

Spectrum of Genetic Variants in Hereditary Breast and Ovarian Cancer Syndrome in Tunisian Patients: A Clinical Study with Literature Review

Spectre des variants génétiques dans le syndrome héréditaire des cancers du sein et de l'ovaire chez des patients tunisiens : Etude clinique et revue de la littérature

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ABSTRACT

Introduction: Hereditary Breast and Ovarian Cancer (HBOC) syndrome is an autosomal dominant trait. It is primarily associated with germline variants in the BRCA1 and BRCA2 genes, which significantly increase the lifetime risk of developing breast and ovarian cancers.

Aim: In this study, we aimed to evaluate the clinical and genetic characteristics of patients suspected of having HBOC syndrome and to determine the prevalence and spectrum of germline pathogenic variants.

Methods: We conducted a retrospective study of patients diagnosed with breast and/or ovarian cancer, with or without a family history of cancer, who underwent genetic testing between 2014 and 2024. A total of 91 patients underwent Sanger sequencing for recurrent BRCA1/2 variants in the Tunisian population and 56 patients underwent NGS-based gene panel sequencing.

Results: We included a total of 147 unrelated patients. Germline heterozygous variant was identified in 33 patients (22.4%) including 26 with isolated breast cancer, 4 with only ovarian cancer, 2 with both breast and ovarian cancers, and one with breast and pancreatic cancers. Among these patients' carriers, 90% had a positive family history of cancer. Variants were found in BRCA1 (n=21), BRCA2 (n=8), ATM (n=3), and RAD51D (n=1).

Conclusion: The identification of constitutional variants in hereditary breast and ovarian cancer genes offers diagnostic, prognostic, and therapeutic benefits which allow for appropriate management of high-risk subjects.

Keywords: BRCA1, BRCA2, hereditary, Variant, germinal, panel

RÉSUMÉ

Introduction: Le syndrome héréditaire du cancer du sein et de l'ovaire (HBOC) est un trait autosomique dominant. Elle est principalement associée à des variants germinaux des gènes BRCA1 et BRCA2, qui augmentent significativement le risque à vie de développer des cancers du sein et de l'ovaire.

Objectif: Dans cette étude, nous avons visé à évaluer les caractéristiques cliniques et génétiques des patients suspectés de porter le syndrome HBOC et à déterminer la prévalence et le spectre des variants pathogènes de la lignée germinale.

Méthodes: Nous avons mené une étude rétrospective sur 147 patientes diagnostiquées avec un cancer du sein et/ou de l'ovaire, avec ou sans antécédents familiaux de cancer, qui ont subi des tests génétiques entre 2014 et 2024. Au total, 91 patients ont subi un séquençage Sanger pour des variants BRCA1/2 récurrents dans la population tunisienne et 56 patients ont subi un test multigénique basé sur NGS.

Résultats: Un variant hétérozygote germinale a été identifié chez 33 patientes (22,4 %), dont 26 atteintes d'un cancer du sein isolé, 4 uniquement d'un cancer de l'ovaire, 2 d'un cancer du sein et de l'ovaire, et une avec des cancers du sein et du pancréas. Parmi les porteurs de ces patients, 90 % présentaient des antécédents familiaux positifs de cancer. Des variantes ont été trouvées dans BRCA1 (n=21), BRCA2 (n=8), ATM (n=3) et RAD51D (n=1).

Conclusion: L'identification de variantes constitutionnelles dans les gènes héréditaires du cancer du sein et de l'ovaire offre des bénéfices diagnostiques, pronostiques et thérapeutiques qui permettent une prise en charge appropriée des sujets à haut risque.

Mots clés: BRCA1, BRCA2, héréditaire, variant, germinal, panel

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INTRODUCTION

Breast cancer is the most common cancer and the leading cause of cancer-related mortality among women worldwide, while ovarian cancer ranks as the sixth most frequently diagnosed cancer globally(1,2). In Tunisia, the incidence of female breast cancer is notably increasing, reaching 39.12 cases per 100,000 women(3).

The majority of breast and ovarian cancers are sporadic. Only 10-15% of all breast and ovarian cancers are attributed to a genetic predisposition(2). The main genes involved in hereditary predisposition to breast and ovarian cancers are BRCA1 and BRCA2, which play a crucial role in the repair of DNA double-strand breaks via homologous recombination. Germline variants in these genes account for approximately 25% of hereditary breast and ovarian cancers (HBOC) syndrome(4).

Pathogenic and likely pathogenic variants in BRCA1 and BRCA2 are rare and are transmitted in an autosomal dominant manner with incomplete penetrance. They are associated with a very high risk of developing breast and ovarian cancers and a lower risk of other cancer types. Among carriers of BRCA1 variants, the cumulative risk by age 80 is estimated at 72% for breast cancer and 44% for ovarian cancer. For BRCA2 carriers, the cumulative risk by age 80 is approximately 69% for breast cancer and 17% for ovarian cancer (5).

Other susceptibility genes have been implicated in HBOC syndrome, including PALB2, CHEK2, ATM, RAD51C, RAD51D, and BRIP1. Most of these genes are involved in DNA repair pathways. The frequency of germline variants varies by geographic and ethnic background. Most of these variants are private, found within a single family or a small number of families. However, several founder and recurrent variants have been described in multiple countries (6,7).

