Study of DNA repair pathways in breast cancer with or without germline *BRCA1/2* mutations

Doctoral thesis dissertation presented

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To apply for the degree of doctor at the University of Barcelona

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ABBREVIATIONS

- **AF:** Allele Frequency
- **ACMG:** American College of Medical Genetics and Genomics
- **AI:** Aromatase Inhibitor
- **AMP:** Association for Molecular Pathology
- **BCS:** Breast-Conserving Surgery
- **BER:** Base Excision Repair
- **CBC:** Contralateral Breast Cancer
- **CHIP:** Clonal Hematopoiesis of Indeterminate Potential
- **CNV:** Copy Number Variation
- **CPM:** Contralateral Prophylactic Mastectomy
- **DSB:** Double-Strand Break
- **ER:** Estrogen Receptor
- **ESMO:** European Society for Medical Oncology
- **ET:** Endocrine Therapy
- **gPV:** germline Pathogenic or Likely Pathogenic Variant
- **HBOC:** Hereditary Breast and Ovarian Cancer Syndrome
- **HER2:** Human Epidermal Growth Factor Receptor 2
- **HER2–:** Human Epidermal Growth Factor Receptor 2–Negative
- **HR:** Hazard Ratio
- **HR+:** Hormone Receptor–Positive
- **HR+/HER2–:** Hormone Receptor–Positive, HER2-Negative Breast Cancer
- **HRD:** Homologous Recombination Deficiency
- **HRR:** Homologous Recombination Repair
- **IARC:** International Agency for Research on Cancer
- **IDFS:** Invasive Disease-Free Survival
- **IHC:** Immunohistochemistry
- **LAFV:** Low Allele Frequency Variant
- **LOF:** Loss of Function
- **LOH:** Loss of Heterozygosity
- **MLPA:** Multiplex Ligation-dependent Probe Amplification
- **MRI:** Magnetic Resonance Imaging
- **NCCN**: National Comprehensive Cancer Network
- **NER:** Nucleotide Excision Repair
- **NGS:** Next-Generation Sequencing
- **NHEJ:** Non-Homologous End Joining
- **OR:** Odds Ratio
- **OS:** Overall Survival
- **PARPi:** Poly (ADP-ribose) Polymerase Inhibitors
- **PCR:** Polymerase Chain Reaction

- **pCR:** Pathologic Complete Response
- **PR:** Progesterone Receptor
- **PRS:** Polygenic Risk Score
- **PV:** Pathogenic or Likely Pathogenic Variant
- **RRM:** Risk-Reducing Mastectomy
- **RRSO:** Risk-Reducing Salpingo-Oophorectomy
- **SERM:** Selective Estrogen Receptor Modulator
- **TNBC:** Triple-Negative Breast Cancer
- **US:** Ultrasound
- **VAF:** Variant Allele Frequency
- **VUS:** Variant of Uncertain Significance

THESIS IN COMPENDIUM OF PUBLICATIONS

The thesis consists of one general objective and 9 specific objectives and three articles:

1. **First publication:**

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Low Allele Frequency Variants Identified on Germline Multi-Gene Panel Testing for Cancer Predisposition Can Suggest the Presence of Constitutional Mosaicism.
Clinical Cancer Research. 2025;31(13):2814–2823.
Impact factor (JCR 2025): 10.2
Quartile: Cancer Research (Q1); Oncology (Q1)

2. **Second publication:**

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ESMO Open. 2025;10(4):104543.
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Quartile: Q1

3. **Third publication (not published, under-review):**

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RESUM DE LA TESIS

Títol: Estudi de les vies de reparació del DNA en càncer de mama amb o sense mutacions germinals en *BRCA1/2*

Introducció: El càncer de mama continua sent la neoplàsia més freqüent entre les dones a escala mundial mundial i una de les principals causes de mortalitat associada al càncer. Els avenços en el coneixement molecular del càncer de mama han permès una millor estratificació del risc, una aproximació diagnòstica més precisa i el desenvolupament d'estratègies terapèutiques personalitzades. En aquest context, la genètica germinal s'ha convertit en un element clau tant en la pràctica clínica com en la recerca translacional.

L'estudi genètic germinal permet identificar variants patogèniques o probablement patogèniques en gens associats a la predisposició hereditària al càncer de mama i d'ovari, especialment aquells implicats en les vies de reparació de l'ADN i en altres processos biològics rellevants. No obstant això, l'ús de panells multigènics ha incrementat la complexitat interpretativa, especialment en relació amb les variants de baixa freqüència al·lèlica (low-allele-frequency variants, LAFVs), que poden reflectir mosaicisme constitucional o altres fenòmens biològics.

S'estima que aproximadament entre el 5% i el 10% dels casos de càncer de mama presenten variants germinals patogèniques. Les mutacions germinals en *BRCA1* i *BRCA2* representen el model millor caracteritzat de càncer de mama hereditari i una proporció significativa dels casos associats a defectes en la recombinació homòloga. La seva identificació té implicacions clíniques directes en les estratègies de prevenció i en la selecció de tractaments sistèmics, incloent la quimioteràpia amb platins i els inhibidors de la poli(adenosina difosfat–ribosa) polimerasa (PARPi).

Hipòtesi: La caracterització precisa de les alteracions germinals en les vies de reparació de l'ADN en el càncer de mama, incloses aquelles variants que requereixen una interpretació complexa com les LAFVs, pot millorar l'avaluació del risc, optimitzar el maneig clínic i refinar la presa de decisions terapèutiques. En particular, les variants patogèniques en gens implicats en la recombinació homòloga poden definir subgrups de pacients amb característiques clíniques, pronòstiques i terapèutiques diferenciades. Així mateix, l'estudi d'aquestes alteracions en diferents contextos poblacionals i assistencials pot aportar informació rellevant sobre la seva aplicabilitat clínica en la pràctica real.

