Double heterozygous mutation in RAD50 and ATM genes in a Peruvian family with five cancer types: a case report

Doble mutación heterocigota en genes RAD50 y ATM en una familia peruana con cinco tipos de cáncer: reporte de un caso

Mutação dupla heterozigótica nos genes RAD50 e ATM em uma família peruana com cinco tipos de câncer: relato de caso

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A Peruvian middle-age woman with breast cancer and a family with other five relatives with five different cancer types was genetically studied. We found a double mutation in 2 different genes which helps our physician team and the patient to choose a treatment following the post-test genetic counseling.

Se estudió mediante un panel multi-génico el caso de una mujer peruana de mediana edad con cáncer de mama y el de su familia con otros cinco parientes con cinco tipos diferentes de cáncer. Encontramos una doble mutación en 2 genes diferentes que ayudó a nuestro equipo médico y al paciente a elegir un tratamiento siguiendo el consejo genético posterior a la prueba.

Key Concepts:

A) What is known about the subject?
While it is known that genetic studies help to improve our understanding of the cancer disease and it is recommended to perform genetic studies for cases with patient's personal or family history of cancer, here in Latin-America it is still a privilege-related procedure.

B) What does this work contribute?
Here we studied a case of a middle-age woman with breast cancer and 5 cancer types in her family history. We found a double heterogenous mutation in RAD50 and ATM genes, genetic information that helps our physician team and the patient to choose a treatment following the post-test genetic counseling.

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Abstract:

Introduction: Cancer is the second leading cause of death worldwide, with 70% of cancer deaths occurring in low- or middle- income countries. To mitigate the mortality of this disease, it is recommended the evaluation of multiple high-penetrance genes. **Methods**: We used a multi-gene panel testing to identify germline variants in a unique case of a breast cancer patient with a family history of five different neoplasm types. The patient, at the age of 50 years, was diagnosed with a high-grade cribriform ductal carcinoma *in situ* in her left breast. **Results**: We identified two heterozygous mutations, one classified as pathogenic/likely pathogenic in *RAD50* and the other classified as a variant of uncertain significance (VUS) in *ATM*. **Conclusion**: In conclusion, the use of the multi-gene panel leads to the identification of a double heterozygous mutation in *RAD50* and *ATM* in a breast cancer patient from a Peruvian family with several cancer types. This data helps our physician team and the patient to choose a treatment following the post-test genetic counseling.

Keywords: breast neoplasms; medical genetics; genetic testing.

Resumen:

Introducción: El cáncer es la segunda causa principal de muerte en todo el mundo, y el 70% de las muertes por cáncer ocurren en países de ingresos bajos o medianos. Para mitigar la mortalidad de esta enfermedad, se recomienda la evaluación de múltiples genes de alta penetrancia. **Métodos**: Utilizamos una prueba de panel de múltiples genes para identificar variantes de la línea germinal en el caso de una paciente con cáncer de mama con antecedentes familiares de cinco tipos diferentes de neoplasias. La paciente, a la edad de 50 años, fue diagnosticada de carcinoma ductal cribiforme de alto grado *in situ* en su mama izquierda. **Resultados**: Identificamos dos mutaciones heterocigotas, una clasificada como patogénica / probablemente patogénica en RAD50 y la otra clasificada como variante de significado incierto (VUS) en ATM. **Conclusión**: En conclusión, el uso del panel multigénico condujo a la identificación de una doble mutación heterocigota en RAD50 y ATM en una paciente con cáncer de mama de una familia peruana con varios tipos de cáncer. Estos datos ayudan a nuestro equipo médico y al paciente a elegir un tratamiento siguiendo el asesoramiento genético posterior a la prueba.

Palabras clave: neoplasias de la mama; genética médica; pruebas genéticas.

Resumo:

Introdução: O câncer é a segunda principal causa de morte em todo o mundo e 70% das mortes por câncer ocorrem em países de baixa ou média renda. Para mitigar a mortalidade por esta doença, a avaliação de vários genes de alta penetrância é recomendada. **Métodos**: Usamos um teste de painel multigênico para identificar variantes da linha germinativa em uma paciente com câncer de mama com história familiar de cinco tipos diferentes de neoplasias. A paciente, com 50 anos de idade, foi diagnosticada com carcinoma ductal cribriforme de alto grau in situ em sua mama esquerda. **Resultados**: Identificamos duas mutações heterozigotas, uma classificada como patogênica / provavelmente patogênica em RAD50 e a outra classificada como variante de significância incerta (VUS) em ATM. **Conclusão**: Em conclusão, o uso do painel multigênico levou à identificação de uma mutação dupla heterozigótica em RAD50 e ATM em uma paciente com câncer de mama de uma família peruana com vários tipos de câncer. Esses dados auxiliam nossa equipe médica e o paciente na escolha do tratamento após o aconselhamento genético pós-teste.

