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ORIGINAL ARTICLE

BRCA1 and *BRCA2* Germline Mutation Analysis in Hereditary Breast/Ovarian Cancer Families from the Aures Region (Eastern Algeria): First Report

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Abstract

Breast cancer is currently the leading cause of cancer morbidity and mortality among Algerian women. In this study, we aimed to investigate the mutation spectrum of *BRCA1* and *BRCA2* genes in hereditary breast/ovarian cancer (HBOC) families from the Aures region (eastern Algeria). High risk breast/ovarian cancer families were selected from overall 1162 consecutive patients collected from cancer registry of anticancer center of Batna. Breast cancers were diagnosed between 2011 and 2015. Recurrent mutations on *BRCA1* and *BRCA2* previously found in Algerian patients were screened using PCR-direct sequencing in 113 HBOC families. In addition, for the first time in Algeria, HBOC patients were analyzed by NGS using a cancer panel of 30 hereditary cancer genes or *BRCA1/2* genetic test. Six distinct deleterious mutations in *BRCA1* and *BRCA2* and a new VUS in *PALB2* were detected in ten patients. Two distinct *BRCA2* pathogenic variants c.1813dupA and c.8485C > T detected in two young female triple negative breast cancer (TNBC) patients, respectively, with a family history of male breast cancer, are reported here for the first time in Algerian population. Interestingly, we also detected a *BRCA* exon 15 deletion in two unrelated young female TNBC patients with strong family history of breast/ovarian cancer. Our study showed differences in the distribution of the mutation spectrum of *BRCA* genes between the Aures region and the north central region of Algeria. Our results will contribute in the implementation of genetic counseling and testing for patients and families at risk of hereditary breast and ovarian cancer.

Keywords Algerian women · Aures region · HBOC · BRCA1 · BRCA2 · Genetic testing · NGS · Cancer panel

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Abbreviation

Array–CGH	Array Comparative Genomic Hybridization
ER	Estrogen Receptor,
CISH	Chromogenic In Situ Hybridization,
HER2	Human Epidermal growth factor Receptor 2,
HBOC	Hereditary Breast and Ovarian Cancer
Ki67	Antigen Ki67
IDC	Invasive Ductal Carcinoma,
IHC	Immunohistochemistry
ILC	Invasive Lobular Carcinoma,
LGR	Large Genomic Rearrangement
MC	Mixed Carcinoma (invasive ductal and invasive lobular)
MLPA	Multiplex Ligation Probe Amplification
NGS	Next Generation Sequencing
PR	Progesterone Receptor

TNBC Triple Negative Breast Cancer
VUS Variant of Uncertain Significance

Introduction

Breast cancer is the most common cancer in Algerian women. Data demonstrated an increase in breast cancer incidence and mortality in Algeria, over the two last decades [1, 2]. To date, gene expression based molecular classification and immunochemistry studies have revealed that breast cancer is highly heterogeneous disease and has led to a molecular classification of breast carcinomas as luminal A, luminal B, HER2 and TNBC [3–5]. Since 2008, the routine ER/PR and HER2 immunohistochemical testing in public and private quality-controlled laboratories helps improve clinical, prognostic, and therapeutic outcomes in women with breast cancer in Algeria [6].

Germline mutations in the *BRCA1* and *BRCA2* genes result in hereditary breast and ovarian cancer syndrome (HBOC). Such mutations are associated with very elevated risks of breast cancer (lifetime risk approximately 70% by age 80 years) and ovarian cancer (45% for *BRCA1* and 20% for *BRCA2* mutation carriers) [7]. The prevalence of *BRCA* mutations and clinical characteristics associated with these mutations in Algerian population have not been well studied. To date, in Algeria like in most of emerging countries, there is a limited access in clinical genetic services mainly due to the costs, the availability of genetic testing and the lack of public insurance for coverage of genetic services [8–12]. The Aures is a large Berber-speaking region in the eastern part of Algeria, home of the Chaoui people who belong to the Berbers, the autochthonous habitants of North Africa [13]. One of the main objective of our present study is to implement affordable genetic testing in the Aures region.

In this first study, we aimed to analyze the mutational spectrum of *BRCA1* and *BRCA2* genes in HBOC patients from the Aures region. We screened for the prevalence of *BRCA1* and *BRCA2* recurrent mutations already detected in hereditary breast / ovarian cancer families from north central region of Algeria [14], in a cohort of 113 HBOC patients. In addition, for the first time in Algerian population, we screened HBOC patients by NGS using a cancer panel of 30 hereditary cancer genes or Color *BRCA1/2* genetic test (https://www.color.com).

Patients and Methods

Study Population

113 HBOC patients (109 women and 4 men) were selected from 1162 consecutive breast cancer patients at the anticancer center of Batna (eastern Algeria) during 2015–2016 (Table 1).

Table 1 Clinicopathological features of consecutive breast cancer patients of our study

Characteristics	Patients N (%) =1144
Overall mean age	48.5 year
Age	
<40y	274 (23.95)
40–49	408 (35.66)
50–59	269 (23.51)
60–69	116 (10.13)
≥70	77 (6.73)
Menopausal status	
Premenopausal	664 (58.04)
Postmenopausal	480 (41.96)
TNM stage (All cases *N* = 710)	
T1 N0 M0 (Stage I)	95 (10.83)
T2-T3 N0 M0 (Stage II)	174(19.84)
T4anyNM0 or AnyTN3M0 (Stage III)	505 (57.58)
AnyTNM1 (Stage IV)	87 (9.92)
Unclassified	16 (1.83)
Histological grade	
I	51 (4.45)
II	758 (62.25)
III	299 (26.13)
IV	36 (3.14)
Histological type	
IDC	1040 (90.90)
ILC	104 (9.10)
Ki −67 (All case *N* = 603)	
<20%	284 (47.09)
≥20%	319 (52.91)
Molecular subtypes	
Luminal A	541 (47.29)
Luminal B	251 (21.94)
HER2+	109 (9.52)
Triple negative	243 (21.24)
Family history of breast and /or ovarian cancer (All cases *N* = 1162)	
Yes	113 10.64
No	1049 89.36