The genetic heterogeneity of HBOC syndrome has historically made germline testing for these cancers difficult, time-consuming, and expensive using conventional sequencing methods. The advent of next-generation sequencing (NGS) technologies has enabled the simultaneous analysis of multiple genes across large patient cohorts with improved efficiency and reduced turnaround time.

To date, implementation of comprehensive genetic testing services for hereditary cancer syndromes in a developing country such as Tunisia will have a major impact on the clinical management of the patients and families at risk particularly in terms of early diagnosis, treatment and prevention. In this context, the objective of this study was to evaluate the clinical and genetic characteristics of patients suspected of having HBOC syndrome and to determine the prevalence and spectrum of germline pathogenic variants using various sequencing techniques.

METHODS

Study subjects

We conducted a retrospective and descriptive study

of patients referred for oncogenetic evaluation at the Genetic Department at Mongi Slim Hospital in La Marsa, Tunisia, who underwent molecular testing for genetic predisposition between January 2014 and December 2024. This study included all patients diagnosed with breast and/or ovarian cancer, with or without a family history of cancer.(8) Patients were selected according to clinical criteria adapted from the 2024 recommendations of the National Comprehensive Cancer Network (NCCN) for hereditary breast and ovarian cancer genetic testing (8):

- Breast cancer diagnosed before age 45.
- Co-occurrence of breast and ovarian cancer in the same individual.
- Bilateral breast cancer, regardless of age.
- Male breast cancer, regardless of age.
- High-grade serous ovarian carcinoma, regardless of age.
- Triple-negative breast cancer diagnosed before the age of 60 years.
- Breast cancer did not meet the above criteria, with one or more of the following: (a) three or more relatives with breast cancer regardless of age within the same parental lineage, (b) at least one close blood relative with ovarian cancer or breast cancer diagnosed before the age of 50, or bilateral breast cancer, or breast cancer in a male, and (c) at least one first- or second-degree relative affected by pancreatic cancer regardless of age.

Genetic Analysis

Constitutional molecular analysis of breast and ovarian cancer susceptibility genes was performed for all patients included in this study. Genomic DNA was extracted from peripheral blood samples using the Qiagen Blood Midi Kit (Qiagen). DNA quality and concentration were assessed by NanoDrop™ One (Thermo Fisher Scientific).

Two different sequencing approaches were employed to detect genetic predisposition to breast and ovarian cancers:

- Sanger sequencing, targeting recurrent BRCA1/2 variants previously identified in the Tunisian population
- Next-generation sequencing (NGS) using a panel of susceptibility genes

The choice of sequencing technique was left to the patients, depending on their socioeconomic status.

Screening for recurrent BRCA1/2 variants in the Tunisian population

Sanger sequencing has been performed at the Department of Genetics, Mongi Slim Hospital, La Marsa, Tunisia and it was used as a targeted method to analyze specific exons of the BRCA1 and BRCA2 genes. The study was restricted to exons 4, 16, 19, and part of exon 10-2 of BRCA1(NM_007294.4), as well as exons 10 and 16 of BRCA2(NM_000059.4), focusing on variants previously reported as recurrent in the Tunisian population(9–12). Given that Sanger sequencing is a stepwise, targeted approach, the initial choice of gene for analysis was guided by family history of cancer or geographic origin. The analysis of BRCA2 was only initiated when there was

a family history of prostate cancer, pancreatic cancer, or male breast cancer (13). For genetic counseling purposes, only variants classified as pathogenic (class 5) or likely pathogenic (class 4), according to the American College of Medical Genetics and Genomics (ACMG) classification criteria, were considered (14).

Gene Panel Sequencing

Different types of breast and ovarian cancer susceptibility panels were offered, ranging from restricted panels (BRCA1/2) to extended panels including up to 102 genes. Sequencing was performed using either the Illumina NextSeq500 or the Ion Torrent Personal Genome Machine platforms. This testing was carried out across multiple laboratories: a private laboratory in France, a private laboratory in Germany, and the Georges-François Leclerc Cancer Center (France). The selection of a targeted or extended panel was based on financial considerations and the patient's socioeconomic level.

The identified variants were compared with publicly available variant databases, including ClinVar, to determine whether they had been previously reported. In addition, the scientific literature was reviewed to identify prior publications reporting these variants and their associated clinical interpretations.

All patients provided written informed consent prior to genetic testing. The study was approved by the local ethics committee of Mongi Slim Hospital, La Marsa, Tunisia. Informed consent was obtained following a dedicated genetic counseling consultation, during which the objectives of the genetic analysis and its potential clinical and familial implications were explained.

When a pathogenic or likely pathogenic variant was identified in an index case, targeted genetic testing was proposed to unaffected relatives for cascade screening.