Objectius: L'objectiu principal d'aquest estudi és avaluar la utilitat clínica, els reptes interpretatius i les implicacions terapèutiques de les variants germinals patogèniques implicades en les vies de reparació de l'ADN en pacients amb càncer de mama.

Mètodes: Aquest treball es basa en l'anàlisi retrospectiva de diverses cohorts de pacients amb càncer de mama sotmeses a estudis genètics germinals mitjançant panells multigènics.

El primer estudi va incloure individus procedents d'un laboratori de referència internacional (Ambry Genetics) i es va centrar en la identificació i caracterització de variants de baixa freqüència al·lèlica, definides com aquelles amb freqüències al·lèliques entre el 10% i el 30%, així com en les seves associacions clíniques amb el diagnòstic de qualsevol tipus de càncer, incloent el càncer de mama, així com amb l'edat, l'origen ètnic i la sospita de mosaicisme constitucional.

El segon estudi es va desenvolupar a partir d'una cohort espanyola amb càncer de mama, amb una recollida detallada de dades clíniques, patològiques i de tractament, amb l'objectiu d'analitzar l'impacte de les variants germinals patogèniques, especialment en gens relacionats amb la recombinació homòloga i els gens associats a la síndrome de Lynch.

El tercer estudi va avaluar l'impacte de les alteracions germinals en gens de reparació de l'ADN en el context del tractament de primera línia amb inhibidors de les quinases dependents de ciclina 4 i 6 en pacients amb càncer de mama avançat receptor hormonal positiu i HER2-negatiu. Aquest estudi es va dur a terme en el marc d'una col·laboració multicèntrica estatal a través del grup SOLTI. Les variants germinals es van classificar segons criteris estandarditzats de patogenicitat. Es van realitzar anàlisis descriptives i comparatives per avaluar diferències clíniques entre pacients portadores de variants patogèniques i pacients sense alteracions germinals identificades, així com associacions amb la resposta al tractament sistèmic i la supervivència.

Principals resultats: En el primer estudi es van incloure 363.405 individus. Les LAFVs es van identificar com un hallatge infreqüent en els estudis genètics germinals (0,8%). Malgrat la seva baixa prevalença, una proporció rellevant es va detectar en gens com *TP53, NF1, CHEK2, ATM* i *BRCA1*. Les LAFVs es van identificar amb més freqüència en individus d'edat avançada i en persones amb diagnòstic de càncer, especialment càncer de mama, en comparació amb portadors de variants heterozigotes convencionals. Les proves complementàries van confirmar mosaicisme constitucional en una proporció significativa de casos (11 de 62; 17,7%), posant de manifest la necessitat d'una interpretació acurada.

El segon estudi va incloure 912 pacients i va mostrar que una proporció significativa de pacients amb càncer de mama presentava variants germinals patogèniques (14,1%), amb predomini de *BRCA1* (24%) i *BRCA2* (31,8%). Aquestes pacients van ser diagnosticades a una edat més jove i van presentar amb més freqüència malaltia bilateral, subtipus triple negatiu i estadis més avançats en el moment del diagnòstic. La identificació de variants patogèniques va influir clarament en les decisions quirúrgiques i en l'ús de teràpies sistèmiques dirigides, especialment PARPi.

El tercer estudi va incloure 233 pacients amb càncer de mama receptor hormonal positiu/HER2-negatiu: 116 amb variants patogèniques en un gen de recombinació homòloga i 117 controls aparellats amb estudi germinal negatiu. Les pacients amb variants patogèniques en *BRCA2* tractades amb inhibidors de CDK4/6 van presentar una supervivència lliure de progressió significativament inferior en comparació amb els controls, especialment en el context de malaltia endocrinosensible. Les anàlisis moleculars exploratòries van identificar una elevada prevalença de pèrdua d'heterozigositat de RB1 en tumors de portadores de *BRCA2*, suggerint mecanismes biològics associats a resistència precoç al tractament.

Conclusions: Aquest treball posa de manifest la rellevància clínica de les alteracions germinals en les vies de reparació de l'ADN en el càncer de mama i destaca la necessitat d'una interpretació integrada dels resultats genètics. La identificació de variants patogèniques, incloses les de baixa freqüència al·lèlica, té un impacte directe en el maneig clínic, la selecció de tractaments i les estratègies de prevenció. Aquests resultats donen suport a la integració sistemàtica de la genètica germinal en l'abordatge del càncer de mama i a l'aplicació d'enfocaments multidisciplinaris per optimitzar la medicina de precisió.

Paraules clau: càncer de mama; genètica germinal; reparació de l'ADN; *BRCA1/2*; variants al·lèliques de baixa freqüència.

INTRODUCTION

I. GENERAL INTRODUCTION

1.1 Cancer as a genetic disease

Cancer is a genetic disease and one of the leading causes of disease and death worldwide. According to data from the International Agency for Research on Cancer (IARC), there were approximately 18.1 million new cancer diagnoses in 2020, excluding non-melanoma skin cancers. Projections indicate that this number could growth to 28 million annual cases by 2040(1) **(see Figure 1)**.