Her2$^+$ ER$^-$, *PR*$^-$ Human Epidermal growth factor Receptor 2$^+$, *IDC* Invasive Ductal Carcinoma, *ILC* Invasive Lobular Carcinoma, *N* number of cases, *TNBC* Triple-negative breast cancer

The cancer registry of the anticancer center of Batna covered an area of 7 provinces among 48 of Algeria (Fig. 1).

Patient and tumor information included: age at diagnosis, menopausal status, receptor status, Ki67 index, TNM stage, histological type, tumor histological grade, breast feeding, parity, and family history of breast/ovarian cancer. The clinical stage of breast cancer was determined according to the 7th edition of the TNM manual [15]: T1 N0 M0 (Stage I), T2-

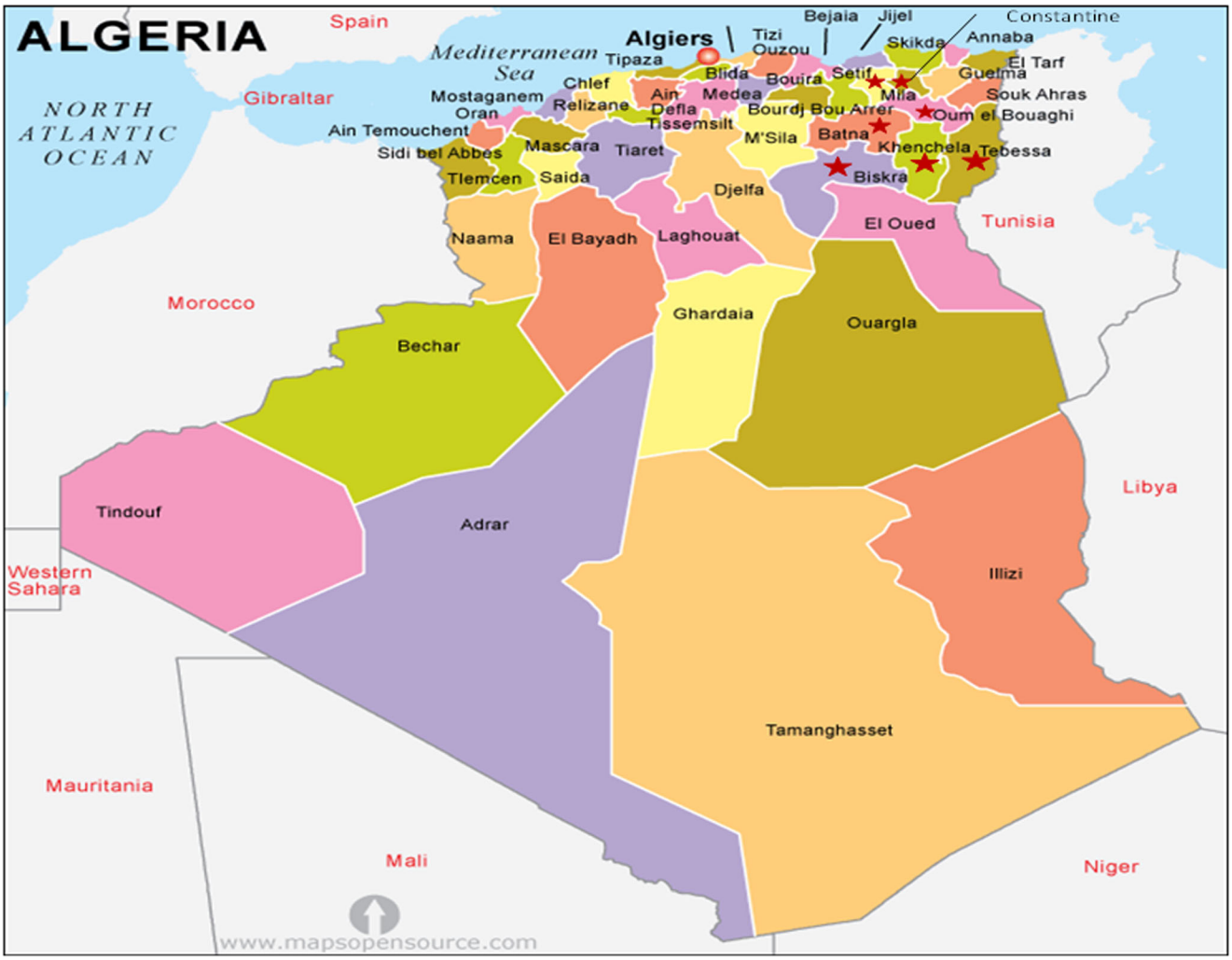

Fig. 1 Map showing the 7 Algerian provinces of the Aures region (indicated by star symbol) covered by the cancer registry of Anti-Cancer Center of Batna (eastern Algeria) where the HBOC patients included in our study were diagnosed and treated

T3 N0 M0 (Stage II), T4anyNM0 or AnyTN3M0 (Stage III) and AnyTNM1 (Stage IV). The patients and their families included in the present study were referred through the Anti-Cancer Center of Batna (Aures region, eastern Algeria). The following selection criteria of patients and affected family members were used:

(a) women with a history of two or more relatives on the same side of the family with breast and/or ovarian cancer and male relatives with prostate cancer along three generations at any age, (b) TNBC phenotype, (c) two or more cases of breast and/or ovarian cancer in first degree relatives, (d) cases of bilateral breast cancer, (e) breast or ovarian cancer before the age of 40, (f) male relatives with breast cancer. Pedigree information was required to encompass at least three generations. Clinical characteristics of study population are presented in Table 3. Prior collecting blood, all selected patients and relatives were informed about the objectives of our study and their DNA samples would be analyzed for mutations in genes associated with hereditary breast/ovarian cancer. All patients and the relatives signed informed consent and ethical approval was obtained from appropriate institutions.