RESULTS

Clinical results

We included a total of 147 unrelated patients (145 women and 2 men). The demographic characteristics of these subjects are detailed in Table 1. The median age at diagnosis in our cohort was 42 years. The mean age at diagnosis of breast or ovarian cancer was 44 years (range: 24–74). Specifically, the mean age at diagnosis was 41 years (range: 24–69) for breast cancer and 53 years (range: 38–74) for ovarian cancer. Approximately 62% (91/147) of patients were diagnosed at or before the age of 45, and 21.7% (32/147) were younger than 36 years at diagnosis (Figure 1).

The majority of patients (136/147; 92.5%) presented with an isolated breast or ovarian cancer. A personal history of both breast and ovarian cancer was noted in 3.4% (5/147) of cases. Additional multiple primary cancers included two cases of synchronous breast and pancreatic cancers, one male patient with breast and prostate cancer, one female patient had breast and endometrial cancer, one

case of breast and thyroid cancer, and one case of breast and bladder cancer. Among breast cancer cases, 88% (109/124) were unilateral, including one multifocal tumor, whereas 12% (15/124) were bilateral, either synchronous (10/15) or metachronous (5/15). Among ovarian cancer cases, five out of 28 were bilateral (17.9%).

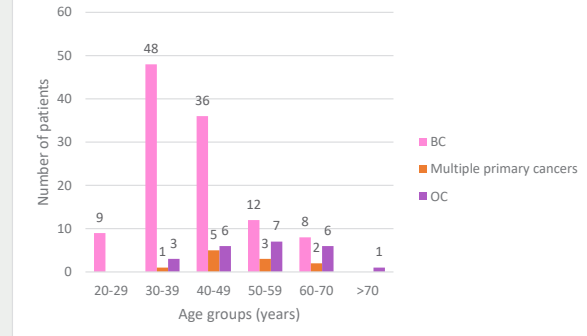


Figure 1. Distribution of patients by age at diagnosis and cancer type. BC: breast cancer, OC: ovarian cancer.

A family history of any cancer was reported in 119/147 (80.9%) patients. Among them, 69% (101/147) had at least one first- or second-degree relative affected by breast and/or ovarian cancer, with 22.8% (23/101) having three or more affected relatives. Twenty-eight patients (19%) had no family history of cancer, and were included in the study based on early-onset disease.

Table 1. Demographic and clinical characteristics of the study population

	Number of cases (%) (N=147)
Gender	
Female	145 (98.6%)
Male	2 (1.4%)
Age at diagnosis (years)	
Median	42
Range	24-74
<36*	32 (21.7%)
≤45	91 (61.9%)
Family history with	
BC and/or OC	101 (68.7%)
Any cancer	119 (80.9%)
Histological type	
BC (n=124)	IDC
	121 (97.6%)
	IDC and Lobular carcinoma
	2 (1.6%)
	DCIS
	1 (0.8%)
OC	HGSC
	25 (90%)
	LGSC
(n=28)	2 (7%)
	Clear cell carcinoma OC
	1 (3%)
BC molecular subtype (n=124)	
	Triple-negative
	35 (52.2%)
	Luminal
	32 (47.8%)
	Not available
	57 (45.9%)

BC: breast cancer; OC: ovarian cancer; IDC: Invasive Ductal Carcinoma; DCIS: ductal carcinoma in situ; HGSC: High-Grade Serous Carcinoma; LGSC: low-grade serous carcinoma; <36*: this threshold was selected to define early-onset primary breast cancer, which is frequently associated with a higher likelihood of hereditary predisposition, particularly involving BRCA1 and BRCA2 pathogenic or likely pathogenic variants.

Among the 124 breast cancer cases, the vast majority (99.1%, 123/124) were classified as invasive ductal carcinoma (IDC), including two cases with mixed ductal and lobular histology, whereas a single case of ductal carcinoma in situ (DCIS) was identified. Immunohistochemical data were available for 67 of the 124 patients (54%), revealing a predominance of the triple-negative subtype, observed in 52% (35/67) of cases.

Regarding ovarian cancers, 25 of the 28 cases were classified as high-grade serous carcinoma, while two were identified as low-grade serous carcinoma and one case with clear cell carcinoma.

Genetic Testing Results

A total of 33 patients (22.4%) were found to carry a germline heterozygous pathogenic or likely pathogenic variant, including 26 with isolated breast cancer, 4 with isolated ovarian cancer, 2 with both breast and ovarian cancers, and 1 with breast and pancreatic cancers. Among these 33 patients, 30 (90%) had a positive family history of cancer (Figure 2). All 29 breast cancers were invasive ductal carcinomas, including one with an additional lobular component, and 16 exhibited a triple-negative phenotype.

