Research Article

Identification of Novel BRCA1 Germline Deleterious Variant Among a Tunisian Family

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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...
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 tissue. Blood samples were obtained from the proband and her alive relatives 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), Leiden 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).
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.
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). MutPred-LOF 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, nonsense-mediated 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 19581 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.
ii.  BRCA1 mutation c.915T>A seems to be associated with an aggressive clinical course.
iii. Nonsense mutations in BRCA1 exon 11 are subject to nonsense-mediated 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 Merzini, Khaled Rahal, Samia Hannachi, Ilmen 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

REFERENCES

1. Miki Y, Swensen J, Shattuck Eidsen D, Futreal P, Harshman K et al. (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66-71. [Crossref]
2. Wooster R, Bignell G, Lancaster J, Swift S, Seal S et al. (1995) Identification of the breast cancer susceptibility gene BRCA2. *Nature* 378: 789-792. [Crossref]

3. Schwartz GF, Hughes KS, Lynch HT, Fabian CJ, Fentiman IS et al. (2009) Proceedings of the international consensus conference on breast cancer risk, genetics, & risk management, April, 2007. *Breast J* 15: 4-16. [Crossref]

4. Riahi A, Kharrat M, Ghourahi ME, Khomsi F, Gamoudi A et al. (2015) Mutation spectrum and prevalence of BRCA1 and BRCA2 genes in patients with familial and early-onset breast/ovarian cancer from Tunisia. *Clin Genet* 87: 155-160. [Crossref]

5. Riahi A, Chabouni Bouhamed H, Kharrat M (2017) Prevalence of BRCA1 and BRCA2 large genomic rearrangements in Tunisian high risk breast/ovarian cancer families: implications for genetic testing. *Cancer Genet* 210: 22-27. [Crossref]

6. Welch PL, Owens KN, King MC (2000) Insights into the functions of BRCA1 and BRCA2. *Trends Genet* 16: 69-74. [Crossref]

7. Deng CX, Brodie SG (2000) Roles of BRCA1 and its interacting proteins. *Bioessays* 22: 728-737. [Crossref]

8. Perez Valles A, Martorell Cebolla M, Nogueira Vazquez E, Garcia J, Fuster Diana E (2001) The usefulness of antibodies to the BRCA1 protein in detecting the mutated BRCA1 gene. An immunohistochemical study. *J Clin Pathol* 54: 476-480. [Crossref]

9. Troudi W, Uhrhammer N, Sibille C, Dahan C, Mahfoudh W et al. (2007) Contribution of the BRCA1 and BRCA2 mutations to breast cancer in Tunisia. *J Hum Genet* 52: 915-920. [Crossref]

10. Richards S, Aziz N, Bale S, Bick D, Das S et al. (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med* 17: 405-424. [Crossref]

11. Rebbeck TR, Friebl TM, Friedman E, Hamann U, Hoo D et al. (2018) Mutational spectrum in a worldwide study of 29,700 families with BRCA1 or BRCA2 mutations. *Hum Mutat* 39: 593-620. [Crossref]

12. Clark SL, Rodríguez AM, Snyder RR, Hankins GDV, Boehning D (2012) Structure-function of the tumor suppressor BRCA1. *Comput Struct Biotechnol J* 1: e201204005. [Crossref]

13. Yu X, Chini CCS, He M, Mer G, Chen J (2003) The BRCT domain is a phospho-protein binding domain. *Science* 302: 639-642. [Crossref]

14. Yu X, Chen J (2004) DNA damage-induced cell cycle checkpoint control requires CdhP, a phosphorylation-dependent binding partner of BRCA1 C-terminal domains. *Mol Cell Biol* 24: 9478-9486. [Crossref]

15. Kim H, Huang J, Chen J (2007) CCDC98 is a BRCA1-BRCT domain-binding protein involved in the DNA damage response. *Nat Struct Mol Biol* 14: 710-715. [Crossref]

16. Wang B, Matsuoka S, Ballif BA, Zhang D, Smogorzewska A et al. (2007) Abraxas and RAP80 form a BRCA1 protein complex required for the DNA damage response. *Science* 316: 1194-1198. [Crossref]