Immunohistochemistry

Tumor expression for hormone receptors (ER and PR), HER2 and Ki67 index was evaluated by breast cancer pathologists of the main Algerian health public and private quality-controlled laboratories. Immunohistochemistry staining of hormone receptors (ER and PR) was performed by using the Kit Envision+™ (Dako). HER2 expression was tested by immunohistochemistry by using HercepTest™ kit (code K5204, Dako). Ki67 expression was tested by immunohistochemistry by using EnVision™ FLEX kit (K8000, Dako). The ER assay clone used was 1D5, the PgR assay clone was PgR636 and the detection system was a polymer. The Ki67 assay clone was MIB-1. ER and PR expression was interpreted as positive if at least 1% immunostained tumor nuclei were detected in the sample, according to ASCO/CAP recommendations for immunohistochemical testing of

hormone receptors in breast cancer [16]. HER2 was considered positive if graded 3+ on immunohistochemistry performed according to ASCO guidelines [17]. All other grades (0 to 2+) were considered negative unless chromogenic in situ hybridization (CISH) of 2+ cases confirmed increased gene copy number (Dako DuoCISH ™, code SK108). Ki67 expression was classified as low (<20%) or high (≥20%).

Breast Cancer Subtypes Definitions

The tumors were classified into molecular subtypes and according to immunohistochemical expression profiles of ER, PR and HER2. Breast cancer subtypes definitions were as follow: Luminal A (ER+ and/or PR+, HER2−), Luminal B (ER+ and/or PR+, HER2+), TNBC (ER−, PR−, HER2−), HER2+ (ER−,PR−, HER2+). Molecular subtypes were correlated with the clinicopathological characteristics of the tumors.

DNA Isolation

Genomic DNA was extracted from peripheral blood lymphocytes using Promega Wizard Genomic DNA Purification Kit (Promega, Madison, MI, USA) (Cat. # A1120) and in accordance with the manufacturer's protocols.

Mutation Analysis

BRCA1 and *BRCA2* genes were screened by PCR-Sanger direct sequencing in a cohort of 113 patients (109 women and 4 men) with hereditary breast and/or ovarian cancer, including all exons where a common mutation was previously found in Algerian population, *BRCA1* exons (3, 4 and 10): c.83_84delTG, c.181 T > G, c.798_799delTT and c.2125_2156inA; *BRCA2* exon (10): c.1310_1313delAAGA. PCR and Sanger sequencing were performed as described elsewhere [18].

NGS Analysis

Twelve HBOC patients were analyzed by Color Genomics using a cancer panel of 30 hereditary cancer genes or Color *BRCA1/2* genetic test (Color genomics, Burlingame, San Francisco, USA, https://www.color.com).

In Silico Analysis

To identify no synonymous amino acid changes likely to disrupt *PALB2* gene function, we used six missense prediction tools: PolyPhen2, SIFT, AGVGD, Mutation Taster, Mutation Assessor and Provean.

Nomenclature and Variant Analysis

All nucleotide numbers refer to the wild-type cDNA human sequence of *BRCA1* (NM_007294.3), *BRCA2* (NM_000059.3), *PALB2* (NM_024675.3) as reported in the GenBank database. The description of nucleotide sequence variants is in accordance with HGVS (Human Genome Variation Society) nomenclature (www.hgvs.org/mutnomen). The HGVS approved systematic nomenclature follows the rule where the nucleotide +1 is the A of the ATG translation initiation codon.

Statistical Analysis

Differences between breast cancer subtypes with regard to clinicopathologic parameters were examined using Chi-square (χ^2) test. A P value of <0.05 was considered as statistical significance. IBM SPSS statistics V20 was used for data analysis.

Results

113 patients (109 women and 4 men) with HBOC syndrome selected from 1162 consecutive breast cancer patients were screened for *BRCA1* and *BRCA2* including all exons where a mutation was previously found in Algerian population (Tables 1, 2, 3 and 4 and Figs. 1 and 2 summarize the results). Twelve HBOC patients were analyzed by NGS using a cancer panel of 30 hereditary cancer genes or *BRCA1/2* genetic test. The median age at diagnosis of 1144 patients with breast cancer was 48.5 years (age ranged from 21 to 84 years) (Table 2). Data for receptor status were available for 1144 cases. Luminal A was the most common subtype (47.29%) followed by luminal B (21.94%), TNBC (21.24%), and Her2+ (9.52%) (Table 2). There was a significant difference in the distribution of age at diagnosis among all breast cancer subtypes ($P = 0.04$) (Table 2). The four breast cancer subtypes were more likely to be diagnosed in young women under age of 50 years. For our 109 women with HBOC syndrome, the median age at diagnosis of patients with breast cancer or ovarian cancer was 44.03 years (Table 3). Data for receptor status were available for 96 cases. Luminal A was the most common subtype (50%) followed by TNBC (24%), luminal B (18%), and Her2+ (4%).The four breast cancer subtypes were more likely to be diagnosed in young women under age of 50 years. Data for histological tumor type showed that invasive ductal carcinoma (IDC) was the commonest histological type in all breast cancer subtypes. We noted that tumors with histological grade 2 and 3 and TNM stage II and III were higher in patients for the four breast cancer subtypes (Table 3).

