 67.2% Macedonians, 25.3% Albanians, and 6.2% individuals of other ethnicities, with 52.5% males. In comparison, the BC cohort (median age, 47 years) consisted entirely of females and was predominantly Macedonian (83%). The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Subcommittee of the Macedonian Academy of Science and Arts for Medicine, Pharmacy, Veterinary Medicine and Dentistry (approval number: 03-203/4, date: 10.12.2024), and written informed consent was obtained from all participants. Genomic DNA was extracted from peripheral blood samples using standard protocols. Targeted sequencing of 94 cancer-associated genes, including *ATM* in the BC cohort, was performed using the TruSight Hereditary Cancer Panel (Illumina Inc., San Diego, CA, USA). Sequencing was conducted on the MiSeq platform using paired-end 150-bp reads. Bioinformatic analysis was performed in-house and included read alignment using BWA v0.7.15,[10] variant calling with GATK v3.8,[10] VCF processing with bcftools v1.9,[11] and variant annotation using Ensembl Variant Effect Predictor v112,[12] all based on the hg19 reference genome. Control samples were processed using either the TruSight One clinical exome panel (n = 290) or the Twist Human Core + RefSeq + Mitochondrial WES panel (n = 1,013), with sequencing performed on the MiSeq or NovaSeq 6000 platforms, respectively. All control data were analyzed using an identical in-house pipeline aligned to the hg38 "no_alt" reference genome, as previously described.[13] Variant coordinates derived from hg19 were converted to hg38 using the UCSC LiftOver tool prior to allele frequency comparison. Variants within coding regions and ± 25 bp of flanking intronic sequences were evaluated, and *ATM* was fully covered in both library enrichment approaches. Variant visualization was performed using Integrative Genomics Viewer (IGV), and variant classification followed American College of Medical Genetics and Genomics guidelines.[14] Variant nomenclature followed Human Genome Variation Society recommendations and was based on the *ATM* reference transcript NM_000051.4, with genomic coordinates corresponding to the GRCh38/hg38 reference genome assembly. Sanger sequencing was used to confirm variants in cases with inconclusive or low-confidence NGS results. Copy number variant (CNV) analysis (exon-level deletions/duplications) was not performed in this study.

RESULTS

Pathogenic *ATM* variants were identified in 23 BC cases (23/1211, 1.9%) and 5 controls (5/1303, 0.4%). *ATM* PVs were significantly associated with an increased risk of BC [odds ratio (OR) = 5.02, 95% confidence interval (CI): 1.90-13.26, $p = 0.0006$]. In total, 14 distinct *ATM* PVs were detected, the majority of which were PTVs. Six PVs were recurrent and detected in multiple individuals, collectively accounting for 71.4% (20/28) of all PV carriers in the study population (Table 1). Two PVs were observed exclusively in controls: c.2250G>A, p.(Lys750=), and c.8147T>C, p.(Val2716Ala). All identified PVs have been previously reported in ClinVar and classified as pathogenic or likely pathogenic. Patient-level clinicopathological data for BC patients carrying *ATM* PVs are summarized in Table 2.

Co-occurrence of *ATM* PVs with PVs in *BRCA1* or *BRCA2* was observed in four BC patients. Two patients carried *BRCA1* PVs, p.(Ala1453Glnfs*3) and p.(Tyr978*), and presented with triple-negative BC, both with a positive family history. Two additional patients carried *BRCA2* PVs, p.(Ser1064Leufs*12) and p.(Ala938Profs*21), and had estrogen receptor (ER)-positive tumors, with only one reporting a family history of cancer. All cases were diagnosed at a young age and exhibited early-onset or aggressive disease phenotypes. These findings illustrate the co-occurrence of PVs in multiple BC susceptibility genes.

Associations between *ATM* PVs and clinicopathological characteristics are summarized in Table 3. Although *ATM* PVs were more frequently observed in patients of Macedonian ethnicity, in women diagnosed before the age of 50 years, and in those with a positive family history, these associations did not reach statistical significance. However, a statistically significant association was observed between *ATM* PV carrier status and human epidermal growth factor receptor 2 (HER2)-positive tumors ($p = 0.0189$).