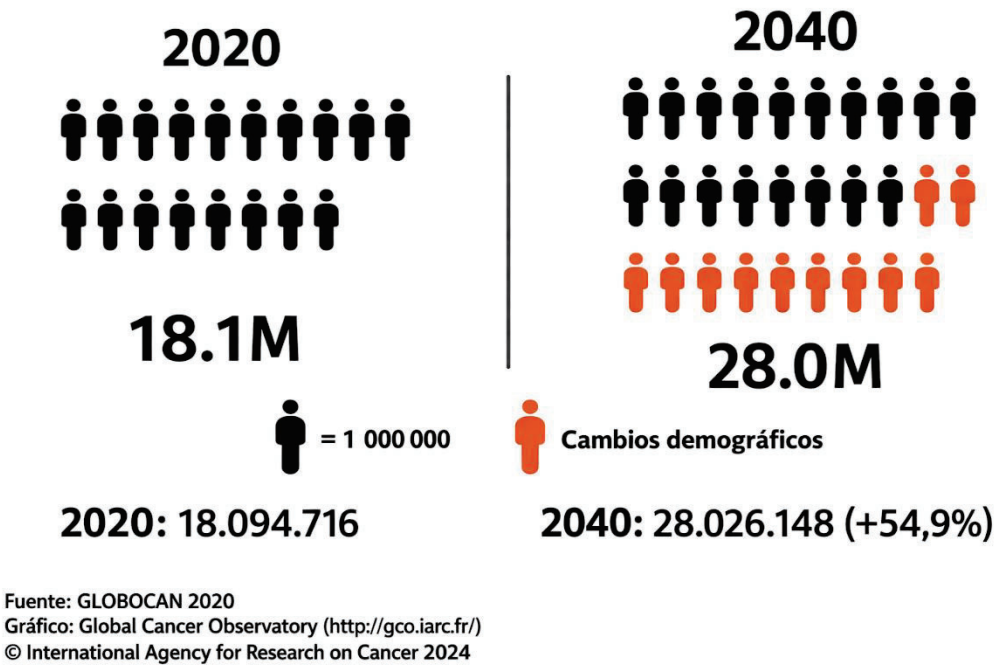


Figure 1. Estimated incidence of cancers in the global population for 2020 and 2040, both sexes (excluding non-melanoma skin cancers) (figure adapted from Globocan 2020(1)).

In Spain, cancer represents a significant public health challenge. According to projections by the Spanish Network of Cancer Registries (REDECAN), approximately 296,103 new cases are expected to be diagnosed by 2025(2). This increase is associated with the aging of the population, demographic growth, and the known risk factors such as smoking, alcohol consumption, obesity, and physical inactivity(3, 4). However, the implementation of early detection programs and certain demographic shifts are expected to help decrease cancer mortality, despite the initial rise in incidence(3, 5-8).

1.2 Multifactorial origin of cancer

Cancer is a multifactorial disease **(see Figure 2),** whose development results from the complex interaction of environmental factors, lifestyle habits, and genetic predisposition.

Based on its origin, cancer can be classified into three main groups(9):

- **Sporadic (75–80%)**: occurs without a defined family pattern, generally linked to environmental or lifestyle factors (diet, alcohol, tobacco).
- **Familial (20%)**: cases cluster within the same family, which may be due to shared (unidentified) genetic factors or common lifestyle exposures (e.g., radon exposure).
- **Hereditary (5–10%)**: caused by inherited pathogenic or likely pathogenic germline variants (gPV) (also referred as mutations) in specific genes associated with an increased cancer risk. These genetic alterations follow different inheritance patterns **(see Figure 3)** and represent a predisposition to developing the disease(10).

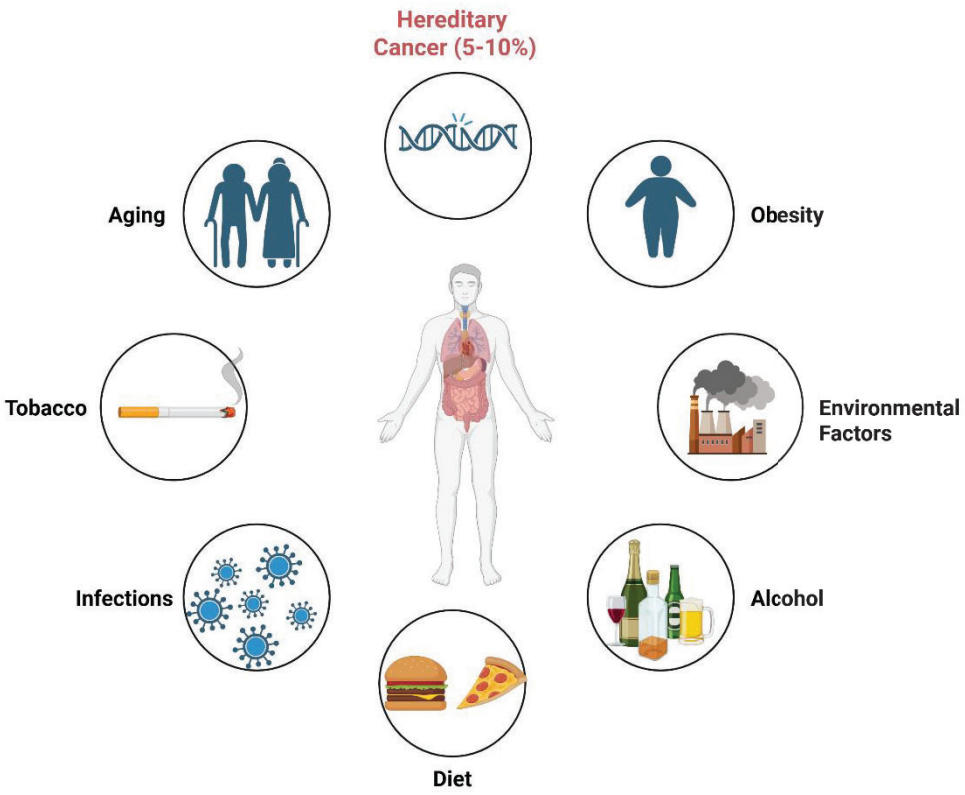


Figure 2. Multifactorial origin of cancer. Risk factors associated with an increased probability of developing cancer (figure created with Biorender)(11).