Table 2 Comparison of Biological, clinical and tumor characteristics by molecular subtypes of consecutive breast cancer patients of our study

Characteristics	Luminal A N (%)	Luminal B N (%)	HER2+ N (%)	TNBC N (%)	*P* value
Total (%)	541 (47.29)	251 (21.94)	109 (9.52)	243 (21.24)	
Age at diagnosis					
<40 y	118 (21.81)	55 (21.91)	30 (27.52)	71 (29.21)	<0.04
40–49	199 (36.78)	102 (40.63)	31 (28.44)	76 (31.27)	
50–59	123 (22.73)	67 (26.69)	28 (25.68)	51 (20.98)	
60–69	58 (10.72)	20 (7.96)	13 (11.92)	25 (10.28)	
≥70	43 (7.94)	7 (2.78)	7 (6.42)	20 (8.23)	
Menopausal status					
Premenopausal	312 (57.67)	148 (58.96)	65 (59.63)	139 (57.2)	0.95
Postmenopausal	229(42.32)	103 (41.03)	44 (40.36)	104 (42.79)	
TNM stage (All cases *N* = 710)					
T1 N0 M0 (Stage I)	20 (6.1)	18 (9.8)	7 (11.66)	6 (4.25)	0.23
T2-T3 N0 M0 (Stage II)	48 (14.67)	17 (9.34)	8 (13.33)	24 (17.02)	
T4anyNM0orAnyTN3M0 (Stage III)	146 (44.64)	88 (48.35)	29 (48.33)	69 (48.93)	
AnyTNM1 (Stage IV)	113 (34.55)	59 (32.41)	16 (26.66)	42 (29.78)	
Histological grade					
I	24 (4.43)	12 (4.7)	6 (5.5)	9 (3.7)	0.08
II	382 (70.6)	164 (65.33)	65 (59.63)	147 (60.49)	
III	119 (21.99)	67 (26.69)	32 (29.35)	81 (33.33)	
IV	16 (2.95)	8 (3.18)	6 (5.5)	6 (2.46)	
Histological type					
IDC	485 (89.64)	226 (90.03)	102(93.57)	227 (93.41)	0.25
ILC	56 (10.36)	25 (9.97)	7 (6.43)	16 (6.59)	

Her2+ ER^-, PR^-, Human Epidermal growth factor Receptor 2+, *IDC* Invasive Ductal Carcinoma, *ILC* Invasive Lobular Carcinoma, *N* number of cases, *TNBC* Triple-negative breast cancer

BRCA1 and BRCA2 Mutations Analysis in Hereditary Breast /Ovarian Cancer Patients Using PCR-Sanger Sequencing

The analysis of DNA samples by PCR-Sanger sequencing for recurrent pathogenic variants already detected in *BRCA1* in Algerian population in our cohort of 113 patients with hereditary breast/ ovarian cancer syndrome revealed that one index case with early ovarian cancer carries the *BRCA1* c.5332 + 1G > A pathogenic variant located in exon 20 (Table 4 and Fig. 2a). In addition, the maternal cousin of the index case and her daughter diagnosed with breast cancer at age 39 and 56, respectively, have been also tested positive for the pathogenic variant *BRCA1* c.5332 + 1G > A (Table 4 and Fig. 2a). The analysis of *BRCA2* identified the rare pathogenic variant c.8940delA/p.Glu2981Lysfs*7 located in exon 22 in breast/ovarian cancer patient (Table 4 and Fig. 2b). The recurrent mutations in *BRCA1* and *BRCA2* genes already reported in HBOC patients from North central region of Algeria have not been detected in our patients.

NGS Analysis

The analysis of two HBOC patients with TNBC phenotype using cancer panel of 30 hereditary cancer genes (Color Genomics) revealed a pathogenic mutation in *BRCA1* gene, a deletion of exon 15: c.4676-?_4986 +?del/p.? in HBOC patient diagnosed with breast cancer at age 27 and ovarian cancer at age 50, respectively (Table 4 and Fig. 2c). The NGS analysis detected also a new VUS on *PALB2* exon 3 c.142A > T/p.Ile48Phe in early onset HBOC patient (Table 4 and Fig. 2d). The analysis of 10 HBOC patients using Color *BRCA1/2* genetic test identified 4 pathogenic variants in *BRCA1* and *BRCA2* genes, respectively: a pathogenic mutation in *BRCA1* gene, a deletion of exon 15: c.4676-?_4986 +?del/p.? in TNBC patient diagnosed with breast cancer at age 41 (Table 4 and Fig. 2g). Moreover, the *BRCA2* frameshift mutation c.1873dupA/p.Ile605Asnfs*11 located in exon 10 has been detected in young female TNBC patient diagnosed at age 33 years (Table 4 and Fig. 2e). We identified the *BRCA2* mutation c.7654dupA/p.Ile2552Asnfs*2 located in exon 16 in young female TNBC patient diagnosed at age

Table 3 Clinicopathological characteristics of 113 HBOC patients screened for *BRCA1* and *BRCA2* germline mutation