Variants of uncertain significance (VUS) were frequently identified in both cohorts. In BC patients, 19 distinct VUS were detected in 27 individuals (2.2%), whereas 22 VUS were identified in 24 controls

TABLE 1. Frequency of Pathogenic *ATM* (NM_000051.4) Variants Among Breast Cancer Patients and Population Controls.

HGVSc	HGVSp	dbSNP	ClinVar ID	Effect	AF_gnomAD	BC cases	Controls
c.67C > T	p.(Arg23*)	rs746235533	232248	Stop gained	3.72E-06	6	/
c.495_496 + 16del	p.?	rs1555059522	487452	Splice donor	6.20E-07	2	/
c.1066-1G > A	p.?	rs876660038	232870	Splice acceptor	/	3	1
c.1564_1565del	p.(Glu522Ilefs*43)	rs587779817	127340	Frameshift	5.45E-05	1	2
c.2250G > A	p.(Lys750=)	rs1137887	3044	Splice, synonymous	4.23E-05	/	1
c.3576G > A	p.(Lys1192=)	rs587776551	3035	Splice, synonymous	2.48E-06	3	/
c.3603del	p.(Phe1201Leufs*6)	rs1057517129	371256	Frameshift	/	1	/
c.3866del	p.(Lys1289Argfs*4)	/	2111936	Frameshift	/	1	/
c.5005G > A	p.(Glu1669Lys)	rs1591693095	800343	Missense, splice	/	1	/
c.6115G > A	p.(Glu2039Lys)	rs864622251	219787	Missense	2.48E-06	1	/
c.6889dup	p.(Gln2297Profs*76)	/	2431274	Frameshift	/	1	/
c.8147T > C	p.(Val2716Ala)	rs587782652	142700	Missense	2.73E-05	/	1
c.8283_8284del	p.(Gln2762Alafs*6)	rs775899653	482713	Frameshift	/	2	/
c.9139C > T	p.(Arg3047*)	rs121434219	3029	Stop gained	1.61E-05	1	/

AF, allele frequency; BC, breast cancer; SNP, single nucleotide polymorphism.

(1.8%). All VUS were missense variants. Additionally, 45 variants with conflicting pathogenicity interpretations were observed in cases and 44 in controls. The c.7475T > G, p.(Leu2492Arg) variant showed substantial classification discordance in ClinVar, with submissions ranging from likely pathogenic to benign. This variant was more frequent in BC cases (1.9%) than in controls (1.1%); however, the association did not reach statistical significance ($p = 0.086$). The OR for this variant was 1.78 (95% CI: 0.91-3.48). The variant appeared more frequent among Albanian than Macedonian BC patients, whereas similar frequencies were observed between ethnic groups in controls. The variant affects a conserved residue within the *ATM* FAT domain and is predicted to be deleterious by multiple *in silico* tools, including AlphaMissense (0.91), REVEL (0.82), CADD (27), PROVEAN (-4.5), and SIFT (0.003). Strong evolutionary conservation at this position was also observed (PhyloP100 = 7.5). Visual inspection of sequencing reads using IGV confirmed the presence of the p.(Leu2492Arg) variant in all carriers. Analysis of seven single nucleotide polymorphisms (SNPs) located within ~50 kb of the *ATM* gene revealed homozygosity for the ancestral haplotype in 26 of 37 (70.3%) carriers of the p.(Leu2492Arg) variant. The remaining 11 carriers harbored the same haplotype in combination with four different haplotypes. The distribution of c.7475T > G variant carriers across ethnic groups and clinicopathological characteristics is presented in Table 4.

DISCUSSION

Heterozygous PVs in *ATM* are well-established moderate risk factors for BC, with meta-analyses and large population-based sequencing studies reporting an approximately 2–5-fold increase in lifetime risk compared with the general population.[15-19] Several studies estimate the lifetime BC risk in heterozygous *ATM* carriers to be 21-24%, whereas the population risk is 12.5% (National Comprehensive Cancer Network Guidelines, Version 2.2026). In the present study, pathogenic *ATM* variants were identified in 1.9% of BC cases and 0.4% of controls, supporting the role of *ATM* as a moderate-penetrance BC susceptibility gene. The observed (OR = 5.02) lies at the higher end of published estimates and approaches the risk reported for certain high-penetrance founder mutations, such as c.7271T > G, p.(Val2424Gly).[20] This relatively high estimate may be influenced by several factors, including the recurrence of specific PVs in our cohort (suggesting possible regional enrichment), differences in case–control structure (e.g., age distribution and sex composition), and the relatively small number of *ATM* PV carriers.