1.3 Mutations and mechanisms of cancer development

Cancer is a genetic disease. Genetic alterations may involve changes in the Deoxyribonucleic acid (DNA) nucleotide sequence or epigenomic modifications, both of which can affect gene expression. An alteration in the DNA sequence is defined as a mutation. Mutations may occur spontaneously or be induced by environmental factors, and they are fundamental drivers of hereditary conditions and cancer. They are categorized by their nature and impact on gene function(12). The main types include(13, 14):

- **Point mutations:** changes in a single nucleotide, which may be *missense* (changing an amino acid), *nonsense* (creating a premature stop codon), or *silent* (no amino acid change).
- **Insertions and deletions (indels):** addition or loss of nucleotides that may disrupt the reading frame (f*rameshift*).
- **Splice-site mutations:** alterations at intron-exon boundaries that can affect mRNA splicing.
- **Copy number variations (CNVs):** duplications or deletions of larger DNA segments, impacting gene dosage.
- **Structural rearrangements:** such as translocations or inversions that can disrupt genes or regulatory regions.

Most of the evidence on cancer-risk mutations involves protein-truncating variants in the DNA (such as *nonsense mutations, frameshifts*, and *deletions*) that lead to loss of the protein function (LOF)(13, 15).

1.4 Inheritance models and knudson's two-hit hypothesis

The offspring of a person carrying a genetic mutation has a 50% probability of inheriting this alteration, implying a higher risk of developing some types of cancer **(see Figure 3)**(15).

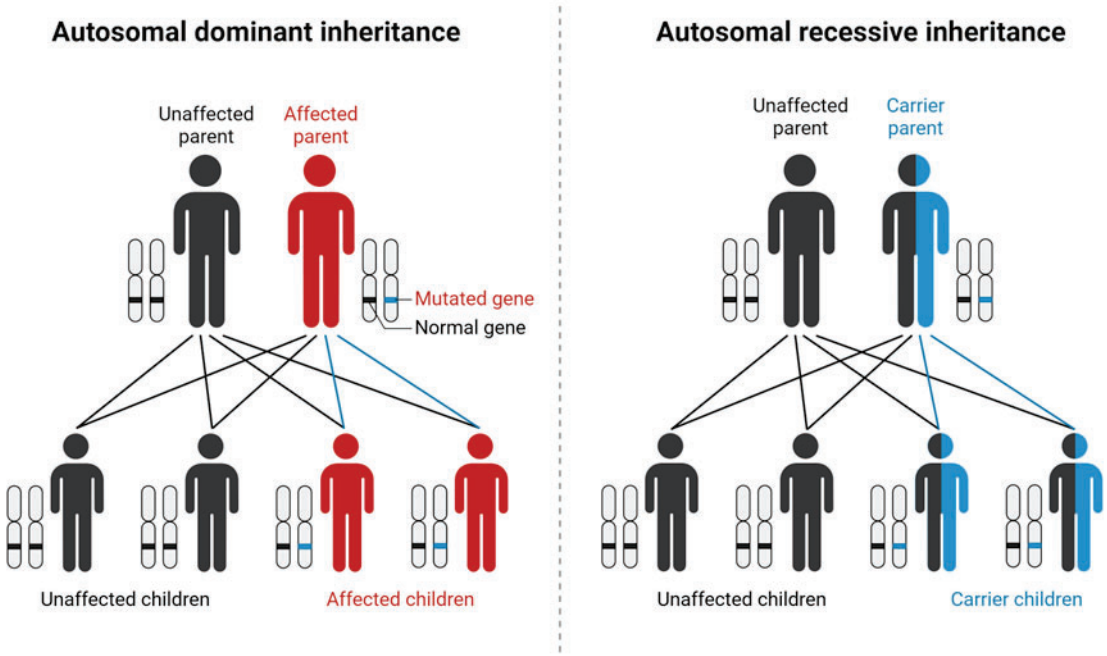


Figure 3. Autosomal dominant and recessive inheritance patterns. Illustrative diagram of genetic transmission in two Mendelian inheritance models: dominant (left, red) and recessive (right, blue), showing the role of affected parents, carriers, and offspring according to genetic status (figure created with Biorender).

We know that cancer results from mutations in specific genes. In 1971, Knudson proposed a mathematical model based on pediatric retinoblastoma cases, illustrating that cancer arises through two genetic hits affecting tumor suppressor genes. In hereditary cancers, individuals inherit the first mutation, requiring only a second somatic event for tumor development. In contrast, sporadic cancer necessitates two independent mutations within the same cell over a person's lifetime. This model also helps explain why hereditary cancers often present at younger ages and are prompt to bilateral involvement, as seen in retinoblastoma among patients with germline pathogenic variants in *RB1*. The Knudson hypothesis has been pivotal in advancing our understanding of cancer genetics and has laid the foundation for genetic testing and targeted therapies **(see Figure 4)(16, 17).**

