

# Oncological Insights from Portuguese Families with Pathogenic Variants in the *RAD51* Gene Family

## Perspetivas Oncológicas de Famílias Portuguesas com Variantes Patogénicas na Família de Genes *RAD51*

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### Abstract

**Overview and Aims:** Among populations with hereditary cancer predisposition, gynecological cancers prevention is particularly relevant. Genetic research has highlighted the role of *RAD51* gene in maintaining genomic stability and its potential role in cancer susceptibility. Our study aimed to characterize families with pathogenic variants in *RAD51* at our center, focusing on the associated clinical outcomes and follow-up care.

**Study Design:** Retrospective study.

**Population:** All families with *RAD51* pathogenic variants, followed in our familial cancer risk clinic, at Hospital Dr. Nélio Mendonça, in Madeira Island, from 30-11-2018 to 30-11-2024.

**Methods:** Data was collected through a review of medical records of all patients who tested positive for *RAD51* pathogenic variant, focusing on baseline characteristics and associated neoplasia. We then conducted detailed family analyses to determine specific cancers in affected individuals, linking those variants to oncological outcomes.

**Results:** Ten index cases with *RAD51* pathogenic variants were identified and all of them were female. Indications for test were: breast cancer (4), ovarian cancer (3), fallopian tube carcinoma (1), colorectal cancer (1) and family history (1). The age at breast cancer diagnosis ranged from 36-55 years, and ovarian cancer diagnosis from 52-82 years. Seven different variants were identified: three in the *RAD51C* gene and four in *RAD51D*. From subsequent family analysis, we tested a total of 36 individuals and 21 of them tested positive for family mutation. Of those, six had previous history of neoplasia: two ovarian cancers, two breast cancers, one case uterine leiomyosarcoma and one bronchial carcinoid tumor.

**Conclusions:** These findings highlight the importance of genetic screening and counseling for individuals from high-risk families to enable early detection and management of elevated cancer risks associated with *RAD51* pathogenic variants. Prophylactic interventions, such as salpingo-oophorectomy and early cancer screening, are crucial for effectively managing these risks.

**Keywords:** *RAD51* pathogenic variants; Breast Carcinoma; Ovarian Carcinoma; Hereditary Cancer; Prevention.

### Resumo

**Introdução e Objetivo:** Nas síndromes de predisposição hereditária para o cancro, a prevenção dos cancros ginecológicos é particularmente relevante. A investigação genética destacou o papel do gene *RAD51* na manutenção da estabilidade

genómica e o seu potencial papel na suscetibilidade ao cancro. Deste modo, o nosso objetivo foi caracterizar famílias com variantes patogénicas no *RAD51*, focando nos desfechos clínicos associados e seguimento.

**Desenho de Estudo:** Estudo retrospectivo.

**População:** Famílias com variantes patogénicas no gene *RAD51*, seguidas na consulta de risco familiar, no Hospital Dr. Nélio Mendonça, na Ilha da Madeira, desde 30-11-2018 até 30-11-2024.

**Métodos:** Os dados foram recolhidos através dos registos médicos de todos os doentes com variantes patogénicas do gene *RAD51*, com foco nas características biodemográficas e nas neoplasias associadas. Posteriormente, foi realizada uma análise familiar detalhada para identificar neoplasias nos indivíduos afetados, relacionando as variantes com os desfechos oncológicos associados.

**Resultados:** Foram identificados dez casos-índice com variantes patogénicas no gene *RAD51*, todos do sexo feminino. As indicações para a realização do teste genético foram: cancro da mama (4), cancro do ovário (3), carcinoma da trompa de Falópio (1), cancro colorretal (1) e história familiar (1). A idade ao diagnóstico de cancro da mama variou entre os 36-55 anos, e ao diagnóstico de cancro do ovário entre os 52-82 anos. Foram identificadas sete variantes distintas: três no gene *RAD51C* e quatro no *RAD51D*. Na análise familiar subsequente, foram testados 36 indivíduos, dos quais 21 apresentaram resultado positivo para a mutação familiar. Entre estes, seis apresentavam história prévia de neoplasia: dois casos de cancro do ovário, dois de cancro da mama, um caso de leiomiossarcoma uterino e um tumor carcinoide brônquico.