Palavras-chave: neoplasias de mama; genética médica; testes genéticos.

INTRODUCTION

Cancer is a disease characterized by malignant transformation and uncontrolled growth of cells due to multiple alterations in their genome, and this public health issue is the second leading cause of death worldwide, with 70% of cancer deaths occurring in low- or middle- income countries[1]. To mitigate the mortality of this disease, it is recommended the evaluation of multiple high-penetrance genes of established clinical utility as possible explanations for a patient's personal or family history of cancer[2].

This report describes the case of a woman with breast cancer, the most frequent fatal cancer in women[3], with a family history of several cancer cases of different types. Interestingly, after performing a multi-gene panel for hereditary cancer, two heterozygous mutations were found in the *RAD50* and *ATM* genes. Both genes are involved in DNA double-strand breaks repair (DSBR) via homologous recombination and *RAD50* is also involved in DSBR via non-homologous recombination[4]. Furthermore, both *RAD50* and *ATM* play key regulatory roles over the telomere maintenance[5]. It is remarkable that variants in these genes are present in a large number of types of cancer and they indeed are attractive targets for precision medicine[6].

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. Informed consent was obtained from all individual participants included in the study.

MATERIALS AND METHODS

The patient of this study is a Peruvian woman with a medical history of uterine myomatosis, colonic polyps, and thyroid cyst. She also used oral contraceptives for two years for anovulation therapy. The patient was diagnosed with a high-grade cribriform ductal carcinoma in situ (DCIS), solid and positive to estrogen and progesterone receptors in her left breast, at the age of 50 years old. The patient underwent a lumpectomy and tamoxifen therapy. Additionally, at the age of 53 years, she was diagnosed with endometrial hyperplasia.

The study of the patient's family tree (Figure 1) revealed several second-degree relatives with neoplasms. A total of five neoplastic cases were reported considering six relatives, including her, in four generations of the patient's family. Interestingly, multiple neoplasm types were reported in this Peruvian family (lung cancer, liver cancer, brain tumor, and endometrial cancer) besides the breast cancer of the patient of this study.

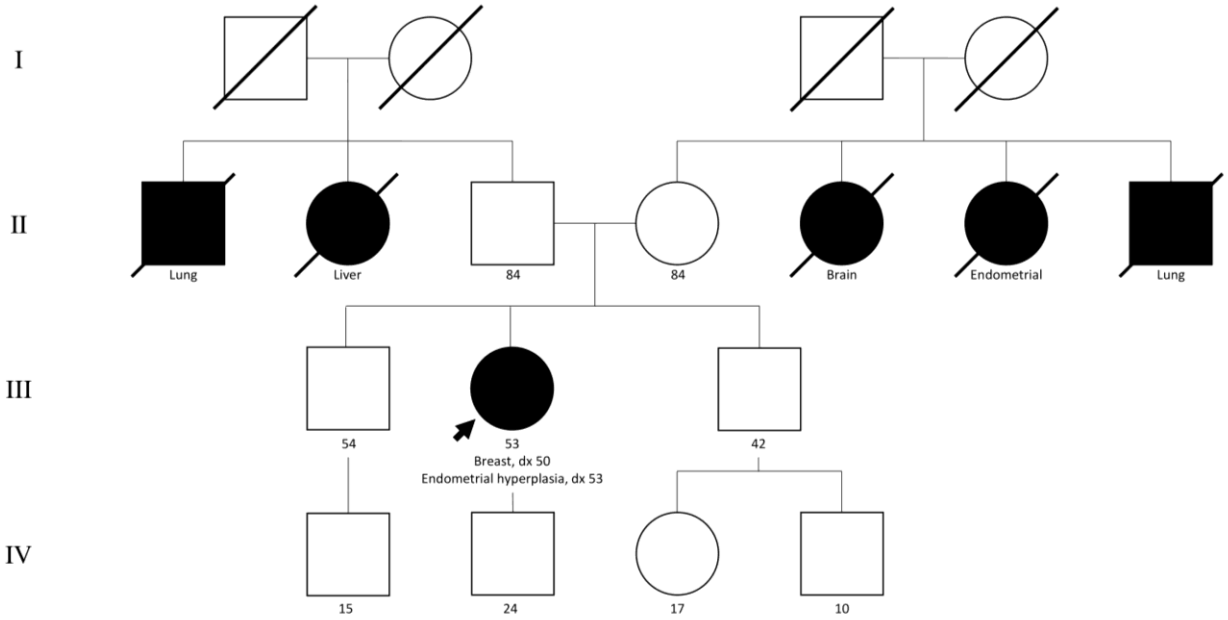

Figure N°1. Patient's family tree. It is indicated the type of cancer and the age of diagnosis when available.

