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# Research Article

# **Identification of Novel** *BRCA1* **Germline Deleterious Variant Among a Tunisian Family**

Hela Sassi<sup>1#\*</sup>, Rym Meddeb<sup>1,2#\*</sup>, Mediha Trabelsi<sup>1,2</sup>, Samia Hannachi<sup>3</sup>, Neila Belguith<sup>1</sup>, Imen Abbes<sup>3</sup>, Khaled Rahal<sup>4</sup>, Karima Mrad<sup>3</sup>, Amel Mezlini<sup>5</sup> and Ridha M'rad<sup>1,2</sup>

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#### ABSTRACT

Inherited predisposition to breast and ovarian cancer are most frequently due to germline mutations in the main genes BRCA1 (OMIM# 113705) and BRCA2 (OMIM# 600185). These inactivating mutations, essentially frameshift and nonsense variation, occurs mainly across conserved regions. The aim of the present study is to report a novel germline BRCA1 mutation identified in a Tunisian family case with early onset of breast and ovarian cancer and to evaluate the genotype phenotype correlation. The proband had high-grade tumors, invasive unilateral ductal carcinoma developed at the age of 38 and a serous ovarian adenocarcinoma after a gap of twelve years. The molecular analysis revealed a novel heterozygous nonsense BRCA1 mutation NM\_007294.4: c.915T>A p.(C305\*) in the proband and her daughter. This mutation leads to a truncated protein which pathogenicity was validated by bioinformatics tools. This variant is subject to nonsense-mediated mRNA decay. We also underlined the immunohistochemistry usefulness by lack of expression of BRCA1 protein in paraffin embedded breast tumor contrasting with normal tissue. Clinical and pathological data tend to be homogeneous and led to the conclusion that there is a genotype phenotype correlation in BRCA1, an element that must be taken into account in genetic counselling. Conclusively, we are the first to report this novel BRCA1 germline likely deleterious variant extending the molecular and clinical spectrum of BRCA1 pathogenic point mutations. Further in vitro functional experiments needs to be established. High-risk individuals carrying this BRCA1 mutation benefit from preventive measures to reduce morbidity.

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# Introduction

Genetic predisposition for breast and ovarian cancer seems to be the most challenging issue in patient's management. The main and most studied genes are *BRCA1* (OMIM# 113705) and *BRCA2* (OMIM#

600185) [1, 2]. About 15-40% of cases with familial aggregation of breast cancer (BC) and up to 90% of families with ovarian and breast cancer have germline mutations in *BRCA* genes [3]. In Tunisia, 29% of hereditary Breast and Ovarian Cancer (HBOC) are linked to mutations in the *BRCA* genes [4, 5]. These tumor suppressor genes play crucial

<sup>&</sup>lt;sup>1</sup>Department of Congenital and Hereditary Diseases, University Tunis El Manar, Charles Nicolle Hospital, Tunis, Tunisia

<sup>&</sup>lt;sup>2</sup>Faculty of Medicine of Tunis, University Tunis El Manar, Laboratory of Human Genetics LR99ES10, Tunis, Tunisia

<sup>&</sup>lt;sup>3</sup>Department of Pathology Anatomy and Cytology, University Tunis El Manar, Salah Azaiez Institute, Tunis, Tunisia

<sup>&</sup>lt;sup>4</sup>Department of Surgery, University Tunis El Manar, Salah Azaiez Institute, Tunis, Tunisia

<sup>&</sup>lt;sup>5</sup>Department of Medical Oncology, University Tunis El Manar, Salah Azaiez Institute, Tunis, Tunisia

 $<sup>^{\#}</sup>Contributed\ equally$ 

<sup>\*</sup>Correspondence to: Hela Sassi, M.D., Medical Doctor, Department of Congenital and Hereditary Diseases, University Tunis El Manar, Charles Nicolle Hospital, 1006, Tunis, Tunisia; E-mail: hella.sassi@gmail.com