The carrier frequency in controls (0.4%) is consistent with the reported European prevalence of 0.2-0.5%.[21,22] Most detected *ATM* PVs were PTVs, accounting for 85.7% of unique variants, in line with previous reports showing that truncating variants predominate due to loss-of-function effects.[23] Six recurrent PVs accounted for 71.4% of all detections, suggesting regional enrichment and highlighting the importance of population-specific risk assessment for *ATM* variants. Available data from Southeastern Europe remain limited and are primarily derived from multigene panel studies. These studies report the presence of *ATM* PVs but do not identify clear founder mutations, instead indicating a heterogeneous spectrum of rare variants.[18,24-27] The co-occurrence of *ATM* PVs with *BRCA1* or *BRCA2* PVs in four early-onset BC cases highlights the value of multigene panel testing, as concurrent germline variants in DNA repair genes may modify cancer risk and phenotype. This is particularly relevant for young patients and those with a positive family history, consistent with current guidelines recommending comprehensive

TABLE 2. Patient-Level Clinicopathological Data of Breast Cancer Patients Carrying Pathogenic *ATM* Variants.

No.	Patient ID	HGVSc	HGVSp	Effect	Ethnicity	Age at diagnosis	Family history for BC/OC	Localization	BC type	T	N	G	Stage	ER pos.	PR pos.	HER2 pos.	TN pos.
1	BC-255	c.67C > T	p.Arg23*	Stop gained	MK	34	Yes	Unilateral	Ductal	1c	1a	3	II A	No	No	No	Yes
2	BC-1767	c.67C > T	p.Arg23*	Stop gained	MK	40	No	Unilateral	Ductal	2	0	NA	IIA	Yes	Yes	Yes	No
3	BC-1997	c.67C > T	p.Arg23*	Stop gained	MK	54	No	Unilateral	NA	NA	NA	NA	NA	NA	NA	NA	NA
4	BC-2230	c.67C > T	p.Arg23*	Stop gained	MK	51	Yes	Unilateral	Lobular	2	1a	2	IIB	Yes	Yes	No	No
5	BC-2416	c.67C > T	p.Arg23*	Stop gained	MK	48	Yes	Unilateral	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	BC-2871	c.67C > T	p.Arg23*	Stop gained	MK	54	Yes	Unilateral	Ductal	2	1	3	IIB	Yes	Yes	NA	No
7	BC-577	c.495_496 + 16del	p.?	Splice donor	AL	33	No	Bilateral	Ductal	4b	0	3	IIIC	Yes	Yes	Yes	No
8	BC-1396	c.495_496 + 16del	p.?	Splice donor	AL	61	Yes	Unilateral	Ductal	2	1	2	IIB	Yes	Yes	No	No
9	BC-656	c.1066-1G > A	p.?	Splice acceptor	MK	28	No	Unilateral	Lobular	2	3	3	IIIC	Yes	Yes	No	No
10	BC-1643	c.1066-1G > A	p.?	Splice acceptor	MK	32	Yes	Unilateral	Ductal	1c	2a	3	IIIA	Yes	Yes	No	No
11	BC-2415	c.1066-1G > A	p.?	Splice acceptor	MK	45	Yes	Unilateral	Ductal	2	2	3	IIIA	Yes	No	Yes	No
12	BC-1492	c.1564_1565del	p.Glu522Ilefs*43	Frameshift	MK	61	Yes	Unilateral	Ductal	2	1	2	IIB	No	No	Yes	No
13	BC-91	c.3576G > T	p.Lys1192=	Splice, synonymous	MK	37	Yes	Unilateral	Ductal	2	0	NA	NA	Yes	Yes	No	No
14	BC-2315	c.3576G > T	p.Lys1192=	Splice, synonymous	MK	37	Yes	Unilateral	Ductal	1c	x	2	IA	No	No	No	Yes
15	BC-2784	c.3576G > T	p.Lys1192=	Splice, synonymous	MK	34	Yes	Unilateral	Ductal	2	3	3	IIIC	Yes	Yes	No	No
16	BC-105	c.3603del	p.Phe1201Leufs*6	Frameshift	MK	40	Yes	Unilateral	Ductal	2	1	NA	NA	Yes	Yes	No	No
17	BC-160	c.3866delA	p.Lys1289Argfs*4	Frameshift	MK	39	Yes	Unilateral	Lobular	2	1	2	IIB	Yes	No	Yes	No
18	BC-1048	c.5005G > A	p.Glu1669Lys	Missense, splice	MK	37	No	Unilateral	Ductal	2	2a	3	IIIA	Yes	Yes	Yes	No
19	BC-1477	c.6115G > A	p.Glu2039Lys	Missense, splice	MK	41	Yes	Unilateral	Ductal	1c	1mi	2	IB	Yes	Yes	No	No
20	BC-1516	c.6889dup	p.Gln2297Profs*76	Frameshift	MK	52	Yes	Unilateral	Ductal	mi	0	3	IA	Yes	Yes	Yes	No
21	BC-865	c.8283_8284del	p.Gln2762Alafs*6	Frameshift	MK	34	Yes	Unilateral	Ductal	2	1a	3	IIB	Yes	Yes	Yes	No
22	BC-2490	c.8283_8284del	p.Gln2762Alafs*6	Frameshift	MK	38	No	Unilateral	Ductal	NA	NA	NA	NA	Yes	Yes	Yes	No
23	BC-1305	c.9139C > T	p.Arg3047*	Stop gained	MK	34	No	Unilateral	Ductal	2	2	2	IIIA	Yes	Yes	No	No

