Screening of BRCA1 and BRCA2 germline mutations in unselected triple-negative breast cancer patients: A series from north of Morocco

Mohammed Mansouri1,2 | Touria Derkaoui1 | Joaira Bakkach1 | Ali Loudiyi2 | Naima Ghailani Nourouti1 | Amina Barakat1 | Jaime Martínez de Villarreal3 | Carlos Cortijo Bringas3 | Mohcine Bennani Mechita1

1Laboratory of Biomedical Genomics and Oncogenetics, Faculty of Sciences and Techniques of Tangier, Abdelmalek Essaâdi University, Tetouan, Morocco
2Oncology Clinic Al Amal of Tangier, Tangier, Morocco
3Genetracer Biotech Laboratory, Santander, Spain

Correspondence
Touria Derkaoui, Laboratory of Biomedical Genomics and Oncogenetics, Faculty of Sciences and Techniques of Tangier, Abdelmalek Essaâdi University, Tetouan, Morocco.
Email: derkaoui.touria.22@gmail.com

Abstract
Background: Triple-negative breast cancer (TNBC) is strongly associated with BRCA1 and BRCA2 germline pathogenic variants. Revised NCCN guidelines recommend that TNBC patients ≤60 years of age should be referred for genetic counseling and consideration of BRCA1/2 testing. The aim of the present study is to characterize germline BRCA1 and BRCA2 variants in a series of TNBC patients from the north of Morocco.

Methods: We analyzed BRCA1 and BRCA2 genes by next generation sequencing in a cohort of 32 patients unselected for family history and age of diagnosis.

Results: Among 32 patients with TNBC, 7 carried a pathogenic BRCA1 or BRCA2 variant (21.87%). Six patients carried a pathogenic BRCA1 variant and one carried a pathogenic BRCA2 variant. Overall, six different pathogenic BRCA1 or BRCA2 variants were identified, including frameshift, nonsense, and missense variants. The BRCA1 missense c.5309G>T, a founder pathogenic variant in the Moroccan population, was identified in two unrelated patients. In addition, six variants of unknown clinical significance were identified.

Conclusions: The understanding of BRCA1 and BRCA2 status in TNBC patients plays an important role in optimizing the preventive and therapeutic management. Despite the small size of our cohort, we believe that this work would help broaden our knowledge of TNBC and BRCA1/2 variants in Morocco.

KEYWORDS
BRCA1, BRCA2, deleterious variants, genetic testing, north of Morocco, triple-negative breast cancer, variants of unknown significance

1 | BACKGROUND

BRCA1 and BRCA2 are the two major breast cancer susceptibility genes. BRCA1 and BRCA2 germline testing is often recommended for...
patients with a strong family history of breast and/or ovarian cancer. Early onset breast cancer and triple-negative breast cancer (TNBC) can also provide an indication for BRCA1/2 testing.

TNBC is defined by the lack of expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2). It accounted for 12% to 17% of all breast cancer cases, but is over-represented in young women. TNBC is associated with aggressive tumor behavior and a worse prognosis. It is a highly heterogeneous subgroup, and generally, several genes appear to be involved. The link between TNBC and BRCA1/2 germline pathogenic variants was more widely described in the literature. In this regard, many studies have established the frequency of BRCA1 and/or BRCA2 deleterious variants in TNBC. This frequency differs according to the populations examined and the selection criteria.

Studies including patients with TNBC selected by age of diagnosis, family history of breast, and/or ovarian cancer or patients referred to genetic counseling have shown a higher rate of germline BRCA1/2 deleterious variants (approximately 50%). Moreover, a study conducted among an unselected cohort of Ashkenazi women with TNBC have identified BRCA1/2 deleterious variants in around 40% of cases. Although, without any selection criteria, the frequency of BRCA1/2 deleterious variants remains higher among TNBC patients. A study including 1824 TNBC patients unselected for age of diagnosis or family history of breast and/or ovarian cancer showed BRCA1/2 germline pathogenic variants in 11.2% of cases. Several other studies have produced frequencies ranging from 9% to 21%. The frequency of these pathogenic variants also differs between BRCA1 and BRCA2 genes. In the TNBC group, most of these variants are located in BRCA1. According to the Consortium of Investigators of Modifiers of BRCA1/2, 68% of tumors arising in BRCA1 carriers were triple negative (TN), compared to only 16% for BRCA2.