**Conclusão:** Estes resultados destacam a importância do rastreio genético e aconselhamento de indivíduos de alto risco, permitindo a deteção precoce e gestão do risco neoplásico associado a este gene. Intervenções profiláticas, como a salpingo-ooforectomia e o rastreio precoce, são cruciais para a gestão do risco.

**Palavras-chave:** Variantes patogénicas *RAD51*; Carcinoma da mama; Carcinoma do ovário; Cancro hereditário; Prevenção.

## INTRODUCTION

Breast and ovarian cancers are significant global health concerns with notable variations in risk across populations. Breast cancer is the most commonly diagnosed cancer worldwide and the leading cause of cancer-related mortality among women<sup>1</sup>. Its risk is influenced by a combination of genetic, environmental, and lifestyle factors, with approximately 1 in 8 women being diagnosed over their lifetime<sup>1</sup>. Ovarian cancer, while less common, poses a considerably higher lethality rate, with about 1 in 87 women affected<sup>2</sup>. Advances in genetic research have increasingly illuminated the role of genetic variants in cancer susceptibility, providing new avenues for early detection, prevention, and personalized treatment strategies. Among these genetic factors, the *RAD51* gene has received increasing attention for its contribution to ge-

netic stability and its potential role in cancer susceptibility<sup>3-6</sup>.

The *RAD51* gene, located on chromosome 15q.15.1, encodes a protein crucial for the homologous recombination repair (HRR) pathway, which repairs double-strand breaks (DSBs) in DNA by facilitating genetic material exchange between homologous strands<sup>4,6</sup>. This process ensures accurate DNA repair, maintaining genomic stability and preventing malignant transformations. *RAD51* is particularly important in rapidly dividing cells, making it significant in cancer biology and therapy<sup>4,6</sup>. In addition to *RAD51* gene, there are several *RAD51* gene paralogs, including *RAD51B*, *RAD51C*, *RAD51D*, *XRCC2*, and *XRCC3*, which are integral components of the HRR pathway<sup>4,6</sup>.

*RAD51C* and *RAD51D* pathogenic variants are associated with an increased risk of hereditary breast cancer in women (20-40% versus average risk of 12.5%),

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especially for triple-negative breast cancer and ovarian cancer (10-20% versus average risk of 1.3%)<sup>3,7-17</sup>. Women with these variants can have a cumulative breast cancer risk of approximately 20-30% by age 70, depending on the specific mutation and family history<sup>14,18</sup>. In case of ovarian cancer, the estimated cumulative risk for *RAD51C* carriers is approximately 1% by age 49, and the risk at age 80 is approximately 6-11%<sup>19</sup>. In *RAD51D* carriers, the estimated cumulative risk of ovarian cancer is approximately 1% by age 49 and is approximately 13-14% at age 80<sup>19</sup>. There are some studies that correlate *RAD51* pathogenic variants and endometrial cancer, however, available data concerning this association could not reach a consensus<sup>5,20</sup>.

*RAD51* gene family is infrequently associated with the development of non-gynecological cancers, such as pancreatic and prostate cancer, and squamous cell carcinoma of the head and the neck, typically occurring in a small percentage of cases, particularly in hereditary forms where they contribute to the broader spectrum of genetic predispositions<sup>21-23</sup>. Other types of cancer, such as colorectal cancer and acute leukemia, have also been associated with this gene, although evidence supporting this relation is limited<sup>23</sup>.