MK, Macedonian; AL, Albanian; PV, pathogenic variants; BC, breast cancer; OC, ovarian cancer; ER, estrogen receptor; PR, progesterone receptor; HER2/neu, human epidermal growth factor receptor 2; TN, triple negative; pos., positive; NA, not applicable; *ATM*, ataxia-telangiectasia mutated.

sequencing in high-risk individuals.

ATM PVs were more frequent among patients of Macedonian origin (2.1%) than among Albanian patients (1.2%) or individuals of other ethnic backgrounds. Of the 23 carriers, 21 were Macedonian and two were Albanian; both Albanian carriers harbored the same variant, c.495_496+16del, which was not detected in Macedonian patients. All *ATM* PVs identified in controls were found exclusively in individuals of Macedonian origin. Together with the high recurrence of specific PVs, these findings support the presence of regional enrichment. Identification of regionally enriched variants has important clinical implications, enabling targeted testing strategies and more efficient cascade screening.

Regarding clinical features, non-significant trends toward earlier age at diagnosis (before 50 years) and a positive family history of breast

TABLE 3. Association of Clinicopathological Characteristics with Pathogenic Variant Carrier Status in Breast Cancer Patients.

	All cases n=1211	PV carriers (n)	PV carriers/ all BC cases (%)	PV non-carriers (n)	BC non-carriers/ all BC cases (%)	OR (95% CI)	*p* value
Ethnicity							
Macedonian	1006	21	2.1	985	97.9	1.74 (0.4-7.48)	0.76
Albanian	165	2	1.2	163	98.8		
Others	40	0	0	40	100		
Age of onset							
≤ 50	724	17	2.3	707	97.7	1.90 (0.75-4.86)	0.18
> 50	481	6	1.2	475	98.8		
No data	6						
Bilateral BC							
Yes	71	1	1.4	70	98.6		0.73
No	1101	22	2	1079	98	1.43 (0.19-10.74)	
No data	39						
Cancer FH							
BC/OC	605	16	2.6	589	97.4	1.91 (0.63-5.81)	0.36
Other	275	3	1.1	272	98.9	0.78 (0.17-3.64)	
No FH	286	4	1.4	282	98.6		
No data	45						
ER							
Positive	767	18	2.3	749	97.7	2.62 (0.77-8.98)	0.12
Negative	331	3	0.9	328	99.1		
No data	113	2					
PR							
Positive	674	16	2.4	658	97.6	1.69 (0.62-4.67)	0.31
Negative	354	5	1.4	349	98.6		
No data	183	2					
HER2							
Positive	225	9	4	216	96	2.92 (1.19-7.13)	0.0189
Negative	781	11	1.4	770	98.6		
No data	205	2					
TN							
Yes	161	2	1.2	159	98.8		
No	836	19	2.3	817	97.7	1.85 (0.43-8.02)	0.41
No data	214	2					

PV, pathogenic variant; BC, breast cancer; OC, ovarian cancer; OR, odds ratio; FH, family history; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor receptor 2, TN, triple negative.