Currently, several studies recommend to search for BRCA1 and BRCA2 variants in all TNBCs, regardless of age or family history. Revised National Comprehensive Cancer Network guidelines have considered that TNBC among women ≤60 years old constitutes an eligibility criterion for genetic counseling.

In this study, we have screened BRCA1 and BRCA2 genes in a cohort of 32 TNBC patients unselected for family history and age of diagnosis. To the best of our knowledge, this is the first study of its kind in the north of Morocco.

### METHODS

#### 2.1 Study population

From January 2010 to December 2015, 331 breast cancer patients were referred to the Oncology Clinic Al Amal of Tangier. Among these, 56 TNBC cases were identified (17%). Thirty-two TNBC patients agreed to participate in this study and provided a blood sample. Patients were selected irrespective of age and family history of breast and/or ovarian cancer.

Informed consent was obtained from all participants. Ethical approval for this study has been granted by the Ethics Committee for Biomedical Research in the Faculty of Medicine and Pharmacy of Rabat (CERB).

#### 2.2 Pathologic data

ER, PR, and HER2 status were determined using immunohistochemistry (IHC). ER and PR were considered negative when +1% of the tumor cells showed positive nuclear staining. For HER2 staining, we considered as negative IHC scores of 0 and +1, or a score of +2 but HER2 fluorescence in situ hybridization negative.

Tumors were classified according to histological classification (WHO 2012). Histological tumor grading was performed using modified Scarff-Bloom-Richardson grading system (mSBR). The stage was determined according to the guidelines of the American Joint Committee on Cancer (AJCC 2010) staging system.

#### 2.3 BRCA1 and BRCA2 analysis

The analysis of BRCA1 and BRCA2 genes had been performed in the Genetracer Biotech Laboratory in Spain, as part of a collaboration with the Laboratory of Biomedical Genomics and Oncogenetics.

Genomic DNA was extracted from 32 blood samples. All coding sequences and intron/exon boundaries of BRCA1 and BRCA2 were screened with the Ion Proton System (Thermo Fisher Scientific). Libraries were prepared with Ampliseq technology, using the Oncomine BRCA1/2 research assay and producing a total of 262 amplicons. These enriched regions were then sequenced with 100 pb single-end reads. Sequencing reads were aligned to the hg19 reference human genome using the Ion Torrent Browser 5.6 software and then analyzed with Ion Reporter 5.6 cloud server, using the Oncomine BRCA1/2 research germline - 530 - w3.1 - DNA - single Sample algorithm and decoder platform. The quality of the sequencing process was verified based on the average base coverage depth (between 4351 and 9051 reads per base), on the percentage of reads on-target (between 83% and 97%), and on the uniformity of base coverage (between 88% and 92%) among the sequenced libraries.

All identified variants have been checked in variant databases. These variants were also checked in PubMed. Finally, the results obtained were verified by Sophia DDM algorithm.

#### 2.4 Statistical analysis

The comparison between BRCA1/2 deleterious variants carriers and noncarriers was undertaken using the χ² test or Fisher’s exact test. A P-value of less than .05 was considered statistically significant. Statistical analysis was performed using the SPSS 20 software.
RESULTS

In the current study, 32 female TNBC patients had a BRCA1 and BRCA2 genetic test by next generation sequencing. The age of diagnosis ranged from 28 to 60 years, with an average age of 45 years. All patients had a high mSBR grade (2 or 3). Three patients had a personal and/or family history of breast or ovarian cancer in a first- or second-degree relative. Sixteen patients had no variant at the coding regions of BRCA1 and BRCA2. Four patients had only benign or likely benign variants. Four patients had variants of unknown clinical significance (VUS) without other deleterious variants. Finally, pathogenic BRCA1 or BRCA2 variants were found in seven patients with TNBC (21.87%).