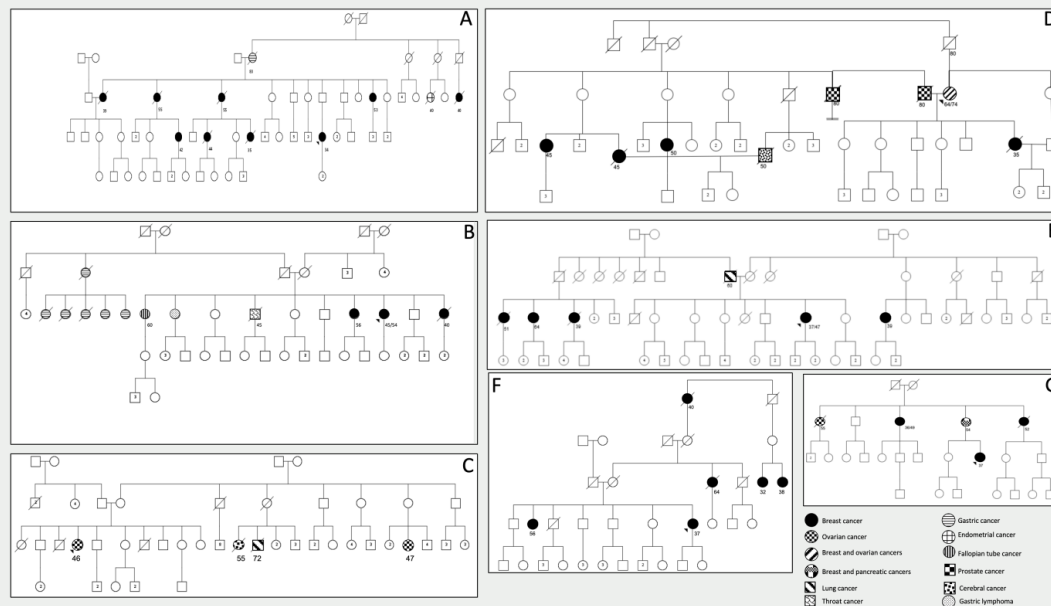


Figure 2. Pedigrees spanning three or more generations of index cases carrying pathogenic or likely pathogenic variants in ATM c.6115G>A (A), BRCA2 c.2451del (B), RAD51D c.694C>T (C), BRCA1 c.5266dup (D), BRCA1 c.211dup (E), BRCA2 c.7654dup (F), and BRCA2 c.1310_1313del (G). All ages are expressed in years.

Of the 147 patients included in this study, 91 underwent Sanger sequencing and 56 were analyzed using TGS. Germline pathogenic or likely pathogenic variants were detected in 18/91 (19.8%) of the Sanger-tested group and in 15/56 (26.8%) of the TGS-tested group. Variants were identified in BRCA1(NM_007294.4) (n=21), BRCA2(NM_000059.4) (n=8), ATM (n=3), and RAD51D (n=1). A total of 15 distinct pathogenic and likely pathogenic variants were identified across BRCA1 (n=8), BRCA2 (n=4), ATM (NM_000051.4) (n=2), and RAD51D (NM_002878.4) (n=1) (Figure 3). All these variants were protein-truncating variants, except for one missense variant in the ATM gene. Clinicopathological characteristics and genetic testing results for these 33 subjects are shown in Table 2

The mean age at diagnosis of breast and/or ovarian cancer was 38 years among BRCA1 variant carriers and 48 years among BRCA2 variant carriers.

Among the 21 BRCA1 variant carriers, 16 had isolated breast cancer, including 4 bilateral cases; 3 had isolated ovarian cancer, one of which was bilateral; and 2 had both breast and ovarian cancers. Nineteen of the 21 had a positive family history of cancer.

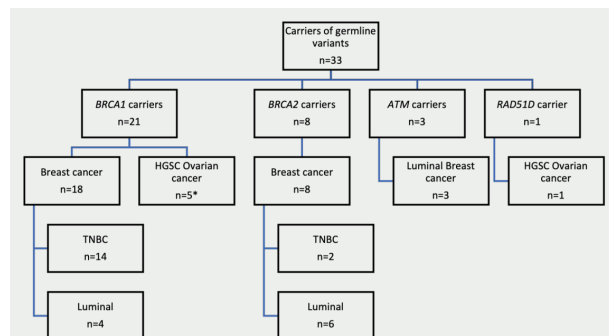


Figure 3. Summary of clinical and histopathological characteristics of the 33 patients carrying germline pathogenic or likely pathogenic variants.

HGSC: High-grade serous carcinoma; TNBC: Triple-negative breast cancer; 5*: two patients with concomitant breast and ovarian cancers

In our cohort, 16 out of 35 patients with TNBC were carriers of a germline pathogenic or likely pathogenic variant. Conversely, among the 18 breast cancer cases associated with a germline BRCA1 variant, 14 exhibited a triple-negative phenotype (Figure 3).

Table 2. Clinicopathological characteristics and spectrum of germline variants in the 33 patients