Rym Meddeb, M.D., Department of Congenital and Hereditary Diseases, Faculty of Medicine of Tunis University Tunis El Manar, Charles Nicolle Hospital, Laboratory of Human Genetics LR99ES10, 1006, Tunis, Tunisia; E-mail: meddeb\_rym@yahoo.fr

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roles by ensuring genomic stability, maintaining its integrity through DNA repair, transcriptional regulation and cell proliferation [6, 7]. *BRCA1* seems to be the most defective gene [4]. *BRCA1*'s transcription provides a main 7.8 kb transcript from 22 coding exons producing a full-length protein. The exon 11, formerly nominated exon 10, represents about 60% of the coding sequences. Not only it is the central exon of *BRCA1* protein but also has a large size with 3426bp [1, 7]. In the present case, we report a Tunisian family with HBOC harbouring a novel germline *BRCA1* mutation, the usefulness of studying BRCA1's expression in normal and tumoral tissues and evaluate the genotype phenotype correlation.

#### **Patient and Methods**

This study was conducted in the oncogenetic department according to the declaration of Helsinki and was approved by the local ethics committee of the hospital. Our patient was referred for clinical suspicion of HBOC. An extensive pedigree was established gathering all the cancers in the family and mentioning the onset age of the first cancer for each individual.

# I Histological and Immunohistochemistry Study

Gross examination and tumor characteristics including tumor size, histological grade, lymph node status, vascular invasion, disease-free interval, recurrence, and development of distant metastasis were evaluated. Classic staining with estrogen receptor (ER), progesterone receptor (PR), HER2 (human epidermal growth factor receptor 2) and proliferation index Ki67 index were performed on paraffin embedded tumor tissue. Samples from normal tissue and paraffin embedded breast tumor were stained for BRCA1 (GenomeME Clone IHC401) and reviewed by independently two pathologists blinded to set BRCA1 protein expression (Table 1).

If nuclear staining was less than 12%, loss of BRCA1 expression in immunohistochemistry was considered and if it was more than 12%, normal BRCA1 expression was concluded according to manufacturer's protocol [8]. Positive control and nuclear staining of stromal cells were

used as an internal positive control. We examined by immunohistochemistry the usefulness of antibody to BRCA1 protein in detecting its expression on paraffin embedded breast tumor and normal tissue.

### II Genetic Study

Blood samples were obtained from the proband and her alive relatives after an informed signed consent. Genomic DNA was extracted from lymphocytes using standard protocols. Sanger sequencing screening of the most frequent Tunisian BRCA mutations have been realised according to manufacturer's protocol and were analysed on ABI 3130 sequencer (Applied Biosystems, Foster City, CA) [4, 9]. The wild-type BRCA1 sequence NM 007294.4, corresponding NP 009225.1, was used to set the nucleotide and amino acid number of the variation. The variant nomenclature was established according to the Human Genome Variation Society (HGVS, Link1) and Mutalyzer 2.0.32 (Link2). The variation was verified in online databases; dbSNP database (Link3). Leinden Open Variation Database (LOVD, Link4), ClinVar (Link5), Universal Mutation Database BRCA1 (UMD BRCA1, Link6) and BRCA Exchange (Link7). Bioinformatics analysis focused on the protein's structure and evolutionary conservation to predict the pathogenicity of nucleotide variation. It was based on a combination of *in silico* predictive online software: Sorting Intolerant From Tolerant (SIFT, Link8), Protein Variation Effect Analyzer (PROVEAN, Link9), Mutation taster (Link10), Mutpred-LOF (Link11), Evolutionary Conserved Regions Browser (Link12), University of California Santa Cruz genome browser (UCSC, Link13) [10].

The predicted functional effect on *BRCA1* protein was tested by PROVEAN and MutPred-LOF with a fixed-cutoff of (-2,5) and g scores higher than 0.5 respectively, to predict whether the variation was deleterious. Both methods phastCons and phyloP were used to determine the grade of conservation of a specific nucleotide. The closer phastCons value is to 1, the more probable the nucleotide is conserved. By contrast, phyloP value varies between -14 and +6 and sites predicted to be conserved are assigned positive scores.