Clinicopathological characteristics		Number of cases N (%)
Mean age	Years: 44,03	$N = 109$
Age at diagnosis	<40	40 (36.69)
	40–49	57(52.29)
	50–59	9 (8.2)
	≥60	3 (2.75)
Breast cancer subtypes (All cases $N = 100$)	Luminal A	50 (50)
	Luminal B	18 (18)
	Her2+	4 (4)
	TNBC	24 (24)
	Unclassified	4 (4)
Menopausal status (All cases $N = 109$)	Premenopausal	66 (60.55)
	Postmenopausal	43 (39.45)
TNM Stage (All cases $N = 100$)	Stage I	18 (18)
	Stage II	34 (34)
	Stage III	25 (25)
	Stage IV	11 (11)
	Unclassified	12 (12)
Histological grade status (All cases $N = 109$)	I	2 (2)
	II	63 (57.79)
	III	42 (38.53)
	IV	2 (1.8)
Histological Type (All cases $N = 100$)	IDC	86 (86)
	ILC	5 (5)
	MC	1 (1)
	Other	8 (8)
Bilateral breast cancer	12	
Male Breast cancer	4	
Hereditary Breast and Ovarian Cancer	12	
Ovarian cancer	9	
Ki-67 status (All cases $N = 100$)	<20	35 (35)
	≥20	54 (54)
	Unknown	11 (11)
Family history with breast and /or ovarian cancer	Yes	113
Age of menarche	Years	13,33
Marital Status (all cases 109)	Married	92 (84.4)
	Single	17 (15.6)
Oral contraception (All cases $N = 109$)	Yes	64 (58.71)
	No	45 (41.29)
Breast feeding(All cases $N = 109$)	Yes	69 (63.3)
	No	40 (36.7)
Parity (All cases $N = 109$)	Yes	95 (87.15)
	No	14 (12.4)

30 years (Table 4 Fig. 2h) and the *BRCA2* non sense mutation c.8485C > T/p.Gln2829* located in exon 19 in young female TNBC patient with bilateral breast cancer diagnosed at age 40 years (Table 4 and Fig. 2f).

Discussion

We have undertaken the analysis of *BRCA1* and *BRCA2* germline mutations in HBOC families from Aures region. In

Table 4 Clinicopathological characteristics of HBOC patients with deleterious *BRCA* mutations and VUS in *PALB 2*

Index case ID	Affected Gene	Exon	Nucleotide change	Amino acid change	Mutation Type	Dx	Y	Clinical phenotype	Grade	TNM	Ki67 (%)	Family history of cancer
0515316 (Pedigree C)	*BRCA1*	15	c.4676-?_4986+? del/p.?	–	Deletion of Exon 15	27 and 50	54	BC–TNBC- OC	III	Stage II	20	Sister 1, BC dx 45y; Sister 2, BC, dx 48y; Sister 3, OC dx? Y55 MC: CCR dx?, dcd 70y
055391 (Pedigree G)	*BRCA1*	15	c.4676-?_4986+? del/p.?	–	Deletion of Exon 15	30	31	BC-TNBC	II	Stage II	20	Sister OC dx 42y, Dcd 44y, MGP OC dx?, Dcd 63y, S1MGP OC dx? Dcd 65y, S2MGP Oc dx? Dcd70y
051511040 (Pedigree A)	*BRCA1*	20	c.5332 + 1G > A	p.Phe1761Asnfs* 14	Skipping of exon 20	40	40	OC	NA	NA	NA	M: OC dx51, dcd 56y **MC1M****: **BC dx56, Y57** MC2M: BC dx51, dcd 54y **DMC1****: **BC dx39, Y 39** DMC2: OC dx?, Y 26
0513612 (Pedigree E)	*BRCA2*	10	c.1813dupA	p.Ile605Asnfs*11	FS	33	36	BC-TNBC	III	Stage III	10	MGP: MBC dx55y, dcd70y HBMGP: MBC dx?, dcd71y MU:PC dx?; MA1: BC dx 45, dcd 48y; Sister 2: BC dx 34y, Y 35
0513493 (Pedigree H)	*BRCA2*	16	c.7654dupA	p.Ile2552Asnfs*2	FS	33	36	BC-TNBC	II	Stage II	70	Sister BC dx 36y, Dcd 40y, D1PA1 BBC dx 54y, Y 57, D2PA CCR dx 53y, Y 57, PA1 BC dx 43y, Dcd 45y, PA2 OC dx 36, Dcd 40y, Father LC dx 49y, Dcd 50y, MGP LC dx 59y, Dcd 70y
0510384 (Pedigree F)	*BRCA2*	19	c.8485C > T	p.Gln2829*	NS	40	51	BBC-TNBC	III	Stage III	30	PGP: MBC dx?, dcd 70y; PA: BC dx?, dcd 40y; F: LC dx? dcd?; MA1: OC dx 53y, Y56, MA2: BC dx38y, dcd 40y; MA3 BC dx35, dcd36y
05151410 (Pedigree B)	*BRCA2*	22	c.8940delA	p.Glu2981Lysfs*7	FS	27?	39	BC-Luminal A-OC	NA	NA	NA	Sister: BC dx 41y, OC dx?, Y43,MA1: BC dx37, dcd 40y MA2: BC dx 40, dcd 60 y MA3: OC dx 38, dcd 40y MA4: BC dx 38, dcd 40y PGP: PC dx 64 y, dcd 65y
0515751 (Pedigree D)	*PALB2*	3	c.142A > T	p.Ile48Phe	MS	30?	30	BC-TNBC-OC	II	NA	80	MGM: OC dx 66, dcd 70 y SMGM: OC dx 60, dcd 70 y Father: GC dx 50y, dcd 54y