17. Chen CF, Li S, Chen Y, Chen PL, Sharp ZD et al. (1996) The nuclear localization sequences of the BRCA1 protein interact with the importin-alpha subunit of the nuclear transport signal receptor. *J Biol Chem* 271: 32863-32868. [Crossref]

18. Perrin Vidoz L, Sinilnikova OM, Stoppa Lyonnet D, Lenoir GM, Mazoyer S (2002) The nonsense-mediated mRNA decay pathway triggers degradation of most BRCA1 mRNAs bearing premature termination codons. *Hum Mol Genet* 11: 2805-2814. [Crossref]

19. Baker KE, Parker R (2004) Nonsense-mediated mRNA decay: terminating erroneous gene expression. *Curr Opin Cell Biol* 16: 293-299. [Crossref]

20. Conti E, Izarralde E (2005) Nonsense-mediated mRNA decay: molecular insights and mechanistic variations across species. *Curr Opin Cell Biol* 17: 316-325. [Crossref]

21. Anczuków O, Ware MD, Buisson M, Zetoune AB, Stoppa Lyonnet D et al. (2008) Does the nonsense-mediated mRNA decay mechanism prevent the synthesis of truncated BRCA1, CHK2, and p53 proteins? *Hum Mutat* 29: 65-73. [Crossref]

22. Chen H, Wu J, Zhang Z, Tang Y, Li X et al. (2018) Association between BRCA Status and Triple-Negative Breast Cancer: A Meta-Analysis. *Front Pharmacol* 9: 909. [Crossref]

23. Foulkes WD, Smith IE, Reis Filho JS (2010) Triple-negative breast cancer. *N Engl J Med* 363: 1938-1948. [Crossref]

24. Rashid MU, Muhammad N, Bajwa S, Faisal S, Tahseen M et al. (2016) High prevalence and predominance of BRCA1 germline mutations in Pakistani triple-negative breast cancer patients. *BMC Cancer* 16: 673. [Crossref]

25. Couch FJ, Hart SN, Sharma P, Toland AE, Wang X et al. (2015) Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unscreened for family history of breast cancer. *J Clin Oncol* 33: 304-311. [Crossref]

26. Gaceb H, Cherbul F, Bakour R, Ould Rous A, Mahfoud F (2018) Clinicopathological and molecular study of triple-negative breast cancer in Algerian patients. *Pathol Oncol Res* 24: 297-308. [Crossref]

27. Muendlein A, Rohde BH, Gasser K, Haid A, Rauch S et al. (2015) Evaluation of BRCA1/2 mutational status among German and Austrian women with triple-negative breast cancer. *J Cancer Res Clin Oncol* 141: 2005-2012. [Crossref]

28. Young S, Pilarski RT, Donenberg T, Shapiro C, Hammond LS et al. (2009) The prevalence of BRCA1 mutations among young women with triple-negative breast cancer. *BMC Cancer* 9: 86. [Crossref]

29. Rebbeck TR, Mitra N, Wan F, Sinilnikova OM, Healey S et al. (2015) Association of type and location of BRCA1 and BRCA2 mutations with risk of breast and ovarian cancer. *JAMA* 313: 1347-1361. [Crossref]

30. Rakha EA, El Sheikh SE, Kandil MA, El Sayed ME, Green AR et al. (2008) Expression of BRCA1 protein in breast cancer and its prognostic significance. *Hum Pathol* 39: 857-865. [Crossref]

31. Ludwig KK, Neuner J, Butler A, Geurts JL, Kong AL (2016) Risk reduction and survival benefit of prophylactic surgery in BRCA mutation carriers, a systematic review. *Am J Surg* 212: 660-669. [Crossref]

32. Daly MB, Pilarski R, Yurgelun MB, Berry MP, Buys SS et al. (2020) NCCN Guidelines Insights: Genetic/Familial High-Risk Assessment: Breast, Ovarian, and Pancreatic, Version 1.2020. *J Natl Compr Canc Netw* 18: 380-391. [Crossref]