All the patients with BRCA1 or BRCA2 pathogenic variants were diagnosed at age ≤60 years. One patient had a medullary carcinoma. A family history of breast and/or ovarian cancer was not associated with BRCA1/2 deleterious variants in this cohort: only one patient had a family history (a second degree relative). Two patients had a personal history of breast or ovarian cancer (Table 1). No statistically significant differences between BRCA1/2 deleterious variant carriers and noncarriers were observed in clinicopathologic and prognostic characteristics.

Most pathogenic variants were found in BRCA1 (six patients vs one), whereas the VUS were found more in BRCA2, particularly in exon 11 (Figures 1 and 2). In all, six different pathogenic variants

| Gene symbol | ID | Nucleotide | Age | Histology | Grade | Stage | Personal history | Family history |
|-------------|----|------------|-----|-----------|-------|-------|-----------------|---------------|
| **BRCA1**   |    |            |     |           |       |       |                 |               |
| GTB691      | NM_007294.3:c.5390C>A | 51  | IDC      | 3      | Early | Ovarian Cancer | No            |
| GTB692      | NM_007294.3:c.798_799del | 28  | IDC      | 3      | Early | No            | No            |
| GTB701      | NM_007294.3:c.2125_2126insA | 31  | IDC      | 2      | Early | No            | No            |
| GTB706      | NM_007294.3:c.5309G>T | 46  | IDC      | 2      | Advanced | No           | No            |
| GTB709      | NM_007294.3:c.5309G>T | 48  | Medullary | 2     | Advanced | No           | No            |
| GTB714      | NM_007294.3:c.116G>A | 34  | IDC      | 3      | Early | No            | No            |
| **BRCA2**   |    |            |     |           |       |       |                 |               |
| GTB708      | NM_000059.3:c.5116_5119del | 38  | IDC      | 3      | Advanced | Breast Cancer | No            |

FIGURE 1  Pathogenic variants and VUS in BRCA1 and their locations on the exons

FIGURE 2  Pathogenic variants and VUS in BRCA2 and their locations on the exons
(Table 1) and six missense VUS or unclassified variants (UVs; Table 2) were identified.

### 3.1 | Deleterious variants

In **BRCA1**, two frameshift (c.798_799del, c.2125_2126insA), two missense (c.116G>A, c.5309G>T), and one nonsense (c.5390C>A) different pathogenic variants were identified. Only the missense c.5309G>T was found in two cases. This pathogenic variant (ClinVar; ENIGMA; LOVD) is located in the BRCT C-terminal BRCA1 domain and leads to the substitution of glycine 1770 by valine. On the other hand, the nonsense c.5390C>A variant was reported in the ClinVar database with only one submission (color: pathogenic), but it has never been cited in PubMed or any other published literature. This variant generates a premature stop codon at position 1797 and leads to a truncated protein with loss of the final 67 amino acids. These amino acids correspond to a part of the BRCT domain of the protein, which has an important role in the repair of DNA, cell cycle control, and tumor suppressor function. In silico tools predict a high level of pathogenicity for this variant (in silico prior probability of pathogenicity: “Score 0.99”).

In **BRCA2**, the frameshift c.5116_5119del was the only pathogenic variant which has been identified. Four bases (AATA) deletion leads to the creation of a premature stop codon at position 1797 and leads to a truncated protein with loss of the final 67 amino acids. These amino acids correspond to a part of the BRCT domain of the protein, which has an important role in the repair of DNA, cell cycle control, and tumor suppressor function. In silico tools predict a high level of pathogenicity for this variant (in silico prior probability of pathogenicity: “Score 0.99”).

### 3.2 | Variants of unknown clinical significance

In **BRCA1**, the missense c.5117G>C was the only variant of unknown significance found in this cohort. This variant involves the alteration of a conserved nucleotide, resulting in an amino acid change from a glycine to an alanine at codon 1706 located at the interface between the two BRCT domains. This VUS was in conjuncture with a deleterious variant (**BRCA1**: c.5309G>T).