Management of *RAD51* pathogenic variants carriers is still in debate. According to the National Comprehensive Cancer Network (NCCN) guidelines, those individuals should start annual mammograms and breast Magnetic Resonance Imaging (MRI) beginning at age 40<sup>7</sup>. The current evidence does not support a universal recommendation for risk-reducing mastectomy (RRM); however, it may be a reasonable option for those with a significant family history or additional risk factors such as atypia or a prior breast cancer diagnosis<sup>7</sup>. Risk-Reducing Bilateral Salpingo-Oophorectomy (RRBSO) is advised between the ages of 45-50, or earlier if there is family history of ovarian cancer, especially if the onset was at a young age, according to the NCCN guidelines and the European Society of Medical Oncology guidelines<sup>7,24</sup>. We also inform women that the use of oral contraceptives could help reduce the risk of ovarian cancer, as seen in women with pathogenic variants in *BRCA1/2*<sup>25</sup>. Currently, there are no guidelines for the prevention of other types of cancers that may be associated with carriers of these variants.

The lack of available data on the Portuguese population regarding pathogenic variants in *RAD51* gene family highlights the importance of this study. Therefore, our work aimed to characterize families with pathogenic variants in *RAD51*, along with the associated outcomes and follow-ups.

## METHODS

A retrospective study was carried out including all families with *RAD51* pathogenic variants, followed in the familial cancer risk clinic, at Hospital Dr. Nélito Mendonça, in Madeira Island, from 30-11-2018 to 30-11-2024.

In our center, *RAD51* testing is included in multigene panels for hereditary cancer risk assessment. Peripheral blood DNA sample is used for *Next-Generation Sequencing* (NGS) (*BRCA1*, *BRCA2*, *CHEK2*, *PALB2*, *TP53*, *ATM*, *BARD1*, *BRIP1*, *CDH1*, *EPCAM*, *MLH1*, *MSH2*, *MSH6*, *NBN*, *PMS2*, *PTEN*, *RAD51B*, *RAD51C*, *RAD51D*, *STK11*, *XRCC*) and for *Multiplex Ligation-dependent Probe Amplification* (MLPA) (to identify *BRCA1/BRCA2* germline founder variants and to test for large genomic rearrangements); *Institute of Pathology and Molecular Immunology of the University of Porto – IPATIMUP – Diagnostics*.

In cases of ovarian cancer, NGS was performed in DNA extracted from ovarian tissue (*ATM*, *BRIP1*, *MLH1*, *MSH2*, *MSH6*, *PALB2*, *PMS2*, *RAD51C* and *RAD51D*; using the *TruSight Hereditary Cancer Panel*, *Illumina – Portuguese Oncology Institute of Porto*). All deleterious variants and variants of uncertain significance identified by NGS were confirmed by Sanger sequencing following a standard protocol. Here, MLPA was also done as above.

After a pathogenic variant is identified in the family, the index case and other individuals at high risk are offered a referral to an oncogenetic appointment. When appropriate, genetic prenatal counseling is also offered.

Data was collected through a review of medical records, encompassing baseline characteristics.

We then focused on identifying and characterizing index cases, particularly examining the presence of germline *RAD51* variants and the associated neoplasia

in these patients. For each index case, we conducted a detailed family analysis, identifying and characterizing relatives who tested positive for *RAD51* pathogenic variants (Familial variant identification by enzymatic amplification of DNA by *Polymerase Chain Reaction* and direct sequencing of the amplified fragments; *IPATI-MUP Diagnostics* or *Joaquim Chaves Laboratories*). This included recording the total number of individuals tested and their results. Additionally, we documented the specific cancers diagnosed in affected individuals within these families.

Due to the study design, we characterized our sample using descriptive statistics. No other statistical tests were performed.

This research project (No. S25002672) was approved by the Ethics Committee of the Hospital Dr. Nélio Mendonça, SESARAM, EPERAM in April 2025.

## RESULTS

We identified ten index cases with *RAD51* pathogenic variants and all of them were female. Indications for test were: four breast cancer (bilateral, triple negative or at young age), three ovarian cancer, one fallopian tube carcinoma, one colorectal cancer with microsatellite instability and one due to family history. The age at breast cancer diagnosis ranged from 36-55 years, and at ovarian cancer diagnosis from 52-82 years. In Table I, we characterize all index cases.