Regarding the multi-gene panel test, we performed a pre-test counseling, the genetic test, and a post-test counseling. The DNA of the patient was extracted from her peripheral blood. A multi-gene panel for hereditary cancer was performed, analyzing the exonic regions, the adjacent intronic regions, and copy number variations (CNV) of 56 genes (Table 1) by next-generation sequencing (NGS): *APC, ATM, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, EPCAM, FH, FLCN, HNF1A, HNF1B, HOXB13, MC1R, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NTHL1, PALB2, PMS1, PMS2, POLD1, POLE, POT1, PRSS1, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, STK11, TP53, TSC1, TSC2, VHL, WT1, XRCC2, and XRCC3*.

RESULTS

Two germline variants were detected in heterozygosis. The variant in *RAD50* (NM_005732.4 c.3715C>T:p.Arg1239Ter) causes a premature stop codon in the exon 24 out of 25, leading to the truncation of the C-terminal 74 amino acids of the RAD50 native protein (Figure 2). This variant is classified as pathogenic/likely pathogenic for breast and ovarian cancer. The variant in *ATM* (NM_000051.3 c.8156G>A:p.Arg2719His) causes a nonsynonymous amino acid change in the protein position 2719, in the kinase PI3/PI4 domain of the ATM protein (Figure 3). However, this variant is classified as a variant of uncertain significance (VUS), further analysis is needed to assess its pathogenicity. Both variants were confirmed by Sanger sequencing.

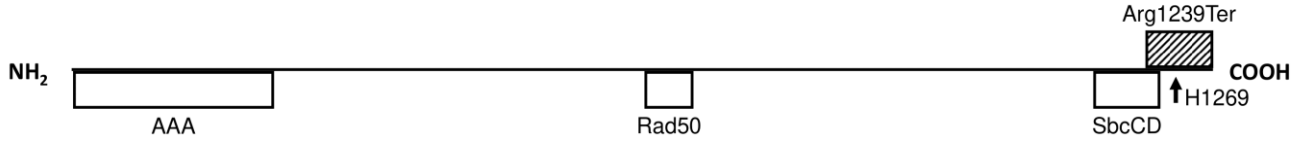

Figure N°2. Diagram of the RAD50 protein showing its main protein families, the variant NM_005732.3c.3715C>T:p.Arg1239Ter (stripped box, which would be lost in the truncated protein), and the 1269 histidine (arrow). AAA: AAA domain. Rad50:Rad50 zinc hook motif. SbcCD: Putative exonuclease SbcCD, C subunit. Figure adapted from: https://www.rcsb.org/pdb/protein/Q92878

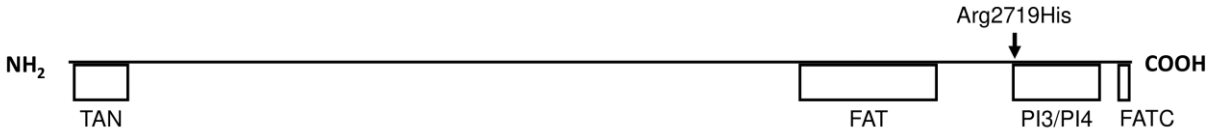

Figure N°3. Diagram of the ATM protein showing its main protein families and the variant NM_000051.3c.8156G>A:p.Arg2719His (arrow). TAN: Tel1/ATM N-Terminal Motif. FAT: "FRAP, ATM and TRRAP" domain. PI3/PI4: Phosphatidylinositol 3- and 4-kinase. FATC: "FRAP, ATM, TRRAP C-terminal" domain. Figure adapted from: http://www.rcsb.org/pdb/protein/Q13315

The study of these genetic variants in the relatives who were diagnosed with cancer has not been possible to carry out since all of them were already dead. Her only son, at the age of 24 years, refused to perform the study for the moment.