Table 1: Antibodies and staining results by immunohistochemistry

Antigen	Antibodies and manufacturers	Dilution	Antigen retrieval	Results in normal breast tissue	Results in tumoral breast tissue
ER	Leica Biosystems Estrogen Receptor Clone 6F11	1:50	EDTA PH6	+	-
PR	Leica Biosystems progesterone receptor Clone 16	1:50	EDTA PH6	+	-
HER2	Monoclonal antibodies CB11	1:40	EDTA PH9	-	-
Ki67 index	Leica Biosystems ki67 MM1	1:50	EDTA PH9	0%	100%
BRCA1	GenomeME Clone IHC401	1:50-100	EDTA PH6	+	-

ER: Estrogen Receptor; PR: Progesterone Receptor; HER2: Human Epidermal Growth Factor Receptor 2; (-) lack of protein expression; (+) positive protein expression.

#### Results

### I Clinical Study

The proband (III.6) was followed at the oncology department since the age of 38 (Figure 1). The clinical examination showed a mass straddling the external quadrants of the right breast of 70x75mm, suspected of malignancy, covered with thickened skin and discreetly inflammatory, associated with a homolateral mobile axillary lymphadenopathy of 20 mm of stiff consistency. The locoregional extension report was normal. After a confirmation biopsy of malignancy, FEC chemotherapy protocol (5-fluorouracil, Epidoxorubicin and Cyclophosphamide) was first prescribed with good response to treatment, followed by a surgical excision of the right lump.

Gross examination found a mass of 45x20x20mm and the histological sections confirmed an invasive ductal carcinoma with an abnormal mitotic activity of 20 mitoses per 10 High Power Field, graded III according to Scarff-Bloom-Richardson grade modified with numerous vascular emboli tumor (Figures 2A & 2B). Immunohistochemical studies in paraffin-embedded tissue sections of breast tumor demonstrated a lacking expression of ER, PR and HER2 and high proliferation index Ki67 (100%) concluded to a high grade triple negative BC tumor (TNBC) (Figures 2C & 2D) (Table 1). Lacking expression of BRCA1 protein was observed in tumor cells, whereas in normal breast tissue, there was a strong nuclear expression (Figures 2E & 2F) (Table 1).

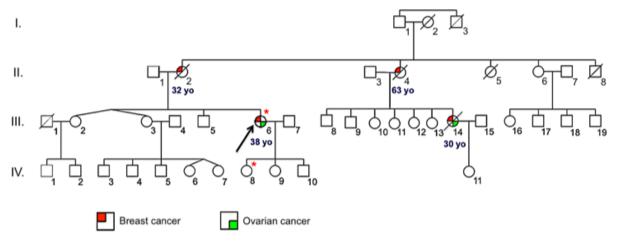


Figure 1: Pedigree of the Tunisian family case.

The black arrow shows our index case. The symbol (\*) indicates patients carrying the novel c.915T>A BRCA1 germline mutation. The onset age of the first cancer for each individual is mentioned in blue (yo: year old).

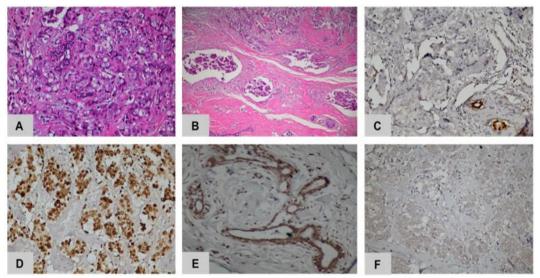


Figure 2: Histological and immunohistochemistry results.

**A)** Invasive high grade ductal carcinoma; **B)** Vascular invasion; **C)** Negative estrogen receptor and progesterone receptor with internal positive nuclear control in normal breast tissue; **D)** High proliferation index Ki67 (nuclear staining); **E)** BRCA1 expression: strong nuclear expression in normal breast tissue; **F)** BRCA1 expression: negative staining in tumor cells.