From all cases we had one death, 36 years after breast cancer diagnosis due to metastatic colorectal cancer. The remaining breast cancer cases are under follow-up and were advised to undergo prophylactic salpingo-oophorectomy between the ages of 45-50. In Table I, we detailed all outcomes.

We identified seven different *RAD51* gene pathogenic variants: three in *RAD51C* and four in *RAD51D*. In Table I, we specify all pathogenic variants observed in our population, and in Figure 1, we present the geographic distribution of these variants.

From the 36 individuals tested for family pathogenic variant, 21 of them tested positive. From those, six had previous diagnosis of neoplasia: two cases of ovarian cancer; two cases of breast cancer; one case of uterine leiomyosarcoma and one case of

carcinoid tumor of the bronchus. We characterize all families with *RAD51* pathogenic variants in Table II.

In line with NCCN guidelines, all positive non-affected females were advised to undergo prophylactic salpingo-oophorectomy between the ages of 45-50 and breast cancer surveillance starting at age 40. Additionally, earlier screening for other associated neoplasia, like colorectal cancer, were initiated to further mitigate risk and ensure timely detection.

## DISCUSSION

Variants in the *RAD51* gene are uncommon genetic changes that are important in DNA repair by homologous recombination, a critical process for preserving genomic stability<sup>3</sup>. Pathogenic variants in *RAD51*, especially in its paralogs *RAD51C* and *RAD51D*, have been associated with a higher chance of developing hereditary breast and ovarian cancers<sup>3</sup>. These alterations hinder the ability of DNA repair, increasing the chances of genetic mutations building up, which can facilitate the growth of cancer<sup>3</sup>. Even though breast and ovarian cancers are linked diseases, *RAD51* pathogenic variants could also play a role in increasing the risk of other types of cancer. This underscores the significance of including them in genetic screenings and cancer prevention plans.

Studies have identified an increased risk of colorectal cancer and, in some cases, peritoneal carcinoma in individuals carrying *RAD51* variants<sup>21-23</sup>. There is limited evidence regarding the association of these variants with lung carcinoid tumors, though some data suggest a link to lung adenocarcinomas<sup>27</sup>. At the same time, while gynecological non-epithelial tumors like uterine sarcomas are associated with genomic instability, including *BRCA* impairment, there is no clear evidence linking *RAD51* variants to an increased risk of sarcoma development; however, there are some clinical cases that found some association between *RAD51D* pathogenic variants and retroperitoneal leiomyosarcoma<sup>28</sup>. Although these associations are less well-characterized, they highlight the potential broader spectrum of cancer risks linked to *RAD51* variants, underscoring the need for further research and consideration of ear-