In **BRCA2**, five missense VUS were identified (c.625C>T, c.4090A>G, c.5634C>G, c.5640T>G, and c.7954G>A). Only the c.4090A>G (p.Ile1364Val) was in conjuncture with a deleterious variant (**BRCA1**: c.116G>A). The c.625C>T (p.Leu209Phe), located in the exon 7, was submitted once in LOVD as unknown significance. This variant was not found in ClinVar or other online databases.

### 4 | DISCUSSION

TNBC is strongly associated with **BRCA1** and **BRCA2** germline deleterious variants. Although, without any selection criteria, the frequency remains higher (9%-21%). We also found an interesting frequency of deleterious variants of these genes (~22%), particularly in **BRCA1** (six vs one). However, the small size of our study reduces the likelihood of finding statistically significant results. This could also explain why there is no significant difference in clinicopathologic and prognostic characteristics between **BRCA1/2** pathogenic variant carriers and noncarriers.

#### 4.1 | Deleterious variants

The spectrum of **BRCA1** and **BRCA2** deleterious variants varies significantly between populations. Some variants have a founder effect and are common in specific populations. In our study, the **BRCA1** missense c.5309G>T pathogenic variant was identified in two patients. This deleterious variant was already reported as a **BRCA1** founder variant in the Moroccan population. Cherbal et al noticed that this variant has also been detected in other unrelated families from Italy and described as founder variant in South and Middle Sardinia, respectively. As Mediterranean countries share a common migration flow history, they concluded that the c.798_799delTT in **BRCA1** could be a Mediterranean founder variant. The **BRCA1** frameshift c.2125_2126insA pathogenic variant has been reported in some French-Canadian cancer families. It has also been identified in Algerian and Moroccan patients with TNBC. Furthermore, **BRCA1** missense c.116G>A corresponds to highly recurrent pathogenic variants. It has been reported

### TABLE 2 | Variants of unknown clinical significance

| Gene symbol | ID | Nucleotide | Protein | Co-occurrences with deleterious variant | In silico tools prior probability of pathogenicity (0.00-1) | Citations |
|-------------|----|------------|---------|----------------------------------------|-----------------------------------------------------|------------|
| BRCA1       | GTB706 | NM_007294.3:c.5117G>C | p.Gly1706Ala | | 0.66 | Laraqui et al 21 |
| BRCA2       | GTB694 | NM_000059.3:c.625C>T | p.Leu209Phe | | - | 0.02 | Gaidrat et al (2012) |
|             | GTB714 | NM_000059.3:c.4090A>G | p.Ile1364Val | | 0.3 | Cherbal et al 20 |
|             | GTB697 | NM_000059.3:c.5634C>G | p.Asn1878Lys | | - | 0.02 | Balia et al 24 |
|             | GTB696 | NM_000059.3:c.5640T>G | p.Asn1880Lys | | - | 0.02 | Fackenthal et al (2012) |
|             | GTB693 | NM_000059.3:c.7954G>A | p.Val2652Met | | 0.29 | Fokkema et al 25 |
|             |        |            |         | | 0.29 | Mesman et al 27 |
in Slovenia and has been suggested as a Slovenian founder variant.28,29 At last, BRCA2 frameshift c.5116_5119del pathogenic variant was described as recurrent in Castile and Leon in Spain, a region that shares a common migration flow history with northern Morocco.30,31

4.2 | Variants of unknown clinical significance

The interpretation of UVs or VUS is highly problematic in genetic counseling. Their impact on protein function is not clear, as well as their contribution in increasing the risk of breast and/or ovarian cancer. In our study, six of these variants were identified and some of them require more attention.

In BRCA1, c.5117G>C variant was observed in the large and broad cohorts of the ExAC project, and has been reported in the literature in affected individuals in North Africa as sporadic and/or familial cases of breast cancer in Moroccan, Algerian, and Tunisian populations.19,21,32,33 Laraqui et al noticed that this is the most frequently detected VUS in Moroccan population.23 However, as in our case, they observed that this variant was in conjunction with a deleterious variant in a Tunisian family, and that this co-occurrence suggests that it could ultimately be neutral. Henouda et al, for their part, considered this variant a favor polymorphism.35 In their opinion, there was no strong evidence that this variant might be disease associated.