TABLE 4. Patient-Level Clinicopathological Data of Breast Cancer Patients Carrying *ATM* c.7475T > G. p.(Leu2492Arg) Variant.

Patient ID	Ethnicity	Age at diagnosis	FH for BC/OC	Localisation	BC type	T	N	G	Stage	ER pos.	PR pos.	HER2 pos.	TN pos.
BC-132	MK	83	Yes	Unilateral	Ductal	1mi	0	3	IA	No	No	No	Yes
BC-284	MK	55	Yes	Unilateral	Ductal	3	3a	3	IIIC	No	No	No	Yes
BC-345	MK	67	No	Unilateral	Ductal	2	0	3	IIA	No	No	No	No
BC-634	MK	57	No	Unilateral	Lobular	1	0	-	IA	No	NA	NA	NA
BC-754	MK	61	Yes	Unilateral	Ductal	1	0	3	IA	Yes	Yes	No	No
BC-773	MK	26	Yes	Unilateral	Ductal	1	3a	3	IIIC	Yes	Yes	Yes	No
BC-783	MK	56	Yes	Bilateral	Lobular	2 (m)	2a	2	IIIA	No	No	No	Yes
BC-897	AL	68	Yes	Unilateral	Ductal	4b	3a	3	IIIC	Yes	Yes	No	No
BC-995	MK	62	No	Unilateral	Ductal	2	0	3	IIA	Yes	Yes	No	No
BC-1173	AL	29	No	Unilateral	Ductal	2 (m)	1a	3	IIB	Yes	Yes	No	No
BC-1181	AL	64	Yes	Unilateral	Ductal	1c (m)	1a	2	IIA	Yes	Yes	No	No
BC-1361	AL	38	No	Unilateral	Ductal	4b (m)	3a	3	IIIC	Yes	Yes	No	No
BC-1380	MK	58	Yes	Unilateral	Lobular	1c	2a	2	IIIA	Yes	Yes	No	No
BC-1567	MK	44	Yes	Unilateral	Ductal	1c	0	3	IA	Yes	Yes	Yes	No
BC-1651	MK	40	Yes	Unilateral	Ductal	2	0	3	NA	Yes	Yes	NA	No
BC-1868	MK	60	No	Unilateral	Lobular	NA	NA	NA	NA	No	No	NA	NA
BC-1869	MK	47	No	Unilateral	Na	NA	NA	NA	NA	Yes	Yes	No	No
BC-2188	AL	33	Yes	Unilateral	Ductal	NA	NA	NA	NA	NA	NA	NA	NA
BC-2383	AL	63	Yes	Unilateral	Ductal	2	3a	3	IIIC	NA	NA	NA	NA
BC-2472	MK	64	Yes	Unilateral	Ductal	2	0	2	IIA	Yes	Yes	No	No
BC-2625	AL	48	No	Unilateral	Lobular	2	1	2	IIB	Yes	Yes	No	No
BC-2631	MK	45	No	Unilateral	Ductal	1c	1c	3	IIA	Yes	No	No	No
BC-2713	MK	61	Yes	Unilateral	Ductal	1	0	2	IIA	Yes	Yes	No	No

MK, Macedonian; AL, Albanian; FH, family history; BC, breast cancer; OC, ovarian cancer; T, tumor size; N, nodes; G, grade; ER, estrogen receptor; PR, progesterone receptor; HER2/neu, human epidermal growth factor receptor 2; TN, triple negative; pos., positive; NA, not applicable; *ATM*, ataxia-telangiectasia mutated.

or ovarian cancer were observed. These effect sizes are consistent with published data, including a meta-analysis reporting a relative risk of 4.94 for *ATM* carriers diagnosed before age 50.[28] The lack of statistical significance is likely due to the limited number of carriers rather than the absence of a true biological association.