**TABLE I. CHARACTERIZATION OF INDEX CASES.**

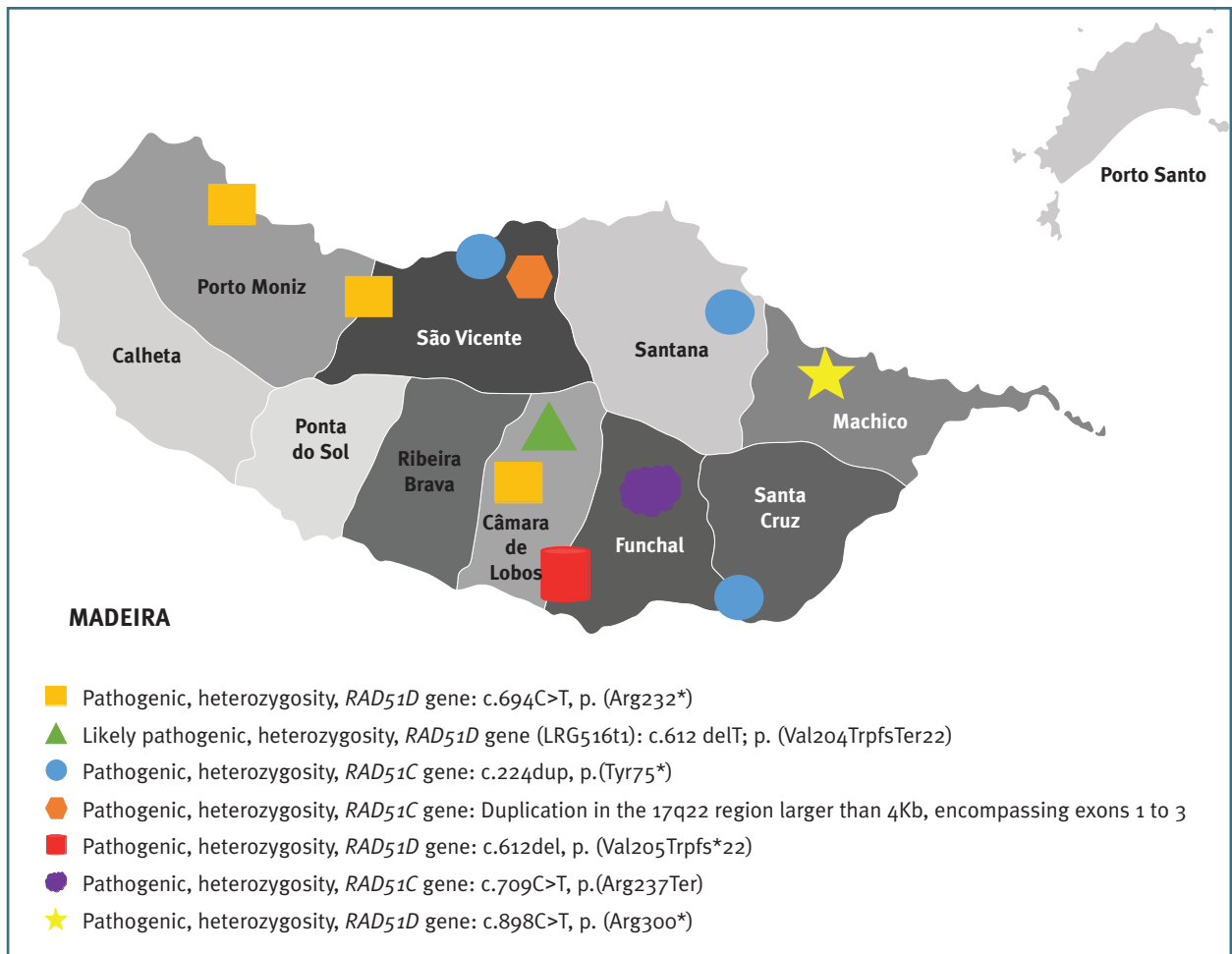
Index case	Age at diagnosis	Sex	Cancer diagnosis year	Genetic test indication	Neoplasia histological type	Pathogenic variant	Result of mutation	Outcome
1	36y	Female	2005	Breast cancer at young age	Unknown	Pathogenic variant, identified in heterozygosity, in the <i>RAD51D</i> gene: c.694C>T, p. (Arg232*)	Premature stop codon	Prophylactic adnexectomy. Follow-up at breast pathology team
2	53y – Left breast cancer; 55y – Right breast cancer	Female	1987-1989	Bilateral breast cancer	Unknown	Likely pathogenic variant, identified in heterozygosity, in the <i>RAD51D</i> gene: c.612 delT, p. (Val204TrpfsTer22)	Premature stop codon	Colorectal cancer with liver metastasis – death in 2022
3	69y	Female	2020	Ovarian cancer	High-grade serous carcinoma of the ovary	Pathogenic variant, identified in heterozygosity, in the <i>RAD51D</i> gene: c.694C>T, p. (Arg232*)	Premature stop codon	Intraoperative diagnosis of Peritoneal carcinomatosis; One year later with disease progression with metastasis to the hepatic hilum and subcapsular region, lombo-aortic and iliac metastasis, and vaginal cuff metastases; Currently under palliative chemotherapy.
4	58y	Female	2020	Ovarian cancer	High-grade serous carcinoma of the ovary	Pathogenic variant, identified in heterozygosity, in the <i>RAD51C</i> gene: c.224dup, p. (Tyr75*)	Premature stop codon	Inoperable recurrence; Peritoneal metastasis; subcapsular liver implants; retrogastric adenomegaly; lombo-aortic adenomegaly; cystic lesion of the abdominal wall; retrorectal/presacral lesion. Currently, the disease is stable and under palliative chemotherapy.