In BRCA2, the c.4090A>G variant is a rare missense change with uncertain impact on protein function. As in our case, Cherbal et al reported this variant in a Moroccan family in co-occurrence with a deleterious variant.20 These co-occurrences let us think that it is rather a neutral variant. On the other side, the BRCA2 c.5634C>G variant has been reported in the literature in individuals affected with hereditary breast and ovarian cancer (HBOC).14,34 However, these reports do not provide unequivocal conclusions about association of the variant with HBOC. Balia et al showed that this variant may be functionally significant based on homologous recombination-based assays, and concluded that this variant could probably be classified as pathogenic even if in silico tools predict a benign effect.34 At last, the BRCA2 c.7954G>A variant has been reported in individuals with breast cancer in LOVD, but it is not present in population databases (ExAC no frequency).35,36 Algorithms developed to predict the effect of missense changes on protein structure and function do not agree on the potential impact of this missense change. However, Mesman et al described how the variant shows a reduction of more than 50% of the HDR function.37 In summary, the available evidence is currently insufficient to determine the role of this variant in disease.

5 | CONCLUSIONS

BRCA1 and BRCA2 deleterious variants confer an excessive risk of developing a second cancer. At the same time, patient’s relatives carrying deleterious variants have an increased risk of breast, ovarian, pancreatic, and prostate cancer. The understanding of BRCA1/2 status plays an important role in optimizing the preventive and therapeutic management. Several studies have recommended to search for these variants in all TNBCs, regardless of age or family history.

Despite the small size of our cohort, we believe that this work would help broaden our knowledge of TNBC and BRCA1/2 variants in Morocco.

ACKNOWLEDGMENTS

We would like to thank all the patients who provided samples for this study. We would also like to thank the members of Generetracer Biotech Laboratory, the team of Biomedical Genomics and Oncogenetics Laboratory and the team of Oncology Clinic Al Amal for their help and support. At last, we would like to thank Laraki A. and BaKir M.Y. for their proofreading.

CONFLICT OF INTEREST

The authors declare that they have no competing interests.

AUTHOR CONTRIBUTIONS

All authors contributed to this paper.

REFERENCES

1. Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med. 2010;363(20):1938-1948. https://doi.org/10.1056/NEJMra1001389.
2. Bauer KR, Brown M, Cress RD, Parise CA, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype. Cancer. 2007;109(9):1721-1728. https://doi.org/10.1002/cncr.22618.
3. Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res. 2007;13(15 Pt 1):4429-4434. https://doi.org/10.1158/1078-0432.CCR-06-3045.
4. Fostira F, Tsitlaidou M, Gogas H, et al. Prevalence of BRCA1 mutations among 284 women with triple-negative breast cancer. J Clin Oncol. 2010;28(15_suppl):1511-1511. https://doi.org/10.1200/JCO.2010.28.15_suppl.151.
5. Couch FJ, Hart SN, Sharma P, et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. J Clin Oncol. 2015;33(4):304-311. https://doi.org/10.1200/JCO.2014.67.141.
6. Bayraktar S, Gutierrez-Barrera AM, Liu D, et al. Outcome of triple-negative breast cancer in patients with or without deleterious BRCA mutations. Breast Cancer Res Treat. 2011;130(1):145-153. https://doi.org/10.1007/s10549-011-1711-z.
7. Robertson L, Hanso H, Seal S, et al. BRCA1 testing should be offered to individuals with triple-negative breast cancer diagnosed below 50 years. Br J Cancer. 2012;106(6):1234-1238. https://doi.org/10.1038/bjc.2012.31.

8. Greenup R, Buchanan A, Lorizio W, et al. Prevalence of BRCA mutations among women with triple-negative breast cancer (TNBC) in a genetic counseling cohort. Ann Surg Oncol. 2013;20(10):3254-3258. https://doi.org/10.1245/s10434-013-3205-1.