DISCUSSION

Several variants in the genes of the MRE11-RAD50-NBS1 (MRN) complex have been associated with a large number of cancer types[6]. It is noteworthy that some of these associated cancers have been reported in second-degree relatives of the patient. In particular, the *RAD50* variant of our patient (NM_005732.4 c.3715C>T:p.Arg1239Ter) has been reported in breast and ovarian cancer[7]. The premature termination codon introduced by this variant leads the translation of a truncated protein without its C-terminal 74 amino acids (Figure 2). This truncation can cause a decrease in the RAD50's ATPase function due to the lack of a D-Loop and to the absence of the 1269 histidine of the native protein, which is proposed as a controller of its functional activation[8]. Although functional studies have not been done for this particular variant, the structural variation that likely disrupt this ATPase catalytic site that would lead to impaired function and the multiple reports in breast and ovarian cancer cases suggest this variant is pathogenic, but additional data are needed to prove it conclusively

For its part, variants of the *ATM* gene have also been associated, even in heterozygosity, with different types of cancer, but especially with breast cancer, knowing that variants of this gene can increase the risk of breast cancer and that the ATM protein (a protein kinase) phosphorylates different tumor suppressor proteins, including BRCA1[9]. In particular, the *ATM* variant of our patient (NM_000051.3 c.8156G>A:p.Arg2719His) is classified as a VUS, despite it causes a nonsynonymous amino acid change in the kinase PI3/PI4 domain of the ATM protein. Although there are several reports of this variant in cancer cases, algorithms developed to predict the effect of missense changes on protein structure and function, like this one, are either unavailable or do not agree on the potential impact of this specific variant so, due to insufficient evidence, it is currently considered a VUS. Further studies and reports are necessary to assess the clinical significance of this variant in cancer[10].

It is remarkable how these two genes, *RAD50* and *ATM*, are directly related since the MRN complex is essential for the activation of the ATM protein[11]. Furthermore, these genes are involved in the DSBR and the telomere lengthening pathways, two key processes when we refer to the hallmarks of cancer. In this sense, it is possible that the effect of the heterozygous variants in *RAD50* and *ATM* found in our patient may complement each other, contributing to the development of cancer, even though they may have a slighter impact, independently. Further analysis looking for the segregation or coexistence of variants in these two genes in this family are recommended since it would help to improve our understanding of their independent contribution and synergy.

The coexistence of mutations in different genes for cancer cases has been previously studied, although not extensively. However, combinations of heterozygous mutations have been recently reported. An example is the report of variants in *ATM* and *BRCA1*[12], among many others. These reports are probably responding to the increasingly extensive use of multi-gene panels for cancer around the world. In addition to the well-established advantages for diagnosis and clinical management, multi-gene panels are also contributing to the identification of more and new pathogenic variants[13]. Their use is highly recommended, especially for developing countries where the numbers of deaths due to cancer are proportionally higher than in developed countries, and the genetics of these populations are less studied as well. Also, we highlight the importance of the pre-test counseling which reduces the patient's distress, improves her risk perception, and increases the predisposition for medical follow-up[14]. In this case, the genetic data from the multi-gene panel helps our physician team and the patient to choose a treatment following the post-test genetic counseling. Two treatment options had been proposed: prophylactic double mastectomy or periodic screening. To share the decision with the patient we showed an educational presentation of basic genetics, up to date information about breast cancer related mutations and possible scenarios regarding her case. After an educational presentation to the patient about breast cancer and possible scenarios regarding her case, considering the evidence supporting that the contralateral risk of DCIS is too low to justify the surgery[15], and according to the National Comprehensive Cancer Network guidelines for high-risk assessment, both the physicians and the patient agreed that she was not a candidate for surgery and should opt for periodic screening.

Finally, she was recommended to perform a personalized risk assessment (she and her relatives), as well as annual screening with physical examination plus breast imaging using 3D mammography for

detecting microcalcifications, breast magnetic resonance imaging, and a transvaginal ultrasound looking for ovarian tumors or endometrial growth.

CONCLUSIONS

In conclusion, to the best of our knowledge, we are reporting the first case of a double heterozygous mutation in the *RAD50* and *ATM* genes in a family with several cancer types, which genetic data helps the patient and physicians to decide between proposed treatments.

Limitaciones de responsabilidad:
La responsabilidad de este trabajo es exclusivamente de los autores.

Conflicto de interés:
Ninguno.

Fuentes de apoyo:
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Originalidad:
Este artículo es original y no ha sido enviado para su publicación a otro medio de difusión científica en forma completa ni parcialmente.

Cesión de derechos:
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Contribución de los autores:
Todos los autores han participado en la concepción del diseño, recolección de la información y elaboración del manuscrito, haciéndose públicamente responsables de su contenido y aprobando su versión final.

REFERENCES

1. Wild CP, Weiderpass E, Stewart BW. World cancer report: cancer research for cancer prevention. Lyon: International Agency for Research on Cancer; 2020. https://digitallibrary.in.one.un.org/TempPdfFiles/5932_1.pdf.