(continues)

TABLE I. CHARACTERIZATION OF INDEX CASES (CONTINUED).

Index case	Age at diagnosis	Sex	Cancer diagnosis year	Genetic test indication	Neoplasia histological type	Pathogenic variant	Result of mutation	Outcome
5	49y	Female	2022	Triple negative breast cancer	Triple negative breast carcinoma	Pathogenic variant, identified in heterozygosity, in the RAD51C gene: c.224dup, p.(Tyr75*)	Premature stop codon	Prophylactic adnexectomy. Follow up with breast pathology team
6	72y	Female	2023	MSI-H CR cancer	Moderately differentiated adenocarcinoma of the hepatic flexure of the colon with associated microsatellite instability	Pathogenic variant, identified in heterozygosity, in the RAD51C gene: Duplication in the 17q22 region larger than 4Kb, encompassing exons 1 to 3	Large duplication	Follow up with surgical and gastroenterology team
7	52y	Female	2024	Fallopian tube carcinoma	High-grade serous adenocarcinoma of the fallopian tubes	Pathogenic variant, identified in heterozygosity, in the RAD51D gene: c.612del, p.(Val205Trpfs*22)	Premature stop codon	Peritoneal carcinomatosis under palliative care
8	82y	Female	2021	Ovarian cancer	High-grade serous carcinoma of the ovary	Pathogenic variant, identified in heterozygosity, in the RAD51C gene: c.709C>T, p.(Arg237Ter)	Premature stop codon	Follow up with gynecological and oncology team
9	34y	Female	NA	Family history	NA	Pathogenic variant, identified in heterozygosity, in the RAD51D gene: c.694C>T, p.(Arg232*)	Premature stop codon	Healthy Follow up with gynecological team
10	44y	Female	2009	Breast cancer at young age	Ductal Carcinoma In Situ	Pathogenic variant, identified in heterozygosity, in the RAD51D gene: c.898C>T, p.(Arg300*)	Premature stop codon	Follow-up at breast pathology team

y- years; MSI-H CR cancer- Microsatellite instability-High colorectal cancer; NA – Not applied



**FIGURE 1.** Geographic distribution of *RAD51* pathogenic variants in Madeira families.

ly screening protocols for these additional cancer types in high-risk individuals.

Due to the small sample size and retrospective nature of our research, it is difficult to definitively establish a causal relationship between *RAD51* variants and these additional cancer types. While our findings suggest potential associations, the limitations of our study prevent us from drawing definite conclusions or extrapolating these results to larger populations.

For those with pathogenic variants in *RAD51* paralogs, we promote an annual mammogram and offer breast MRI starting at age 40. The evidence is insufficient to uniformly recommend RRM, although for those with a concerning family history or other risk factors (e.g., atypia or a diagnosis of breast cancer), it may be reasonable for carriers to consider this option<sup>7</sup>. Screening

for ovarian cancer is not useful for women with moderate risk but could be beneficial for those with high-risk factors such as Hereditary Ovarian Carcinoma (HOC) pathogenic variants or a family history of the disease. Screening with a combination of serial CA-125 and pelvic ultrasound is commonly used for women in this high-risk group. NCCN guidelines note this strategy can be considered but that it has not been shown to decrease ovarian cancer mortality<sup>7</sup>. To the best of our knowledge, there are no other specific guidelines that outline screening and counseling protocols for individuals with *RAD51* pathogenic variants. Research conducted on populations with a high risk of ovarian cancer has indicated that using pelvic ultrasound and CA-125 for screening can lead to earlier stage detection and potentially enhance survival rates<sup>29,30</sup>. However, no