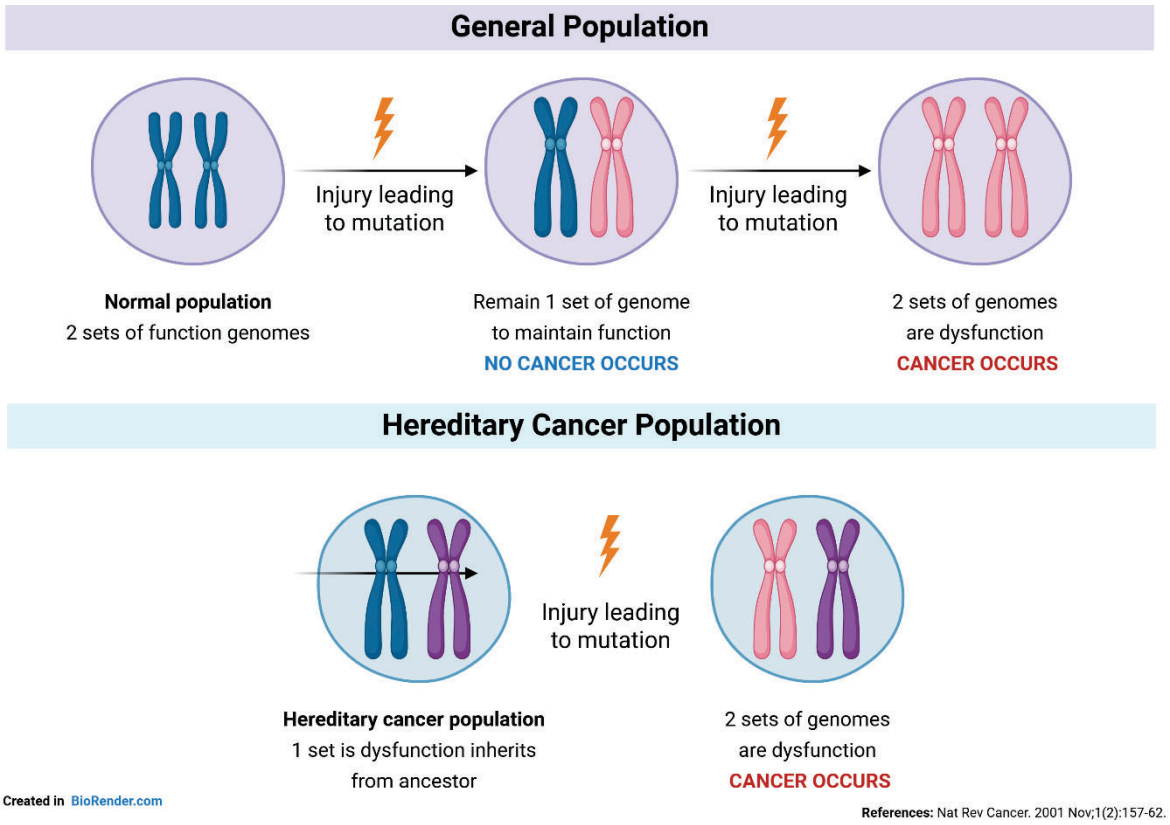


Figure 4. The two-hit model in cancer development(16)**.**
The top panel illustrates the general population: two functional copies (blue) of tumor suppressor genes protect against cancer. A first mutation (pink) inactivates one copy, but function is maintained until a second hit disables the remaining allele, leading to cancer (both pink). The bottom panel shows the hereditary cancer population: individuals inherit one defective allele (purple) and require only a single somatic mutation (pink) for tumor development. Colors indicate gene status: blue = normal, pink = somatic mutation, purple = inherited pathogenic variant (figure adapted from Nat Rev Cancer, 2001 Nov, with Biorender(16)).

Identifying families suspected of having a genetic predisposition to cancer is key to implementing more effective, tailored preventive and surveillance measures. Proper management of these cases requires a personalized risk assessment conducted by professionals properly trained in genetic counseling. This evaluation provides detailed information about an individual's or family's probability of developing cancer, the risk of inheriting the mutation (e.g., reproductive genetic counseling), and available strategies for early detection (e.g., mammograms, colonoscopies), prevention (e.g., chemoprevention, risk-reduction surgeries), and treatment (e.g., immunotherapy, poly (ADP-ribose) polymerase inhibitors (PARPi))(12, 18-20).

II. GERMLINE GENETIC TESTING IN ONCOLOGY

2.1 Indications and hereditary cancer syndromes

In recent years, the indication for germline genetic testing has increased significantly, due to the rising incidence of cancer and the increased need to determine whether its origin is hereditary(18-22). This has significant preventive implications and potential impact on personalized therapeutic decision-making(20, 22-24).

Since the mid-20th century, more than 200 genes associated with increased cancer susceptibility have been described. Germline alterations in these genes are associated with a significantly elevated risk of developing certain cancers, often at younger ages than the general population(25-27).

It is not always possible to identify the genetic alteration responsible for familial cancer. This may be due to technological limitations, the existence of undiscovered genes, or variants of uncertain significance (VUS).

Table 1 summarizes the key hereditary cancer predisposition syndromes along with their associated genes.

Table 1. Key hereditary cancer syndromes (adapted from SEOM textbook)(12).

Disease	Incidence	Gene(s)
Lynch syndrome	1/200-1,000	*MSH2, MLH1, MSH6, PMS2*
Hereditary breast/ovarian cancer (HBOC)	1/500-2,500	*BRCA1, BRCA2, PALB2*
Multiple endocrine neoplasia type 1	2-10/100,000	*MEN1*
Multiple endocrine neoplasia type 2	1/25,000	*RET*
Familial adenomatous polyposis (FAP)	1/8,000-13,000	*APC*
PTEN hamartoma tumor syndrome	1/200,000	*PTEN*
Von Hippel-Lindau syndrome	1/36,000-45,000	*VHL*
Hereditary retinoblastoma	1/13,500-25,000	*RB1*

The criteria for referring patients with cancer or their relatives for germline genetic testing are multiple, and recommendations vary by country and healthcare system.