Patient ID	Sequencing Approach	Affected gene	Exon	Nucleotide change	Amino-acid change	Variant classification	Clinical phenotype	Age at diagnosis (years)	Histological type	Breast cancer molecular subtype	Family history of cancer
P4	Hot Spot BRCA1/2 Variants	BRCA1	10	c.1612C>T	p.(Gln538Ter)	Pathogenic	Bilateral BC	47	IDC	luminal B	Mother:OC
P5	Panel 21 genes	BRCA1	16	c.5030_5033del	p.(Thr1677IlefsTer2)	Pathogenic	BC	41	IDC	Triple-negative	Mother:BC
P13	Hot Spot BRCA1/2 Variants	BRCA1	4	c.211dup	p.(Arg71LysfsTer10)	Pathogenic	Bilateral BC	31	IDC	Triple-negative	Paternal aunt: BC
P17	Panel 21 genes	BRCA1	19	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic	BC	26	IDC	Triple-negative	Paternal cousin: BC
P18	Panel 2 genes	BRCA1	10	c.915T>A	p.(Cys305Ter)	Likely Pathogenic	Bilateral OC	64	HGSC	Not applicable	2 sisters: OC
P30	Panel 11 genes	BRCA2	24	c.9182del	p.(Leu3061Ter)	Likely Pathogenic	BC	44	IDC	luminal	3 paternal cousins, paternal aunt, and maternal cousin: BC; father and paternal uncle: gastric cancer; paternal uncle: prostatic cancer
P34	Hot Spot BRCA1/2 Variants	BRCA1	4	c.211dup	p.(Arg71LysfsTer10)	Pathogenic	BC	37	IDC	Triple-negative	Sister: BC
P36	Panel 21 genes	BRCA1	19	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic	BC	24	IDC	Triple-negative	2 paternal aunts: BC
P41	Panel 13 genes	BRCA1	19	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic	BC	31	IDC	Triple-negative	Paternal aunt: BC
P44	Panel 27 genes	RAD51D	8	c.694C>T	p.(Arg232Ter)	Pathogenic	OC	46	HGSC	Not applicable	Maternal cousin: OC
P48	Panel 27 genes	BRCA2	11	c.2451del	p.(Asn818IlefsTer7)	Likely Pathogenic	Bilateral BC	45/54	IDC	luminal A	Sister: Fallopian tube cancer, brother: gastric lymphoma; brother: throat cancer, 2 sisters: BC; 1 paternal aunt and 5 paternal cousins: gastric cancer
P55	Panel 27 genes	BRCA1	10	c.2418dup	p.(Ala807SerfsTer3)	Pathogenic	Bilateral BC	47/53	IDC	luminal B	Paternal aunt: BC; paternal aunt: endometrial cancer, paternal uncle: gastric cancer
P59	Panel 27 genes	ATM	5	c.492G>A	p.(Trp164Ter)	Pathogenic	BC	32	IDC	luminal	Maternal aunt and maternal cousin: BC
P64	Hot spot BRCA1/2 variants	BRCA1	19	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic	BC	40	IDC	Triple-negative	3 sisters: BC
P65	Hot spot BRCA1/2 variants	BRCA2	16	c.7654dup	p.(Ile2552AsnfsTer2)	Pathogenic	Bilateral BC and Pan cancer	40	IDC	Triple-negative	2 paternal aunts and 1 sister: BC
P66	Hot spot BRCA1/2 variants	BRCA1	19	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic	OC and BC	74/64	HGSC and luminal A IDC		Daughter and 3 paternal cousins: BC; Father: prostatic cancer
P73	Hot spot BRCA1/2 variants	BRCA2	10	c.1310_1313del	p.(Lys437IlefsTer22)	Pathogenic	BC	66	IDC	luminal	Mother and maternal cousin: BC
P74	Hot spot BRCA1/2 variants	BRCA1	19	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic	BC	25	IDC	Triple-negative	Paternal cousin: BC
P75	Hot spot BRCA1/2 variants	BRCA1	4	c.211dup	p.(Arg71LysfsTer10)	Pathogenic	OC	45	HGSC	Not applicable	Paternal cousin: BC
P95	Hot spot BRCA1/2 variants	BRCA2	10	c.1310_1313del	p.(Lys437IlefsTer22)	Pathogenic	BC	64	IDC	luminal	2 sisters and 2 nieces: BC
P96	Hot spot BRCA1/2 variants	BRCA1	19	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic	BC	25	IDC	Triple-negative	Paternal aunt: BC; maternal grandfather: gastric cancer
P97	Panel 70 genes	BRCA2	10	c.1310_1313del	p.(Lys437IlefsTer22)	Pathogenic	BC	37	IDC	luminal	Mother: BC and pancreatic cancer; 1 maternal aunt: OC; 2 maternal aunts: BC
P98	Hot spot BRCA1/2 variants	BRCA2	10	c.1310_1313del	p.(Lys437IlefsTer22)	Pathogenic	BC	55	IDC	Triple-negative	None
P100	Hot spot BRCA1/2 variants	BRCA1	19	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic	BC	30	IDC	Triple-negative	None
P101	Hot spot BRCA1/2 variants	BRCA1	16	c.5030_5033del	p.(Thr1677IlefsTer2)	Pathogenic	BC	38	IDC	Triple-negative	None
P102	Hot spot BRCA1/2 variants	BRCA1	16	c.5030_5033del	p.(Thr1677IlefsTer2)	Pathogenic	OC	42	HGSC	Not applicable	Sister and maternal aunt: BC; brother: cerebral cancer
P123	Hot spot BRCA1/2 variants	BRCA1	10	c.798_799del	p.(Ser267LysfsTer19)	Pathogenic	Bilateral OC and BC	48/53	HGSC and IDC	Triple-negative	Sister: BC and OC; mother: pancreatic cancer; 2 maternal cousins: BC and OC
P129	Hot spot BRCA1/2 variants	BRCA1	19	c.5266dup	p.(Gln1756ProfsTer74)	Pathogenic	BC	30	IDC	Triple-negative	Father and paternal uncle: lung cancer
P137	Panel 102 genes	ATM	5	c.492G>A	p.(Trp164Ter)	Pathogenic	Bilateral BC	47/49	IDC	luminal A	Sister, maternal aunt, maternal cousin: BC; maternal cousin: colon cancer; maternal uncle: leukemia
P138	Panel 27 genes	BRCA1	4	c.211dup	p.(Arg71LysfsTer10)	Pathogenic	Bilateral BC	37/47	IDC	luminal A	1 maternal cousin and 3 paternal cousins: BC; Father: lung cancer
P142	Hot spot BRCA1/2 variants	BRCA2	16	c.7654dup	p.(Ile2552AsnfsTer2)	Pathogenic	BC	37	IDC	luminal B	Sister, maternal aunt, maternal great grandmother, 2 maternal cousins: BC
P143	Panel 27 genes	BRCA1	15	c.4754del	p.(Pro1585GlnfsTer16)	Pathogenic	BC	36	IDC	Triple-negative	Mother and sister: BC; maternal aunt: colon cancer
P146	Panel 27 genes	ATM	42	c.6115G>A	p.(Glu2039Lys)	Likely Pathogenic	BC	34	IDC and Invasive lobular carcinoma	luminal B	4 paternal aunts, 3 paternal cousins: BC; paternal grandmother: gastric cancer; paternal cousin: endometrial cancer