BBC Bilateral Breast Cancer, *BC* breast cancer, *CCR* colorectal cancer, *dcd* deceased, *DMC* Daughter of Maternal Cousin, *DPA* Daughter of Paternal Aunt, *dx* age at diagnosis, *F* Father, *FS* frame shift, *GC* gastric cancer, *HBMGP* Half Brother of Maternal Grand Pa, *M* Mother, *MA* Maternal Aunt, *MC* Maternal Cousin, *MBC* Male Breast Cancer, *MCM* Maternal Cousin of the Mother, *MGM* Maternal Grand Ma, *MGP* Maternal Grand Pa, *MS* missense, *MU* Maternal Uncle, *NA* no data available, *NS* non sense, *OC* ovarian cancer, *S1MGP* Sister of Maternal Grand Pa, *TNBC* Triple Negative Breast Cancer, *SMGM* Sister of Maternal Grand Ma, *PA* Paternal Aunt, *PC* prostate cancer, *PGP* Paternal Grand Pa, *Y* current age, **: Relatives of patient 051511040, carriers of the *BRCA1* mutation c.5332 + 1G > A

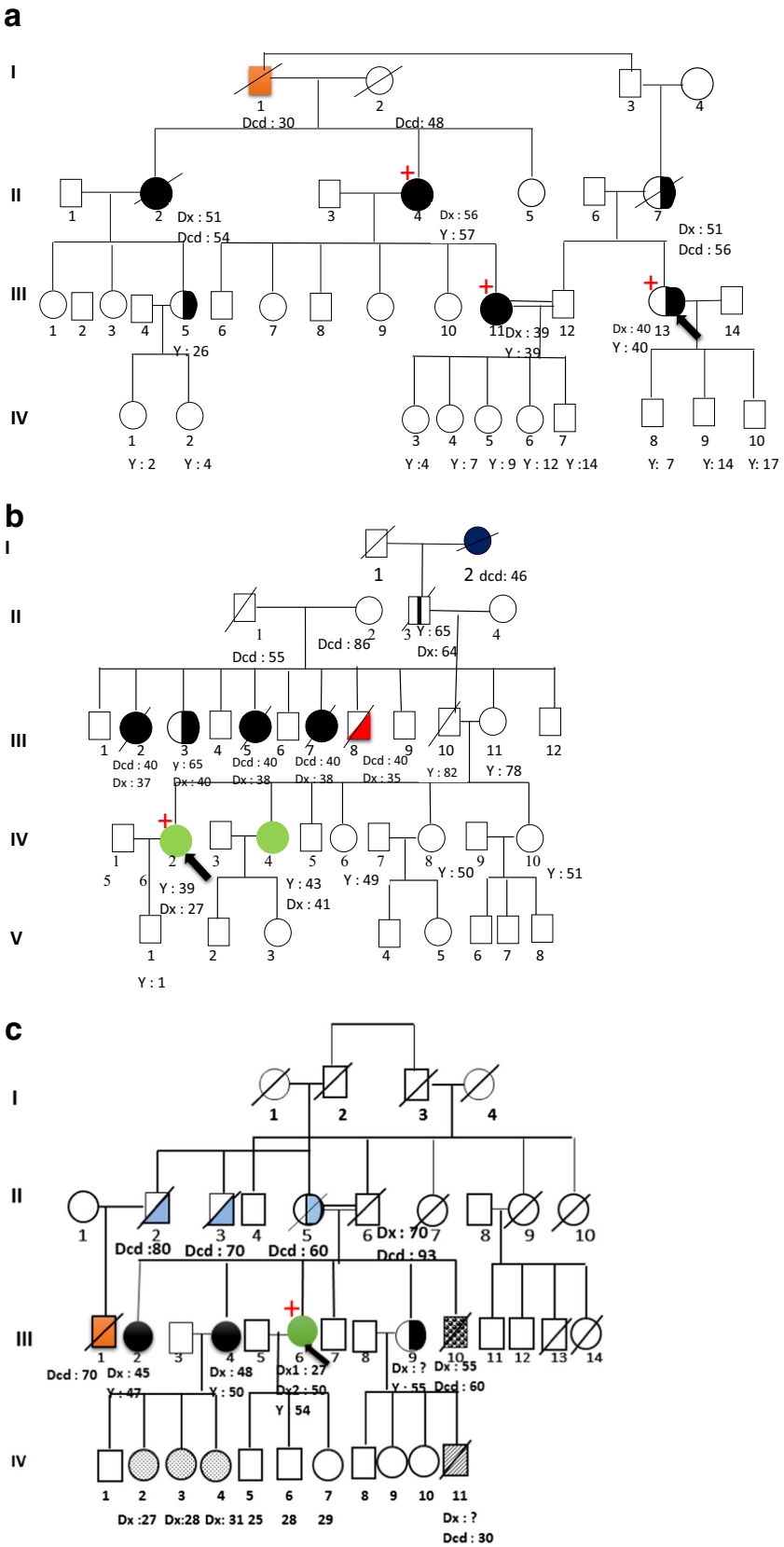

Fig. 2 **a**–**h** Pedigrees of the families with deleterious *BRCA* mutations and VUS in *PALB 2*. (+) mutation carrier