Below are general criteria applicable to all hereditary syndromes(19, 28, 29):

- Several first-degree relatives on the same side of the family are affected by the same cancer or related tumors.
- Early age of tumor onset.

- Occurrence of multiple malignancies in the same individual.
- Bilateral involvement of paired organs, as seen in bilateral breast or kidney cancer.
- Presence of multiple tumor foci (multifocality) in the same organ.
- Diagnosis of rare tumors.
- Associated with congenital defects or anomalies characteristic of certain syndromes.
- Individuals with relatives carrying a gPV in a cancer susceptibility gene.
- People who meet criteria for hereditary cancer syndrome who previously had negative germline genetic testing with a limited panel or single-gene test, are now seeking broader multigene panel testing.
- If a gPV is identified in tumor genomic analysis with clinical implications, should it also be present at the germline level.

2.2 Methods and modalities of germline testing

Over the past decades, advances in sequencing technologies have profoundly transformed oncology and germline genetic testing. The implementation of multigene panels has enabled the identification of multiple genes associated with hereditary cancer predisposition and prompted the development of targeted therapies for various cancer types(19). Many of the genes studied are involved in key cellular pathways such as DNA repair, cell cycle control, and genomic stability(30).

Germline genetic analysis is an essential tool in evaluating patients suspected of having hereditary cancer(31). In current clinical practice, the most used approach is next-generation sequencing (NGS) of targeted multigene panels, complemented, when necessary, by techniques such as Multiplex Ligation-dependent Probe Amplification (MLPA) to detect structural alterations not reliably captured by NGS(32).

Understanding the genetic material analyzed (DNA and RNA) and the techniques employed is fundamental to interpreting and applying germline testing results to patient care(33).

2.2.1 Genetic material: DNA and RNA

Deoxyribonucleic acid (DNA) is the molecule that stores genetic information in eukaryotic cells. It consists of a double helix of nucleotides whose sequence encodes the instructions needed for protein synthesis. From DNA, ribonucleic acid (RNA) is transcribed, playing key roles in gene expression. The main functional types of RNA are(34):

- **Messenger RNA (mRNA):** transfers coding information from the nucleus to the cytoplasm for protein translation.
- **Transfer RNA (tRNA):** carries amino acids to the ribosome during protein synthesis.
- **Ribosomal RNA (rRNA):** part of ribosome structure, where proteins are assembled.

The study is performed using DNA extracted from peripheral blood lymphocytes of the patient within the family who is the most suitable proband—meaning the individual with the highest likelihood of being a carrier. In all scenarios, NGS technology is employed, supplemented by

copy number variation analysis through MLPA or in silico detection systems. Blood provides higher quality and more stable genetic material, remaining the standard in most clinical laboratories, although samples from saliva, buccal swabs, or hair are viable alternatives **(see Figure 5)**(19).

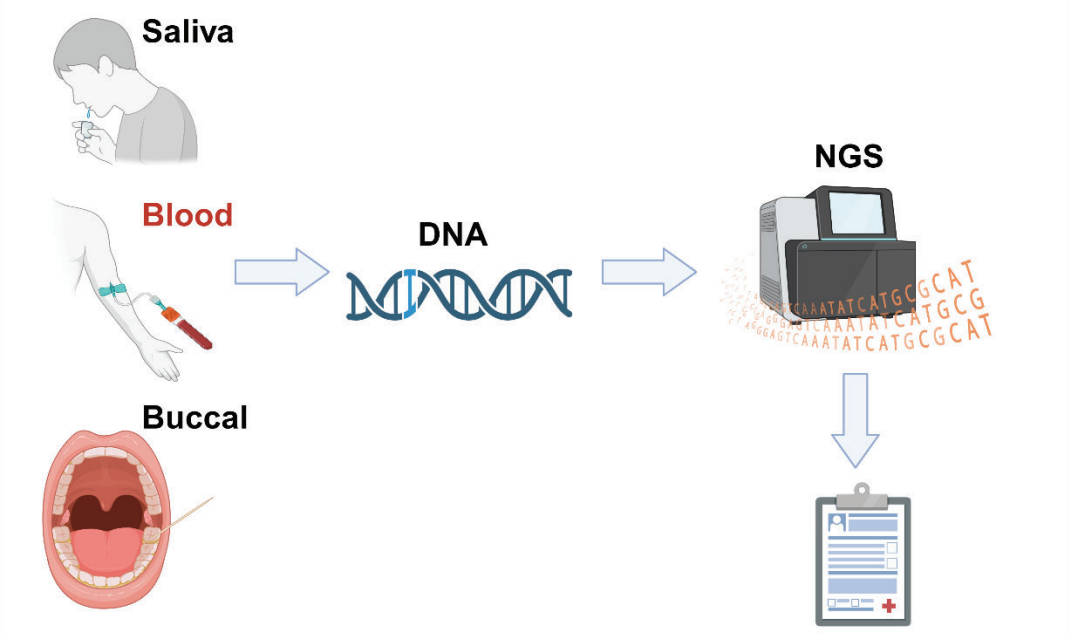


Figure 5. Sources of germline DNA and workflow for genetic testing. DNA for germline analysis is typically extracted from peripheral blood leukocytes, though saliva and buccal swabs are alternative sources. Extracted DNA undergoes NGS to identify germline pathogenic variants, which guide clinical management (figure created with Biorender).