**TABLE II. CHARACTERIZATION OF FAMILIES WITH *RAD51* GENE PATHOGENIC VARIANTS.**

Family number	Heritage	Familiar pathogenic variant	Families' members tested (n)	Positive families' members (n)	Affected families' members (n)	Ongoing results	Neoplasias of affected families' members (n)
1	Seixal/São Vicente	Pathogenic variant, identified in heterozygosity, in the <i>RAD51D</i> gene: c.694C>T, p. (Arg232*)	4	2	1	1	Breast cancer (1)
2	Câmara de Lobos	Likely pathogenic variant, identified in heterozygosity, in the <i>RAD51D</i> gene (LRG516t1): c.612 delT; p. (Val204TrpfsTer22)	6	4	2	–	Carcinoma of unknown primary (ovarian origin probably) (1) Leiomyosarcoma (1)
3	Câmara de Lobos	Pathogenic variant, identified in heterozygosity, in the <i>RAD51D</i> gene: c.694C>T, p. (Arg232*)	12	8	0	–	–
4	Faial/Caniço	Pathogenic variant, identified in heterozygosity, in the <i>RAD51C</i> gene: c.224dup, p.(Tyr75*)	8	2	2	3	Ovarian cancer (1) Breast cancer (1)
5	Boaventura/ /Caniço	Pathogenic variant, identified in heterozygosity, in the <i>RAD51C</i> gene: c.224dup, p.(Tyr75*)	2	2	1	–	Carcinoid tumor of the bronchus (1)
6	Boaventura	Pathogenic variant, identified in heterozygosity, in the <i>RAD51C</i> gene: Duplication in the 17q22 region larger than 4Kb, encompassing exons 1 to 3	0	0	0	–	–
7	Funchal/ /Câmara de Lobos	Pathogenic variant, identified in heterozygosity, in the <i>RAD51D</i> gene: c.612del, p. (Val205Trpfs*22)	2	1	–	1	–
8	Monte	Pathogenic variant, identified in heterozygosity, in the <i>RAD51C</i> gene: c.709C>T, p.(Arg237Ter)	2	2	–	–	–
9	Porto Moniz	Pathogenic variant, identified in heterozygosity, in the <i>RAD51D</i> gene: c.694C>T, p. (Arg232*)	*	*	*	*	*
10	Machico	Pathogenic variant, identified in heterozygosity, in the <i>RAD51D</i> gene: c.898C>T p.(Arg300*)	*	*	*	*	*

\*waiting family risk appointment for detailed family analysis

specific survival benefit for ovarian cancer has been proven<sup>29,30</sup>. In the absence of data on screening for women with non-*BRCA* HOC variants, it is reasonable to consider this approach for this group<sup>29,30</sup>. Surgical risk-reduction with bilateral salpingo-oophorectomy is the mainstay of management for women with HOC variants between the ages of 45-50<sup>7</sup>. Chemoprevention using birth control pills can decrease cancer chances for women with HOC who want contraception but have not completed childbearing or undergone RRBSO<sup>4</sup>. Two research studies indicate that aspirin could lower the risk of ovarian cancer in the general population and this strategy might be beneficial for women with HOC mutations<sup>4</sup>. An analysis combining data from the Ovarian Cancer Association Consortium revealed a slight correlation between aspirin use and a lower risk of ovarian cancer (OR 0.91 95% Confidence Interval: 0.84-0.99)<sup>31</sup>. In a study conducted in Denmark, it was discovered that there was no variation in the risk of epithelial ovarian cancer among occasional aspirin users. However, individuals who used aspirin continuously and over a long period of time had a reduced risk of developing this type of cancer<sup>32</sup>.