9. Comen E, Davids M, Kirchhoff T, Hudis C, Offit K, Robson M. Relative contributions of BRCA1 and BRCA2 mutations to “triple-negative” breast cancer in Ashkenazi women. Breast Cancer Res Treat. 2011;129(1):185-190. https://doi.org/10.1007/s10549-011-1433-2.

10. Gonzalez-Angulo AM, Timms KM, Liu S, et al. Incidence and outcome of BRCA mutations in unselected patients with triple receptor-negative breast cancer. Clin Cancer Res. 2011;17(5):1082-1089. https://doi.org/10.1158/1078-0432.CCR-10-2560.

11. Hartman A-R, Kaldate RR, Sailer LM, et al. Prevalence of BRCA mutations in an unselected population of triple-negative breast cancer. Cancer. 2012;118(11):2787-2795. https://doi.org/10.1002/cncr.26576.

12. Sharma P, Klemm JR, Kimler BF, et al. Germline BRCA mutation evaluation in a prospective triple-negative breast cancer registry: Implications for hereditary breast and/or ovarian cancer syndrome testing. Breast Cancer Res Treat. 2014;143(3):707-714. https://doi.org/10.1007/s10549-014-2980-0.

13. Muendlein A, Rohde BH, Gasser K, et al. Evaluation of BRCA1/2 mutational status among German and Austrian women with triple-negative breast cancer. J Cancer Res Clin Oncol. 2015;141(11):2005-2012. https://doi.org/10.1007/s00432-015-1986-2.

14. Wong-Brown MW, Meldrum CJ, Carpenter JE, et al. Prevalence of BRCA1 and BRCA2 germline mutations in patients with triple-negative breast cancer. Breast Cancer Res Treat. 2015;150(1):71-80. https://doi.org/10.1007/s10549-015-3293-7.

15. Wang C, Zhang J, Wang Y, et al. Prevalence of BRCA1 mutations and responses to neoadjuvant chemotherapy among BRCA1 carriers and non-carriers with triple-negative breast cancer. Ann Oncol. 2015;26(3):523-528. https://doi.org/10.1093/annonc/mdu559.

16. Mavaddat N, Barrowdale D, Andrilis IL, et al. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the consortium of investigators of modifiers of BRCA1/2 (CIMBA). Cancer Epidemiol Biomarkers Prev. 2012;21(3):154-147. https://doi.org/10.1158/1055-9965.EPI-11-0775.

17. Quiles F, Teulé Á, Martinussen Tanstad N, et al. Identification of a founder BRCA1 mutation in the Moroccan population. Clin Genet. 2016;90(4):361-365. https://doi.org/10.1111/cge.12747.

18. Quiles F, Fernández-Rodríguez J, Mosca R, et al. Functional and structural analysis of C-terminal BRCA1 missense variants. PLoS One. 2013;8(4):e61302. https://doi.org/10.1371/journal.pone.0061302.

19. Uhrhammer N, Abdelouahab A, Lafarge L, Felliel V, Ben Dib A, Bignon Y-J. BRCA1 mutations in Algerian breast cancer patients: high frequency in young, sporadic cases. Int J Med Sci. 2008;5(4):197-202.

20. Cherbal F, Bakour R, Adane S, Boualga K. BRCA1 and BRCA2 germline mutation spectrum in hereditary breast/ovarian cancer families from Maghrebian countries. J Ovarian Res. 2013;34(1):1-8. https://doi.org/10.3233/BD-130348.

21. Laraqi A, Uhrhammer N, Lahlou-Amine I, et al. Mutation screening of the BRCA1 gene in early onset and familial breast/ovarian cancer in Moroccan population. Int J Med Sci. 2013;10(1):60-67. https://doi.org/10.7150/ijms.5014.

22. Mahfoudh W, Bououina N, Ahmed SB, et al. Hereditary breast cancer in Middle Eastern and North African (MENA) populations: identification of novel, recurrent and founder BRCA1 mutations in the Tunisian population. Mol Biol Rep. 2012;39(2):1037-1046. https://doi.org/10.1007/s10033-011-0829-8.