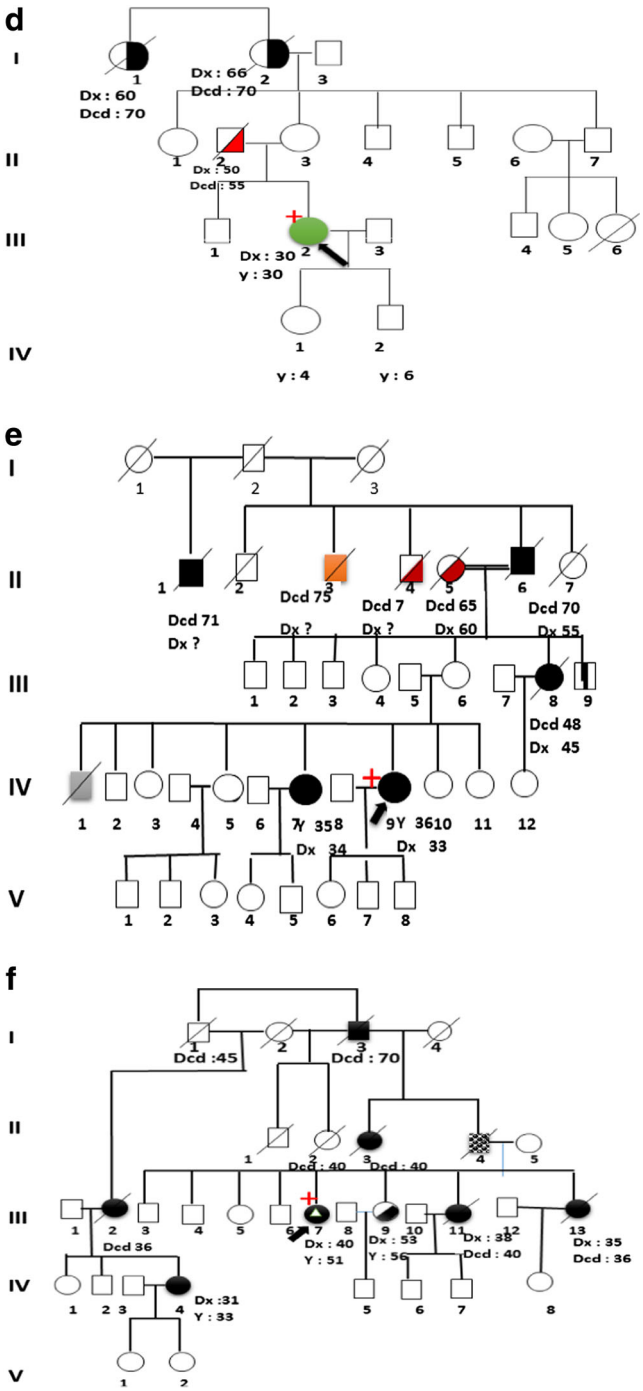

Fig. 2 (continued)

addition, for the first time in Algeria, HBOC patients were analyzed by NGS using a cancer panel of 30 hereditary cancer genes or *BRCA1/2* genetic test.

This current study has identified two pathogenic variants in *BRCA1,* four deleterious mutations in *BRCA2* and one new VUS in *PALB2*, respectively. The *BRCA1* pathogenic variant c.5332 + 1G > A located in exon 20, reported two times in BRCA Share database and 6 times in ClinVar database, has been identified in a patient with early ovarian cancer and 2 s relatives diagnosed with breast cancer. Interestingly, the *BRCA1* exon 15 deletion: c.4676-?_4986 +?del/p.?, reported one time in BRCA Share database, has been detected here in two unrelated TNBC patients with strong family history of hereditary breast/ovarian cancer. The *BRCA1* exon 15 deletion detected in our study has been already reported in two German families with hereditary breast cancer [19]. Investigation aimed at determining the genomic breakpoints of the *BRCA1* exon 15 deletion detected in our two HBOC patients are ongoing. Moreover, we identified the rare deleterious *BRCA2* mutation c.8940delA/p.Glu2981LysfsX7 located in exon 22, reported one time in BRCA Share database and ClinVar database, in a patient with strong family history of breast/ovarian cancer. Interestingly, for the first time in Algerian population, we detected two distinct *BRCA2* pathogenic variants c.1813dupA and c.8485C > T in two young female TNBC patients, respectively, with a family history of male breast cancer. The maternal and paternal Grand Pa of the two probands, respectively, who both developed male breast cancer are assumed to be the most likely candidates to have the pathogenic variant in the *BRCA2* gene. These results are in agreement with previous studies, which showed that the most frequent genetic causes of male breast cancers with family history are *BRCA2* mutations [20, 21]. In addition, we note that the *BRCA2* pathogenic mutation c.1813dupA described for the first time in Algerian population is a founder mutation in Germany [22]. Interestingly, our study showed that the phenotype TNBC in six (06) HBOC patients, carriers of *BRCA1* (2), *BRCA2* (3) pathogenic variants and a VUS in *PALB2* gene (1), is associated with younger age, higher histological grade, a positive family history of breast and/or ovarian cancer. These findings were consistent with previous studies that screened for *BRCA1* and *BRCA2* germline mutations in TNBC patients from various populations in different countries [9, 23–30]. We also identified a new VUS c.142A > T/p.I48F in *PALB2* in a patient with early breast/ovarian cancer. In addition, in Silico analysis using the six following in Silico tools: PolyPhen2, SIFT, AGVD, MutationTaster, MutationAssessor and Provean of the new VUS I148F which is located in exon 3 of *PALB2* in the BRCA1 interaction region, showed conflicting interpretations. PolyPhen2 and SIFT have predicted the new VUS I148F as possibly damaging and affect protein function, respectively. AGVD, MutationTaster, MutationAssessor and Provean have predicted this new variant as benign, polymorphism, medium and neutral, respectively. Further testing of family members of our index case carrier of *PALB2* VUS I148F, and studies for the co-segregation of the variant with the disease in the family and functional studies are needed for the