2.2.2 Molecular techniques (PCR, Sanger, NGS, MLPA, Microarrays)

Diagnostic molecular technologies have evolved from targeted methods to large-scale analysis platforms. The main techniques currently employed in germline genetic testing are:

- **PCR (Polymerase Chain Reaction):** an enzymatic technique enabling exponential amplification of specific DNA regions through cycles of denaturation, primer annealing, and extension. It underlies multiple protocols, including Sanger sequencing and some NGS steps. It is also used for targeted validation of specific variants(35).
- **Sanger sequencing:** also known as classical or direct sequencing, is a technique developed by Frederick Sanger in 1977. It involves using DNA polymerase and chain-terminating dideoxynucleotides (ddNTPs) to synthesize DNA fragments in a PCR reaction. During this enzymatic process, DNA polymerase extends a primer complementary to the target DNA by incorporating a mix of standard nucleotides (dNTPs) and fluorescently labeled ddNTPs. Because ddNTPs lack a 3’ hydroxyl group, their incorporation halts further elongation, generating DNA fragments of varying lengths that terminate at specific nucleotides(36).

These fragments are then separated by capillary electrophoresis and detected by fluorescence. Sequencers use a laser to excite the fluorophores, and a detector captures the emitted light. Specialized software converts this data into an electropherogram, displaying peaks that represent the DNA sequence, with each peak color-coded according to the nucleotide (A = adenine, T = thymine, G = guanine, C = cytosine). This method allows precise determination of whether a DNA fragment is altered and identifies the exact change **(see Figure 6).**

Sanger sequencing is highly accurate but can process only a limited number of samples and is not scalable. It is now primarily used to validate variants detected by NGS, especially when they have clinical significance or intermediate allele frequencies.

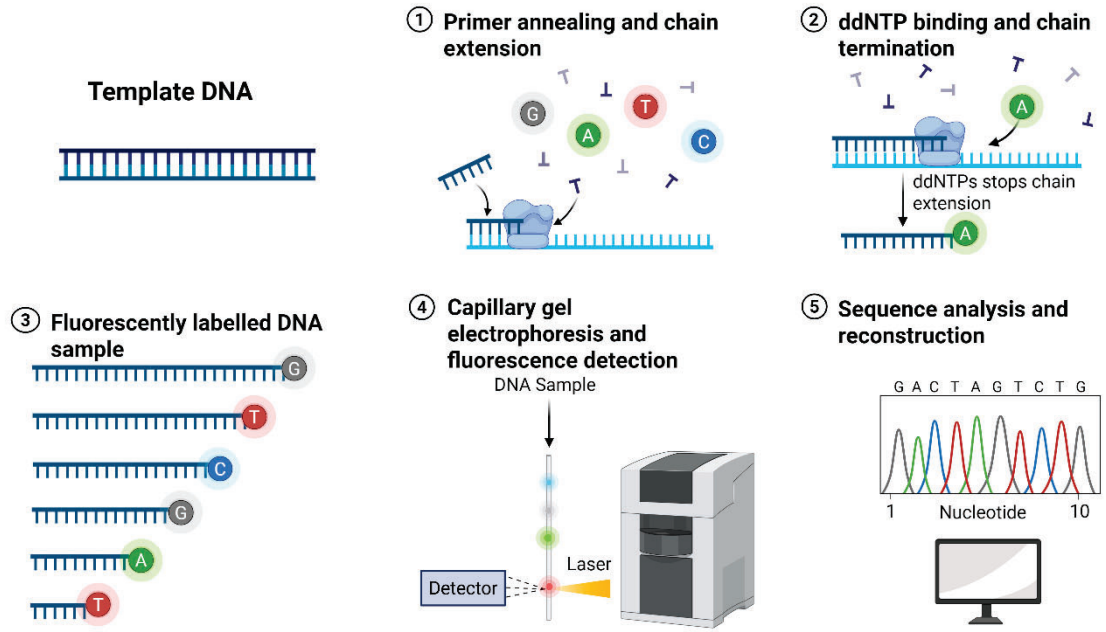


Figure 6. Sanger sequencing workflow.

(1) Primer annealing and DNA strand extension using DNA polymerase.

(2) Incorporation of chain-terminating dideoxynucleotides (ddNTPs) halts elongation at specific bases.

(3) This generates a pool of fluorescently labeled DNA fragments of varying lengths.

(4) Fragments are separated by capillary gel electrophoresis and detected by fluorescence.

(5) Software reconstructs the DNA sequence, visualized as an electropherogram with peaks corresponding to each nucleotide (A = adenine, T = thymine, G = guanine, C = cytosine). Figure created with Biorender.

- **NGS:** Next-generation sequencing enables massively parallel analysis of multiple genes or genomic regions, significantly reducing the cost and turnaround time of genomic sequencing. In clinical practice, targeted multigene panels that include cancer predisposition genes (such as *BRCA1, BRCA2, TP53, CHEK2*, among others) are commonly used. Various NGS platforms differ in their underlying technology or

chemistry, leading to differences in the size of DNA fragments they can sequence, the coverage achieved, and the read depth(37, 38):

• **Coverage:** percentage of the target region sequenced above a minimum threshold.

• **Read depth:** average number of times a specific base is read. High depth (>100–150×) improves diagnostic sensitivity and allows detection of low-frequency variants, which may be relevant in somatic mosaicism or cross-contamination.

- **MLPA:** a complementary technique used when deletions, insertions, or duplications are suspected. It enables the detection of copy number changes in the genome, ranging from small fragments to large regions, and can assess the arrangement of multiple exons or even entire genes. Its high sensitivity for exon-level copy number variations (CNVs) makes it a key tool for diagnosing syndromes characterized by a high rate of structural rearrangements(39).
- **DNA microarrays:** These microarrays consist of a matrix containing thousands of DNA probes with known genetic sequences. They work by hybridization: if the sample DNA is complementary to the probe, it binds, generating a detectable signal. This technology was initially developed to study RNA, enabling large-scale gene expression analysis. Hybridization-based microarrays can detect copy number variations, single nucleotide polymorphisms (SNPs), and gene expression profiles. Today, their use is largely limited to specific applications such as transcriptomic studies or broader genomic characterization in research settings. Interpretation of results is complex and requires specialized software(40).