Although *RAD51* pathogenic variants are mostly associated with an increased risk of breast and ovarian cancers, recent evidence suggests that these variants may also elevate the risk for other types of cancers<sup>23</sup>. Despite the absence of formal screening guidelines for cancers outside the breast and ovarian categories, clinicians are increasingly recognizing the potential value of early detection in these other cancers. As part of our practice, we offer earlier screening for cancers like colorectal cancer to individuals with *RAD51* pathogenic variants and colorectal cancer in the family. This proactive approach aims to identify and manage malignancies at an earlier stage, improving patient outcomes in the absence of specific guidelines.

At our center, during genetic counseling, the risks associated with a biallelic pathogenic variant in the *RAD51C* gene, such as Fanconi Anemia Group O, an autosomal recessive inherited disorder, are also explained, along with the technical possibilities and limitations of Preimplantation Genetic Testing (PGT). If the couple wishes, and following appropriate pre-test genetic counseling of the partner, *RAD51C* gene sequencing is offered. If the result is positive, the couple is re-

ferred to a Medically Assisted Reproduction consultation.

Preimplantation Genetic Testing for Monogenic Diseases (PGT-M) could only be carried out upon prior authorization request, as this gene is not yet included in the current exemption list, according to the specific table updated in June 2024<sup>33</sup>.

To the best of our knowledge, this is the first Portuguese case series to describe the phenotypes associated with pathogenic variants in the *RAD51* gene. Our study provides valuable insights into families with *RAD51* pathogenic variants, shedding light on a topic that has been relatively underexplored in existing research. Considering the lack of information on comparable families with the same mutations, our results highlight the significance of recording these instances to improve comprehension of the genetic cancer risks linked to *RAD51*, specifically non-gynecological cancers. This study demonstrates a potential association between non-gynecological cancers and variants in this gene, addressing a knowledge gap but also underlines the importance of raising awareness and conducting more research on the impacts and situations of families affected by the issue. Furthermore, our research sets the groundwork for future studies, opening the door for more thorough research that could enhance genetic counseling and targeted screening approaches for at-risk individuals.

Our research is limited in terms of generalizability due to the small sample size. The study's retrospective nature also brings in possible biases such as incomplete or inconsistent medical records, which hinders the capture of all necessary data. These factors restrict our capacity to generalize or extend results to wider demographics. However, the research offers important information about the connection between *RAD51* variants and cancer susceptibility, underscoring the necessity for more extensive studies with larger groups of participants to confirm these initial findings.

The Autonomous Region of Madeira is a Portuguese archipelago that includes two inhabited islands: Madeira Island and Porto Santo, with an approximate population of 267,000 people<sup>26</sup>. We should highlight that the sample under investigation is based on a geographically isolated population, which is expected to re-

sult in greater genetic homogeneity. Additionally, the population may exhibit a founder effect, which warrants further exploration in subsequent studies. A genetically more uniform population, residing within the same geographic region, could potentially provide more robust evidence for the phenotypic traits identified. However, for the genes in question, the studied sample demonstrated considerable heterogeneity in variants and geographic distribution (Figure 1), suggesting that the results do not support the presence of a founder effect. Furthermore, establishing a definitive phenotype-genotype relationship remains challenging.

Considering the findings from the present study, it is important to highlight that further research is necessary. Specifically, a national, multicenter study encompassing all identified families would be crucial for enhancing the precision of the phenotype-genotype correlation. A comparative analysis of data from such a national cohort, alongside population-level data, could provide valuable new insights into the non-classical neoplasms identified in our study, which are not typically associated with pathogenic variants in the *RAD51* gene (e.g., colorectal cancer, leiomyosarcoma, carcinoid tumor).

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#### AUTHORS CONTRIBUTIONS

Gonçalo Freitas and Inês Sargaço: Data curation, formal analysis, methodology and writing. Sara Câmara: Conceptualization, resources, formal analysis, supervision, validation and writing review. Ana Sofia Alves: Resources; Rita Freitas: Validation and writing review.

This article was co-authored by Gonçalo Freitas and Inês Sargaço.

#### CONFLICT OF INTEREST

There are no conflicts of interests.

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**RECEIVED:** 03/04/2025

**ACCEPTED:** 21/11/2025