2. Robson ME, Bradbury AR, Arun B, Domchek SM, Ford JM, Hampel HL, Lipkin SM, Syngal S, Wollins DS, Lindor NM. American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility. J Clin Oncol. 2015 Nov 1;33(31):3660-7. doi: 10.1200/JCO.2015.63.0996.

3. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018 Nov;68(6):394-424. doi: 10.3322/caac.21492. Epub 2018 Sep 12. Erratum in: CA Cancer J Clin. 2020 Jul;70(4):313.

4. Gobbini E, Cassani C, Villa M, Bonetti D, Longhese MP. Functions and regulation of the MRX complex at DNA double-strand breaks. Microb Cell. 2016 Jul 27;3(8):329-337. doi: 10.15698/mic2016.08.517.

5. Wu Y, Xiao S, Zhu XD. MRE11-RAD50-NBS1 and ATM function as co-mediators of TRF1 in telomere length control. Nat Struct Mol Biol. 2007 Sep;14(9):832-40. doi: 10.1038/nsmb1286.

6. Syed A, Tainer JA. The MRE11-RAD50-NBS1 Complex Conducts the Orchestration of Damage Signaling and Outcomes to Stress in DNA Replication and Repair. Annu Rev Biochem. 2018 Jun 20;87:263-294. doi: 10.1146/annurev-biochem-062917-012415.

7. Castéra L, Krieger S, Rousselin A, Legros A, Baumann JJ, Bruet O, Brault B, Fouillet R, Goardon N, Letac O, Baert-Desurmont S, Tinat J, Bera O, Dugast C, Berthet P, Polycarpe F, Layet V, Hardouin A, Frébourg T, Vaur D. Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes. Eur J Hum Genet. 2014 Nov;22(11):1305-13. doi: 10.1038/ejhg.2014.16. 8. Hopfner KP, Karcher A, Shin DS, Craig L, Arthur LM, Carney JP, et al. Structural biology of Rad50 ATPase: ATP-driven conformational control in DNA double-strand break repair and the ABC-ATPase superfamily. Cell. 2000;101(7):789–800.

9. Cortez D, Wang Y, Qin J, Elledge SJ. Requirement of ATM-dependent phosphorylation of brca1 in the DNA damage response to double-strand breaks. Science. 1999 Nov 5;286(5442):1162-6. doi: 10.1126/science.286.5442.1162.

10. George Priya Doss C, Rajith B. Computational refinement of functional single nucleotide polymorphisms associated with ATM gene. PLoS One. 2012;7(4):e34573. doi: 10.1371/journal.pone.0034573.

11. Cassani C, Vertemara J, Bassani M, Marsella A, Tisi R, Zampella G, Longhese MP. The ATP-bound conformation of the Mre11-Rad50 complex is essential for Tel1/ATM activation. Nucleic Acids Res. 2019 Apr 23;47(7):3550-3567. doi: 10.1093/nar/gkz038.

12. Andrés R, Menao S, Arruebo M, Quílez E, Cardiel MJ. Double heterozygous mutation in the BRCA1 and ATM genes involved in development of primary metachronous tumours: a case report. Breast Cancer Res Treat. 2019 Oct;177(3):767-770. doi: 10.1007/s10549-019-05343-4.

13. Tsaousis GN, Papadopoulou E, Apessos A, Agiannitopoulos K, Pepe G, Kampouri S, Diamantopoulos N, Floros T, Iosifidou R, Katopodi O, Koumarianou A, Markopoulos C, Papazisis K, Venizelos V, Xanthakis I, Xepapadakis G, Banu E, Eniu DT, Negru S, Stanculeanu DL, Ungureanu A, Ozmen V, Tansan S, Tekinel M, Yalcin S, Nasioulas G. Analysis of hereditary cancer syndromes by using a panel of genes: novel and multiple pathogenic mutations. BMC Cancer. 2019 Jun 3;19(1):535. doi: 10.1186/s12885-019-5756-4.

14. Manahan ER, Kuerer HM, Sebastian M, Hughes KS, Boughey JC, Euhus DM, Boolbol SK, Taylor WA. Consensus Guidelines on Genetic` Testing for Hereditary Breast Cancer from the American Society of Breast Surgeons. Ann Surg Oncol. 2019 Oct;26(10):3025-3031. doi: 10.1245/s10434-019-07549-8.

15. Miller ME, Muhsen S, Olcese C, Patil S, Morrow M, Van Zee KJ. Contralateral Breast Cancer Risk in Women with Ductal Carcinoma In Situ: Is it High Enough to Justify Bilateral Mastectomy? Ann Surg Oncol. 2017 Oct;24(10):2889-2897. doi: 10.1245/s10434-017-5931-2.