The oncological follow-up revealed 12 years later (50-year-old) a right pleural effusion associated with a pelvic mass and peritoneal carcinosis suspecting an ovarian primitive origin since the high level of CA-125: 8265U/mL. The surgical exploration led to a radical hysterectomy with double salpingo-oophorectomy, omentectomy and pelvic lymphadenectomy. The pathological findings concluded to a serous ovarian adenocarcinoma grade III, invading the omentum, left round ligament, the right oophorectomy and the Douglas. The index case (IC) (III.6) was referred to oncogenetic consultation for clinical suspicion of HBOC at the age of 45. In the family history, her mother (II.2) and her maternal aunt (II.4) were diagnosed with BC at the age of 32 and 63 respectively. Her maternal cousin (III.14) has developed an ovary breast syndrome at the age of 30 (Figure 1).

#### **II Genetic Investigation**

DNA sequencing of the IC revealed a novel heterozygous nonsense mutation in exon 11 of *BRCA1* NM\_007294.4: c.915T>A p.(C305\*) creating a premature stop codon (Figures 3A & 3B). It generates a truncated BRCA1 protein with 305 amino acids. The molecular study was carried out on the proband's offspring (IV.8, IV.9 and IV.10) showing the same variation in her daughter IV.8. This mutation was not referenced in any online databases.

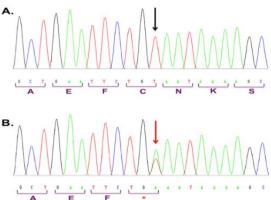


Figure 3: Sequence chromatography of the novel germline BRCA1 mutation c.915T>A.

A) Normal sequence (forward). The black arrow shows the wild-type nucleotide at position 915 in the reference sequence of exon 11 *BRCA1*. **B**) Mutated sequence: *BRCA1* NM\_007294.4: c.915T>A p.(C305\*). The red arrow indicates the single nucleotide substitution at the heterozygous state. The purple letters refers to the amino-acid sequence. The red symbol (\*) indicates the premature stop codon at position 305.

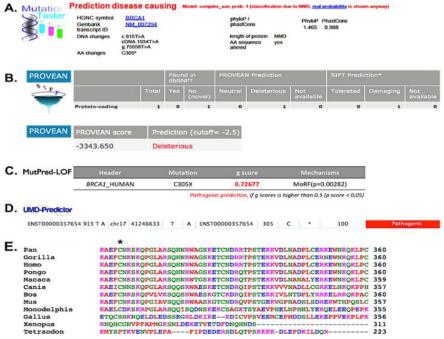


Figure 4: Overview of functional effects of the novel germline BRCA1 mutation p.(C305\*) by bioinformatics prediction tools.

(A) Summary data from mutation taster. (B) PROVEAN and SIFT prediction regarding this novel mutation.

(C) G score established by MutPred LOF tool. (D) Functional effect predicted by UMD Predictor. (E) Alignment of human amino-acid sequence compared to other species. The symbol (\*) indicates the amino-acid Cysteine at position 305.

NMD: nonsense-mediated mRNA decay

# III In silico Prediction

Mutation taster, PROVEAN, MutPred-LOF and UMD predictor revealed that this novel mutation seems to be deleterious (Figures 4A-4E). Nonsense-mediated mRNA decay (NMD) is likely to occur according to mutation taster (Figure 4A). MutPredLOF predicted that there is likely a MoRF (Molecular Recognition Features) after residue 305, with a significant p-value (p=0.00282) (Figure 4C), meaning that there are regions of these residues with intrinsic disorder. Thus, the predicted pathogenicity could at least partially be driven by a loss of a disordered region. The protein sequence in humans compared with other species confirmed the high conservation of the specific amino-acid Cysteine at the position 305 during evolution (Figures 4A & 4E). Analysis of NM\_007294.4:c.915T>A p.(C305\*) with all the bioinformatics tools revealed that it is 100% pathogenic.