Consistent with previous reports, *ATM* PV carriers predominantly presented with ER-positive tumors.[16,28] Notably, a significant association with HER2-positive status was observed (OR = 2.92, $p = 0.0189$). However, because of the relatively small number of carriers, multivariable adjustment for age and ethnicity was not performed; therefore, this finding should be interpreted with caution. In addition, the limited sample size resulted in wide confidence intervals, which may affect the precision of the estimated effect size. Recent meta-analyses have reported enrichment of germline *ATM* PVs in hormone receptor–positive/HER2-positive BC compared with triple-negative disease.[29] Supporting this, analyses of HER2-positive tumors have identified *ATM* as one of the most frequently mutated genes, with potential implications for response to trastuzumab therapy.[30] In our cohort, 39% of *ATM* PV carriers were HER2-positive, and most of these tumors were also ER-positive, corresponding to the Luminal B subtype, which is associated with a poorer prognosis.[31] This observation may reflect biological heterogeneity within *ATM*-associated BCs, in which *ATM* dysfunction may interact with HER2-driven tumorigenesis. However, confirmation in larger, population-matched cohorts is required to determine whether a true association between *ATM* PVs and HER2-positive disease exists.

VUS were exclusively missense and were detected at comparable frequencies in BC cases (2.2%) and controls (1.8%). Of particular interest was the *ATM* c.7475T > G, p.(Leu2492Arg) (rs56399857) variant, which was more frequent in BC cases than in controls (1.9% vs. 1.1%), corresponding to a nearly twofold but not statistically significant increase in risk (OR = 1.78, $p = 0.086$). Its allele frequency in our control cohort (0.54%) was substantially higher than that reported in gnomAD (Genome Aggregation Database; 0.016% globally and 0.021% in non-Finnish Europeans). Balkan populations are underrepresented in global reference datasets such as gnomAD, which may result in underestimation of the true background frequency of certain variants in this region. The variant appeared more frequent among Albanian than Macedonian BC patients, whereas similar frequencies were observed between ethnic groups

in controls; however, the small number of carriers limits definitive conclusions. Published data on the prevalence of this variant in Balkan populations remain limited, although it has been reported at lower frequencies in Greece, Slovenia, and Bulgaria.[32,33] In the GeneBass database, the variant shows a nominal association with BC ($p = 2.43 \times 10^{-3}$), although the reported allele frequencies remain substantially lower than those observed in our cohort. The elevated frequency observed in both cases and controls supports population-specific enrichment and suggests that p.(Leu2492Arg) may represent a regionally enriched variant in the Macedonian population rather than a clearly disease-associated allele. Analysis of seven SNPs within a ~50 kb region surrounding *ATM* suggested that the p.(Leu2492Arg) variant is linked to an ancestral haplotype. However, analysis of phased haplotypes and additional markers in larger cohorts will be required to more accurately assess the potential founder origin of this variant. This finding further highlights the importance of population-matched control datasets when interpreting missense variants in moderate risk genes such as *ATM*. The *ATM* p.(Leu2492Arg) variant has been reported in several cancer types, including colorectal, prostate, chronic lymphocytic leukemia, glioblastoma, breast, and ovarian cancers as well as in hereditary cancer syndromes.[26,34,35] The variant is located within the highly conserved FAT domain of *ATM* and affects a residue with strong evolutionary conservation. Multiple *in silico* prediction tools consistently indicate a deleterious effect; however, such predictions alone are insufficient to establish pathogenicity in the absence of functional validation, which is currently lacking. Given its presence in healthy populations, repeated detection in cancer cohorts, and conflicting ClinVar classifications, the most appropriate classification remains a VUS. Further population-based and functional studies are required to clarify its clinical relevance.

Several limitations of this study should be acknowledged. The control cohort was not fully matched to the BC cohort in terms of age and sex, as controls were primarily individuals referred for genetic testing for non-oncological conditions and therefore had a younger median age and included both males and females. However, because *ATM* PVs are germline alterations present from birth, their carrier frequency is unlikely to be strongly influenced by age or sex distribution. In addition, the absence of CNV analysis may have led to a slight underestimation of the true prevalence of pathogenic *ATM* variants, as approximately 5-10% of such variants are deletions or duplications involving part or the entire *ATM* gene.

Overall, our findings highlight the importance of population-specific analyses for interpreting *ATM* variants and suggest that regional genetic architecture may significantly influence variant frequencies and risk estimates in BC.

Ethics Committee Approval: The study was conducted according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Subcommittee of the Macedonian Academy of Science and Arts for Medicine, Pharmacy, Veterinary Medicine and Dentistry (approval number: 03-203/4, date: 10.12.2024).

Informed Consent: Written informed consent was obtained from each patient.

Data Sharing Statement: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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