BC: breast cancer; OC: ovarian cancer; IDC: Invasive Ductal Carcinoma; HGSC: High-Grade Serous Carcinoma; Pan cancer: Pancreatic cancer

All BRCA2 variant carriers were diagnosed with breast cancer, including two bilateral cases. One patient also presented with a synchronous pancreatic head carcinoma. A positive family history of cancer was documented in seven of the eight cases. All tumors were invasive ductal carcinomas (IDC), comprising six luminal and two TNBC subtypes.

Three recurrent BRCA1 variants were identified: c.211dup, c.5030_5033del and c.5266dup. The c.5266dup variant was the most frequent, identified in 9 unrelated patients with breast cancer, including 1 patient with both breast

and ovarian cancer. The c.211dup and c.5030_5033del variants were identified in 4 and 3 patients, respectively. Among the four BRCA2 variants, c.1310_1313del was detected in four unrelated patients with breast cancer. Two novel variants, c.9182del and c.2451del, not previously reported in public databases (LOVD, ClinVar, BRCA Exchange) or the literature, were also identified. These correspond to a nonsense and a frameshift variant, respectively, and were classified as likely pathogenic according to ACMG criteria (PVS1, PM2) (Figure 4).

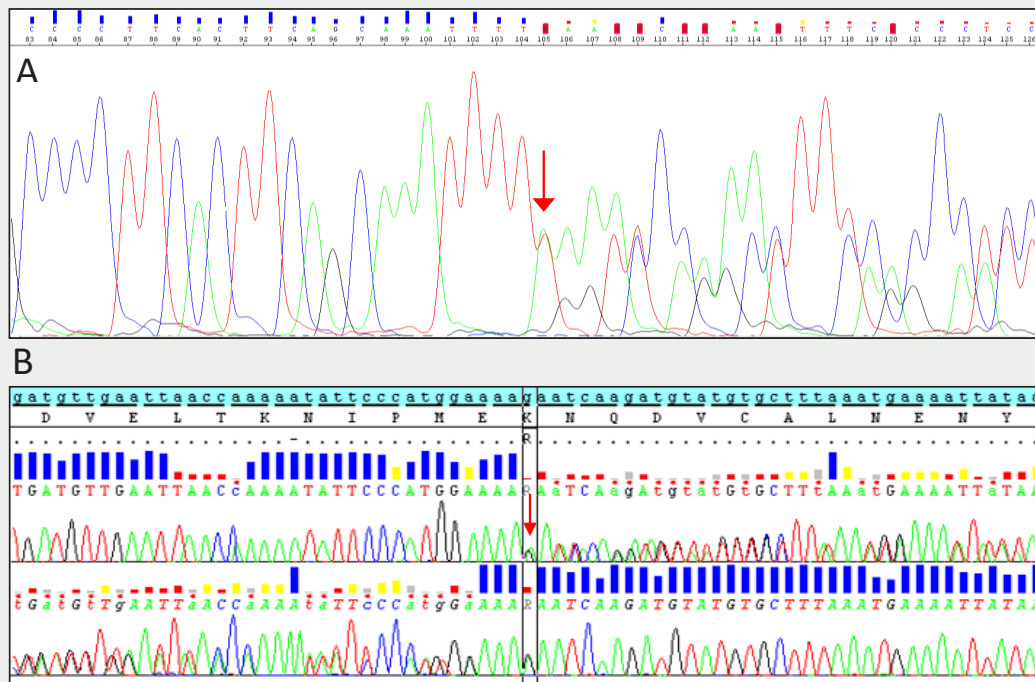


Figure 4. Partial Sanger sequencing chromatograms illustrating the two novel BRCA2 variants. A: c.9182del (Forward sequence) and B: c.2451del (Forward and Reverse sequences). The position of the variant is indicated by a red arrow.