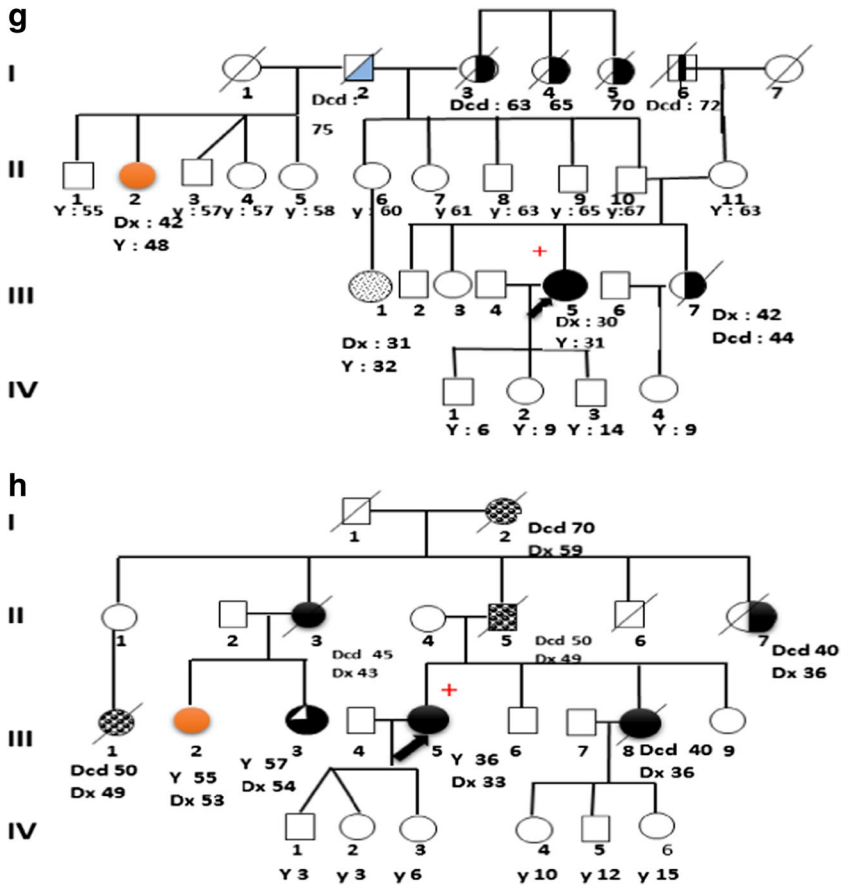

Fig. 2 (continued)

classification of this VUS. Moreover, the six germline mutations detected in *BRCA* genes in women from the Aures region have not been identified in previous studies that screened *BRCA1* and *BRCA2* germline mutations in patients from north central region of Algeria [14, 18, 31]. As Algeria is a country of continental dimension with admixed population of Berber descent with Sub-Saharan African, European and Middle East elements [13], we could not rule out the fact that we have geographic differences in the distribution of the mutation spectrum of *BRCA1* and *BRCA2* in Algeria. Indeed, we note that the pathogenic mutations described in this report differ from that seen in Moroccan and Tunisian populations despite the fact that they share the same genetic background with Algerian population [32, 33]. These findings could suggest a large *BRCA1* and *BRCA2* mutational spectrum in Maghrebian populations. To date, such results about the geographic differences of the distribution of the mutational spectrum of *BRCA* genes have been already reported in various populations from different countries [34–36].

There are potential limitations of our current study, which should be considered. Sample size of the included study population is small. Although in our present study, we have screened for *BRCA1* and *BRCA2* recurrent mutations which are population-specific and occur with high frequency in breast and/or ovarian cancer patients with a family history of cancer, a limitation of our current study is several exons of *BRCA1* and *BRCA2* gene have not been sequenced. That means the frequency of *BRCA* genes mutations in our HBOC patients from Aures region is underestimated. We are also aware that *BRCA1* and *BRCA2* mutations analysis was performed mostly by PCR- Sanger sequencing and we did not screen for LGR (large genomic rearrangement, large exonic deletions or duplications) by using MLPA or Array-CGH. Screening of all coding exons of *BRCA* genes including flanking intronic regions in a large series of hereditary breast and/or ovarian cancer patients by using NGS analysis of cancer panel of hereditary cancer genes will allow the assessing of the prevalence of *BRCA1* and *BRCA2* mutations in women from the Aures region.

One of the strengths of the present study is this report was the first genetic testing for *BRCA1* and *BRCA2* germline mutations in HBOC patients from the Aures region using PCR-direct Sanger sequencing and NGS with 30 hereditary cancer genes panel or *BRCA1/2* genetic test. The mutational profile knowledge of *BRCA* genes in HBOC families from the Aures

region will contribute to the implementation of cost-effective strategies for the prevention, identification and treatment of hereditary breast and ovarian cancer.

Conclusions

Our data provide insight into the genetics of HBOC syndrome in the Aures region (eastern Algeria). Our study showed differences in the distribution of the mutation spectrum of *BRCA1* and *BRCA2* between the Aures region and the north central region of Algeria. Further prospective studies should reaffirm our findings. The screening of *BRCA1* and *BRCA2* genes in large cohort of patients will help to know about the frequency, the spectrum, the contribution and the prevalence of *BRCA* genes mutations. These studies will help to implement affordable genetic testing and to improve the clinical management and better risk assessment of hereditary breast and ovarian cancer.

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Compliance with Ethical Standards

Conflicts of Interest The authors declare non conflict of interest.

Ethical Approval All patients tested for *BRCA1* and *BRCA2* germline mutations and screened by PCR-direct sequencing and NGS analysis, respectively, signed written informed consent. The study was approved by the institutional review boards and ethical approval was obtained from appropriate institutions (USTHB, CAC Batna, FNRSDT and CNEPRU D01N01UN160420130007, 113 participants, start date: 4/16/2015, end date: 9/28/2016).

Informed Consent Informed consent was obtained from all individual participants included in the study.

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