#### Discussion

In this study, we present a Tunisian family with HBOC at an early onset. A novel heterozygous nonsense mutation NM\_007294.4:c.915T>A p.(C305\*) in *BRCA1* gene was identified, whose transcript is likely leading to nonsense-induced mRNA decay (NMD). Rebbeck *et al.* (2018) defined the mutational spectrum in a worldwide study of 29,700 families with female *BRCA* mutations carriers. These inactivating mutations of *BRCA1*, occurring across the entire coding and non-coding region, are heterogeneous and the major mutations types in *BRCA1* were frameshift (57,5%) followed by nonsense variation (19%) [11]. Actually, hundreds of *BRCA1* mutations have been found throughout exon 11 (UMD *BRCA1* database).

Different literature arguments support the pathogenicity of the new mutation. In fact, several pathogenic nonsense mutations with a premature stop codon in exon 11 upstream and downstream the new mutation c.915T>A have been reported (UMD *BRCA1* database). Some of these mutations were illustrated in (Figure 5).

Furthermore, this mutation occurs at exon 11 which covers a large part of the *BRCA1* gene and leads to a 305-amino-acids-*BRCA1* truncated protein instead of 1,863 amino acids in the wild-type protein removing domains of high importance [1, 7].

The conserved domains of interest of the protein in our study are the two Nuclear Localization Sequences amino acids NLS1 (Amino acids 501-507), NLS2 (Amino acids 607-614) which are encoded by exon 11, and the C-terminal BRCT domain [7, 12-17]. The respective functions of these domains consist on migration of BRCA1 from cytosol to nucleus and response to DNA damage by interacting with proteins required in G2/M transition checkpoint [12-17]. We should highlight that the amino acids encoded by *BRCA1* exon 11 have binding site for DNA-double strand breaks repair protein (Rad 50, Rad 51 and PALB2), cycle cell control protein (Rb protein) and transcription factor Myc reducing the oncogenic activity of c-Myc (Figure 5) [12]. The p.(C305\*) *BRCA1* mutation lead to the loss of a considerable part of exon 11, the NLS amino acids and BRCT domains producing a likely non-functional protein.

The bioinformatics prediction tools used in this study were in favour of the pathogenic character of this mutation. Furthermore, nonsensemediated mRNA decay (NMD) is likely to occur according to Mutation Taster. The NMD pathway is a conserved evolutionarily surveillance mechanism degrading mRNAs with a premature termination codon avoiding its interference in normal cellular pathways [18-20]. Olga Anczuków *et al.* tested in nonsense *BRCA1* mutation the ability to detect amounts of truncated proteins in lymphoblastoid cell lines. No BRCA1 truncated protein was detected, suggesting that it is highly unstable thus destroyed by NMD [21]. Experimental studies in the novel variation c.915T>A should test the NMD process.

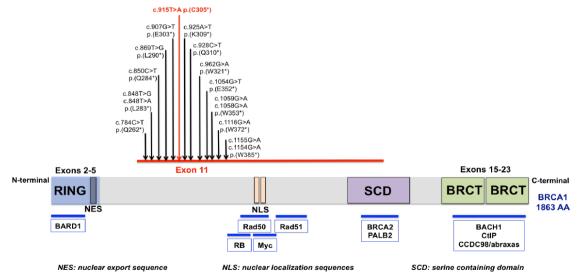


Figure 5: Schematic overview of BRCA1 protein's structure and its conserved regions (NP\_009225.1).

The red line refers to the schematic spanning region of exon 11 and the blue lines indicates the binding site for each protein. The black arrows show the schematic location of some of the different mutations reported in the literature. The novel Tunisian mutation c.915T>A p.(C305\*) is in red.

As well, in this Tunisian family, detection of this mutation in relative's diagnostic with BC or ovarian cancer would have been an additional argument in favour of the segregation of this mutation with the disease. Several studies reported different disease phenotypes among patients carrying mutations in exon 11 of *BRCA1* compared to other *BRCA1* germline mutations [22-28]. Rebbeck *et al.* (2015) reported a total of 19 581 females carrying *BRCA1* mutation of which 1132 had a nonsense mutation in exon 11, 796 BC and 336 ovarian cancer, with a mean age at cancer diagnosis 40, 4 and 50, 2-year-old respectively, likewise our IC who had an early onset age of cancer (BC: 38 years old, ovarian cancer: 50-years-old) [29]. We have to highlight that in the same family, earlier onset age cancer have been noticed (30 and 32-years-old).