The two ATM variants were identified in the heterozygous state in three female patients, all diagnosed with isolated estrogen receptor (ER)-positive breast cancer. Two of the three had at least three first- or second-degree relatives with breast cancer. In addition, a family history of colon cancer and leukemia was reported in one family, while another family presented with gastric and endometrial cancers (Table 2).

The RAD51D c.694C>T variant was identified in one patient diagnosed with high-grade serous ovarian cancer at 46 years of age, who had one second-degree relative with ovarian cancer.

All patients were informed of their germline testing results during a dedicated oncogenetic consultation. Carriers of variants were provided with appropriate genetic counseling, and presymptomatic testing was offered to at-risk relatives. Appropriate surveillance and preventive strategies were proposed to all unaffected carriers of pathogenic variants, and psychological support was systematically offered.

DISCUSSION

The median age at diagnosis in our study was 42 years, consistent with previously reported Tunisian cohorts, which range between 41 and 45 years (9–11,15,16). This is also comparable to findings from Algerian and Moroccan populations, in which the median age at diagnosis is approximately 38 years (17,18). In our cohort, 61.9% of patients were diagnosed at or before the age of 45, and 21.7% were diagnosed before age 36, which aligns with data from the literature (19–21). The mean age at diagnosis in our cohort (years) was lower than that reported in studies from developed countries (years) (22–24). This may be explained by the selection of younger women for genetic testing in North African countries.

The co-occurrence of breast and ovarian cancer in the same patient is highly suggestive of an underlying genetic predisposition (24). In our study, such an association was identified in five patients, two of whom carried a germline pathogenic variant.

In our cohort, a germline pathogenic or likely pathogenic variant was identified in 22.4% patients. In the literature, the detection rate of germline variants in breast and

ovarian cancers varies considerably depending on selection criteria and molecular techniques used. In Tunisian cohorts, the variant detection rate varies from 10.16% (25) to 44.68% (26). Most studies included patients with breast or ovarian cancer and a suggestive family history, usually defined as the presence of breast or ovarian cancer in first- or second-degree relatives. In some cases, family history was considered a mandatory criterion for genetic testing (9,11,15,19). The study by Jandoubi et al. (2024) reported the highest detection rate (44.68%) (26), which may be explained by selection bias, as it employed strict inclusion criteria highly suggestive of hereditary cancer (e.g., diagnosis at ≤ 36 years, multiple primary cancers, at least three affected relatives, etc.). That study included 94 patients for genetic analysis, with 79 tested using NGS technologies. More recently, most published studies on HBOC syndrome have adopted the broader criteria recommended by the NCCN, particularly regarding age at diagnosis (≤ 45 years) (8). In our study, we adopted similarly broad inclusion criteria, in line with NCCN recommendations.

In Algerian and Moroccan cohorts, the frequency of germline variants ranges from 5.7% to 25.64%. The low detection rate reported by Laraqui et al. (2013) may be explained by the predominant inclusion of sporadic breast cancer cases, as well as the relatively high age limit for breast cancer diagnosis (≤ 45 years) used in the study (27). In contrast, Tazite et al. (2012) mainly included patients with a family history of breast or ovarian cancer, which explains the higher detection rate reported (25.64%) (16). In our study, the use of gene panel sequencing yielded a higher diagnostic yield (26%) compared with targeted Sanger sequencing (19%), highlighting the improved sensitivity of gene panel sequencing in detecting pathogenic variants beyond the classical BRCA1/2 regions and in additional susceptibility genes (ATM, RAD51D). Overall, these findings underscore the growing utility of NGS-based testing in the implementation of precision oncology in Tunisia.

Eighty-seven percent of our patients with germline variants had a family history of breast or ovarian cancer, consistent with previous findings. Indeed, the prevalence of BRCA1/2 pathogenic variants is higher in individuals with a family history, early-onset breast cancer, or bilateral breast cancer (28–31). In our cohort, the mean age at diagnosis was lower in BRCA1 variant carriers (38 years) than in BRCA2 carriers (48 years), in line with studies showing that BRCA1 variants are more frequently associated with earlier onset of breast or ovarian cancer than BRCA2 variants (24).

The prevalence and spectrum of BRCA1/2 pathogenic and likely pathogenic variants observed in our cohort are consistent with findings reported in other Maghreb populations. In North Africa, BRCA1 variants are generally more frequent than BRCA2 variants, accounting for approximately 60–70% of all BRCA-related cases, as summarized by Cherbal et al. (2012) (32).

There is a strong association between BRCA1 alterations and the TNBC phenotype. Women carrying germline BRCA1 pathogenic variants are significantly more likely to develop TNBC than non-carriers. In line with previous

reports showing 14.8–37% of TNBC cases linked to BRCA1 variants (33,34), 45% of TNBC patients in our cohort harbored a germline pathogenic or likely pathogenic variant, mainly affecting BRCA1.