23. Tazzite A, Jouhadi H, Nadifi S, et al. BRCA1 and BRCA2 germline mutations in Moroccan breast/ovarian cancer families: novel mutations and unclassified variants. Gynecol Oncol. 2012;125(3):687-692. https://doi.org/10.1016/j.ygyno.2012.03.007.

24. Castéra L, Krieger S, Rousselin A, et al. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. Eur J Hum Genet. 2014;22(11):1305-1313. https://doi.org/10.1038/ejhg.2014.16.

25. Belanger MH, Dolman L, Arcand SL, et al. A targeted analysis identifies a high frequency of BRCA1 and BRCA2 mutation carriers in women with ovarian cancer from a founder population. J Ovarian Res. 2015;8:1. https://doi.org/10.1186/s13048-015-0124-8.

26. Gaceb H, Cherbal F, Bakour R, Ould-Rouis A, Mahfouf H. Clinicopathological and molecular study of triple-negative breast cancer in Algerian patients. Pathol Oncol Res. 2017;24:1-12. https://doi.org/10.1007/s12253-017-0242-2.

27. Jouafi F, Laarabi FZ, Marchoudi N, et al. First application of next-generation sequencing in Moroccan breast/ovarian cancer families and report of a novel frameshift mutation of the BRCA1 gene. Oncol Lett. 2016;12(2):1192-1196. https://doi.org/10.3892/ol.2016.4739.

28. Krajc M, Zadnik V, Novaková S, et al. Geographical distribution of Slovenian BRCA1/2 families according to family origin: Implications for genetic screening. Clin Genet. 2014;85(1):59-63. https://doi.org/10.1111/cge.12119.

29. Kais Z, Chiba N, Ishaoka C, Parvin J. Functional differences among BRCA1 missense mutations in the control of centrosome duplication. Oncogene. 2012;31(6):799-804. https://doi.org/10.1038/onc.2011.271.

30. Infante M, Durán M, Lasa A, et al. Two founder BRCA2 mutations predispose to breast cancer in young women. Breast Cancer Res Treat. 2010;122(2):567-571. https://doi.org/10.1007/s10549-009-0661-1.

31. Blay P, Santamaria I, Pitiot AS, et al. Mutational analysis of BRCA1 and BRCA2 in hereditary breast and ovarian cancer families from Asturias (northern Spain). BMC Cancer. 2013;13:243. https://doi.org/10.1186/1471-2407-13-243.

32. Cherbal F, Salhi N, Bakour R, Adane S, Boualga K, Mailet P, BRCA1 and BRCA2 unclassified variants and missense polymorphisms in Algerian breast/ovarian cancer families. Dis Markers. 2012;32(6):343-353. https://doi.org/10.3233/DMA-2012-0893.

33. Henouda S, Bensalem A, Reggad R, Serrar N, Rouahab L, Pujol P. Contribution of BRCA1 and BRCA2 germline mutations to early Algerian breast cancer. Dis Markers. 2016;2016:7869095. https://doi.org/10.1155/2016/7869095.

34. Balla C, Galli A, Caligo MA. Effect of the overexpression of BRCA2 unclassified missense variants on spontaneous homologous recombination in human cells. Breast Cancer Res Treat. 2011;129:1001. https://doi.org/10.1007/s10549-011-1607-y.

35. Fokkema IF, Taschner PE, Schaafsma GC, Celli J, Laros JF, den Dunnen JT. LOVD v2.0: the next generation in gene variant databases. Hum Mutat. 2011;32(5):557-563. https://doi.org/10.1002/humu.21438.

36. National Center for Biotechnology Information. ClinVar; [VCV000462460.2]. https://www.ncbi.nlm.nih.gov/clinvar/variation/VCV000462460.2. Accessed December 9, 2019.

37. Mesman RLS, Calléja FMGR, Hendriks G, et al. The functional impact of variants of uncertain significance in BRCA2. Breast Cancer Res Treat. 2015;150(1):71-80. https://doi.org/10.1007/s12253-014-0242-9.

38. MANSOURI ET AL.