Mutations in the central of *BRCA1* gene tend to cause more ovarian cancer than BC compared to variation at the N and C termini of *BRCA1* [29]. The IC III. 6 had both breast and ovarian cancer considering the loss of exon 11 and the C-terminal BRCT domain. Thus, the clinical data associated to exon 11 mutations tend to be homogeneous. As for the pathological data, most of BRCA1 mutations located in exon 11 have been correlated with high grade TNBC, large tumor size (>2cm) and poor prognosis, as our patient, leading to a genotype phenotype correlation [22-28].

Rakha et al. evaluated the prognostic significance of BRCA1 expression by immunohistochemistry study on paraffin embedded tissue in large series of 1940 invasive BC [30]. Like our case, strong nuclear staining in normal breast tissue and absent staining in ductal carcinomas had been frequently observed on not specific type (66%), with high grade, advanced stage, large tumor size, advanced lymph node stage, vascular invasion and triple negative phenotype [30]. Lack of expression in BRCA1 protein in tumor tissue contrasting with its normality in safe breast tissue reflects the loss of heterozygosity in tumor cells thus proving the causality of this novel germline BRCA1 variant in the tumor phenotype.

The identification of this novel mutation allowed us to give an appropriate genetic counselling to the family, to test high risk relatives including the proband's offspring and to propose, on the one hand, personalized care for IC and on the other hand, preventive measures for her daughter according to the recent recommendations such a bi-annual clinical examination, annual surveillance with breast MRI, prophylactic mastectomy and bilateral salpingo-oophorectomy [31, 32].

#### Conclusion

All available bioinformatics arguments classify such variant as deleterious disease-causing mutation. The BRCA1 immunohistochemistry study is still usefulness to set the causality of novel *BRCA1* variants in tumoral phenotype. Furthermore, functional assays at the transcriptional and proteomics levels are required in the germline DNA to understand the loss of function effect of NM\_007294.4:c.915T>A p.(C305\*) mutation and better know its implication in HBOC. Thus, an integrated strategy helps for a comprehensive assessment of pathogenicity. Once the molecular diagnosis is done, an appropriate genetic counselling could be provided, and a personalized care could be established.

### Highlights

- i. c.915T>A p.(C305\*) is a novel Tunisian nonsense germline mutation in *BRCA1*.
- BRCA1 mutation c.915T>A seems to be associated with an aggressive clinical course.
- Nonsense mutations in BRCA1 exon 11 are subject to nonsensemediated mRNA decay.
- iv. Integrated strategies help for a comprehensive assessment of pathogenicity.

#### **Author Contributions**

Guarantor of integrity of the entire study: Ridha M'rad, Rym Meddeb, Hela Sassi; Study concepts and design: Rym Meddeb; Literature research: Hela Sassi, Rym Meddeb, Ridha M'rad; Clinical studies: Ridha M'rad, Rym Meddeb, Amel Mezlini, Khaled Rahal, Samia Hannachi, Imen Abbes, Karima Mrad; Experimental studies: Hela Sassi, Rym Meddeb; Data analysis: Hela Sassi, Rym Meddeb, Mediha Trabelsi, Neila Belguith; Statistical analysis: N/A (not apply); Manuscript preparation: Hela Sassi, Rym Meddeb, Ridha M'rad; Manuscript editing: Rym Meddeb, Mediha Trabelsi.

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# **Ethical Approval**

This study was approved by the local ethics committee of the hospital.

# **Funding**

None.

#### **Conflicts of Interest**

None.

#### **Abbreviations**

BC: Breast Cancer

**HBOC:** Hereditary Breast Ovarian Cancer **NMD:** Nonsense-Mediated mRNA Decay **TNBC:** Triple-Negative Breast Cancer

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