Our results confirm the predominance of BRCA1 variants, particularly c.5266dup, in agreement with data from other Tunisian cohorts and Maghreb populations (9–12,19,32). The c.5266dup variant, recognized as the most common pathogenic BRCA1 mutation worldwide, represents 20.4% of all BRCA1 variants identified in Tunisian cohorts. The recurrence of this variant in unrelated Tunisian families strongly suggests a potential founder effect. Further haplotype analysis would be necessary to elucidate its ancestral origin in Tunisia. However, some private or rare variants appear to be specific to the Tunisian population, underscoring its genetic heterogeneity. To our knowledge, the BRCA1:c.211dup variant has only been identified in Tunisian patients, mainly from the Cap Bon region. Such geographic restriction suggests a possible founder effect. Additional haplotype or genealogical studies would be useful to verify whether these cases share a common ancestral allele.

The c.1310_1313del variant in BRCA2 is considered a North African recurrent variant, with a high prevalence, representing 50% of all BRCA2 pathogenic variants in the region (35). It is located within the breast cancer cluster region (BCCR) of BRCA2. In our study, it was found in four patients with breast cancer, one of whom had a maternal aunt with ovarian cancer.

The c.9182del and c.2451del variants in BRCA2 are novel, having not been previously reported in databases or the literature. The c.2451del variant is also located in the BCCR of BRCA2 and was identified in a patient with bilateral breast cancer and a strong family history of breast, fallopian tube, and gastric cancers. Although c.9182del lies outside the BRCA2 cluster region, it was detected in a patient with unilateral breast cancer and a significant family history of breast and gastric cancers.

In our cohort, two ATM variants were detected in three patients with ER-positive breast cancer, consistent with previous findings (36) indicating that ATM variants are predominantly associated with ER-positive tumors. All three patients had a strong family history of cancer, including breast, colorectal cancers, and leukemia, although no pancreatic cancer was reported. In contrast, the literature indicates that ATM pathogenic variants confer an increased risk of pancreatic ($\times 4$) and prostate cancers ($\times 2.5$) (36). Despite its association with breast cancer susceptibility, ATM is considered a moderate-risk gene and is not yet included in current clinical management guidelines.

We also identified the RAD51D c.694C>T variant in a patient with serous ovarian cancer and a second-degree relative with ovarian cancer. The association between RAD51D pathogenic variants and ovarian cancer has been demonstrated in multiple studies (13,37).

Identifying pathogenic variants or likely pathogenic in hereditary breast and ovarian cancer genes is crucial for personalized patient management and familial cascade testing. Early detection of carriers allows for tailored

surveillance and risk-reducing strategies, ultimately improving cancer prevention and clinical outcomes. This molecular genetic testing facilitates targeted therapies and identifies at-risk relatives.

Our study is distinguished by several specific characteristics that set it apart from previously published Tunisian studies. To our knowledge, this is the largest cohort reported so far that integrates both Sanger sequencing and next-generation sequencing (NGS) for variant detection. In addition, most of our patients were selected in accordance with the NCCN guidelines for hereditary cancer testing. Our analysis identified previously unreported variants in BRCA2 gene, contributing to the expansion of the mutation spectrum in the North African population.

This study has several limitations. First, no pathogenic variant was identified in 114 of the 147 patients, despite strong personal and/or family histories suggestive of hereditary cancer predisposition. This may be partly explained by the molecular strategy used, as the initial screening based on recurrent Tunisian BRCA1/2 variants did not cover the entire coding regions of these genes. In such situations, comprehensive gene panel sequencing recommended to improve variant detection and refine risk assessment. In addition, large genomic rearrangements were not systematically assessed in the BRCA-negative cases. This limitation may have contributed to missing certain pathogenic variants, since large deletions or duplications in BRCA1/2 genes represent a known mechanism of hereditary breast and ovarian cancer predisposition and can be detected only through dedicated techniques such as MLPA(38). Furthermore, the relatively limited sample size reduces the statistical power of the study and restricts the extrapolation of our results to the broader Tunisian population. Another limitation of this study is its monocentric design, as all patients were recruited from a single tertiary genetics center. This may limit the representativeness of the cohort and the generalizability of the findings to the overall Tunisian population.

CONCLUSION

Our study provides valuable insight into the germline variant landscape of breast and ovarian cancer patients in Tunisia, emphasizing the significance of using broader selection criteria aligned with international guidelines. We confirmed that family history, early age at diagnosis, and tumor subtype remain strong predictors of germline variant detection. With a detection rate of 22.4%, our results are consistent with those of other regional studies and underscore the recurrence of BRCA1 c.5266dup and BRCA2 c.1310_1313del variants in Tunisian and selected North African cohorts. Notably, we identified two novel BRCA2 variants, expanding the variant spectrum in this population. The presence of ATM and RAD51D variants also supports the added value of gene panel sequencing beyond BRCA1/2. Our findings reinforce the need for comprehensive genetic screening strategies to optimize hereditary cancer detection and inform clinical

management in underserved populations. Identification of pathogenic or likely pathogenic variants enables familial cascade screening, facilitating the detection of asymptomatic carriers who may benefit from personalized surveillance, risk-reducing strategies, and appropriate genetic counseling.

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