The current diagnostic approach to hereditary cancer is based on NGS as the primary technique for detecting point mutations and small insertions/deletions, complemented by MLPA for structural rearrangements. PCR and Sanger sequencing are used for validation or specific contexts, while microarrays are increasingly limited in use. This integrated strategy maximizes diagnostic sensitivity and enables precise genetic characterization of the patient.

2.3 INTERPRETATION OF GERMLINE GENETIC RESULTS

2.3.1 Variant classification system (ACMG-AMP, Sherloc)

Once the sample is collected and processed using the previously described techniques, the ordering clinician receives a report detailing the results and providing recommendations(19, 28). The detection of gPV in cancer-associated genes through genetic testing enables the diagnosis of hereditary cancer syndromes(18).

These reports often include terms such as pathogenic or likely pathogenic variants, variants of uncertain significance (VUS), benign or likely benign variants, and/or variant allele frequency (VAF), which can be challenging to interpret. Advances in NGS technologies and the expanding knowledge of cancer-related genes have also contributed to increasingly complex results(41).

The possible outcomes of germline genetic testing include:

• **Positive:** a germline pathogenic or likely pathogenic variant is identified, providing informative results that indicate an increased risk for one or more cancers.

• **Not informative:** no germline pathogenic variants are detected in any of the genes analyzed.

• **VUS:** a variant is identified in a cancer-associated gene (or other gene), but its impact on protein function and its relationship to the disease remain unclear.

To determine the pathogenicity of a variant and its potential implications for patient and family management, multiple evidence-based variables must be considered. Various frameworks have been proposed; notably, in 2015 the American College of Medical Genetics and Genomics (ACMG), together with the Association for Molecular Pathology (AMP), introduced a standardized system for the clinical interpretation of sequence variants(41, 42).

Variant classification under the ACMG-AMP 2015 guidelines is based on a comprehensive assessment of multiple variables that collectively inform the pathogenicity or benignity of a genetic change. These include population data (allele frequency in general and disease-specific cohorts), computational predictions (in silico tools assessing impact on protein function or splicing), and functional evidence from well-established laboratory assays(41).

Additionally, the evaluation incorporates segregation data within families, de novo occurrence in affected individuals with unaffected parents, and phenotypic data that examines consistency between the patient's clinical features and known disease presentations(41, 42). The guidelines also consider the type and location of the variant (e.g., whether it affects a mutational hotspot or a critical functional domain), prior published reports or databases indicating pathogenicity or benignity (ClinVar; https://www.ncbi.nlm.nih.gov/clinvar/), and the presence of the variant in trans or in cis with other pathogenic variants in recessive conditions. For genes associated with autosomal recessive syndromes—such as *BRCA2, PALB2,* and *BRCA1*, linked to Fanconi anemia, or *ATM*, associated with ataxia-telangiectasia—identification of a homozygous or compound heterozygous pathogenic configuration in an affected individual may support pathogenic classification(43-45). For instance, if a variant is found in trans with a known pathogenic variant in a child diagnosed with Fanconi anemia or ataxia-telangiectasia, this clinical context can provide strong phenotypic evidence toward pathogenicity, especially when supported by functional data and consistency with the known molecular mechanisms of disease. By weighing these diverse lines of evidence (each assigned a level of strength) the ACMG framework enables a standardized, transparent, and reproducible approach to variant interpretation.

The final variant classification results from combining these criteria according to pre-specified logical rules(41, 42):

- **Pathogenic:** a variant classified as pathogenic has robust evidence demonstrating that it causes disease. This category requires multiple strong or very strong lines of evidence, such as functional studies, segregation data, and well-established disease

mechanisms, consistently supporting its deleterious role. Pathogenic variants are directly linked to disease risk or manifestation and are actionable in clinical care.

- **Likely Pathogenic:** a variant classified as likely pathogenic has substantial, although not definitive, evidence indicating it is disease-causing. It generally meets a combination of strong, moderate, and supporting criteria but lacks the full weight required for a pathogenic designation. Such variants are typically managed clinically with caution like pathogenic variants but may warrant additional familial studies or functional validation.
- **Variant of Uncertain Significance (VUS):** a VUS is a variant for which current evidence is insufficient or conflicting, preventing a clear classification as either pathogenic or benign. These variants highlight the limits of existing knowledge and underscore the need for further data from functional assays, segregation analyses, or larger population studies. When a VUS is identified, clinical management should be guided by the individual's personal and family history. Importantly, VUS classifications should be re-evaluated every 1 to 2 years to assess potential reclassification, as any upgrade or downgrade may have implications for the carrier's clinical management.
- **Likely Benign:** a variant classified as likely benign has evidence suggesting it does not cause disease, often based on high population frequency relative to the disease prevalence or multiple supporting benign criteria. While not definitively proven to be harmless, it is unlikely to be clinically significant and does not typically influence patient management.
- **Benign:** a benign variant has conclusive evidence demonstrating it does not cause disease. This is most often based on an allele frequency that is far too high to be consistent with a rare genetic disorder, or on multiple lines of strong benign evidence. Such variants are considered non-contributory to the patient's condition.

The ACMG-AMP guidelines for variant classification were improved by the Sherloc points system **(see Figure 7)**(42):

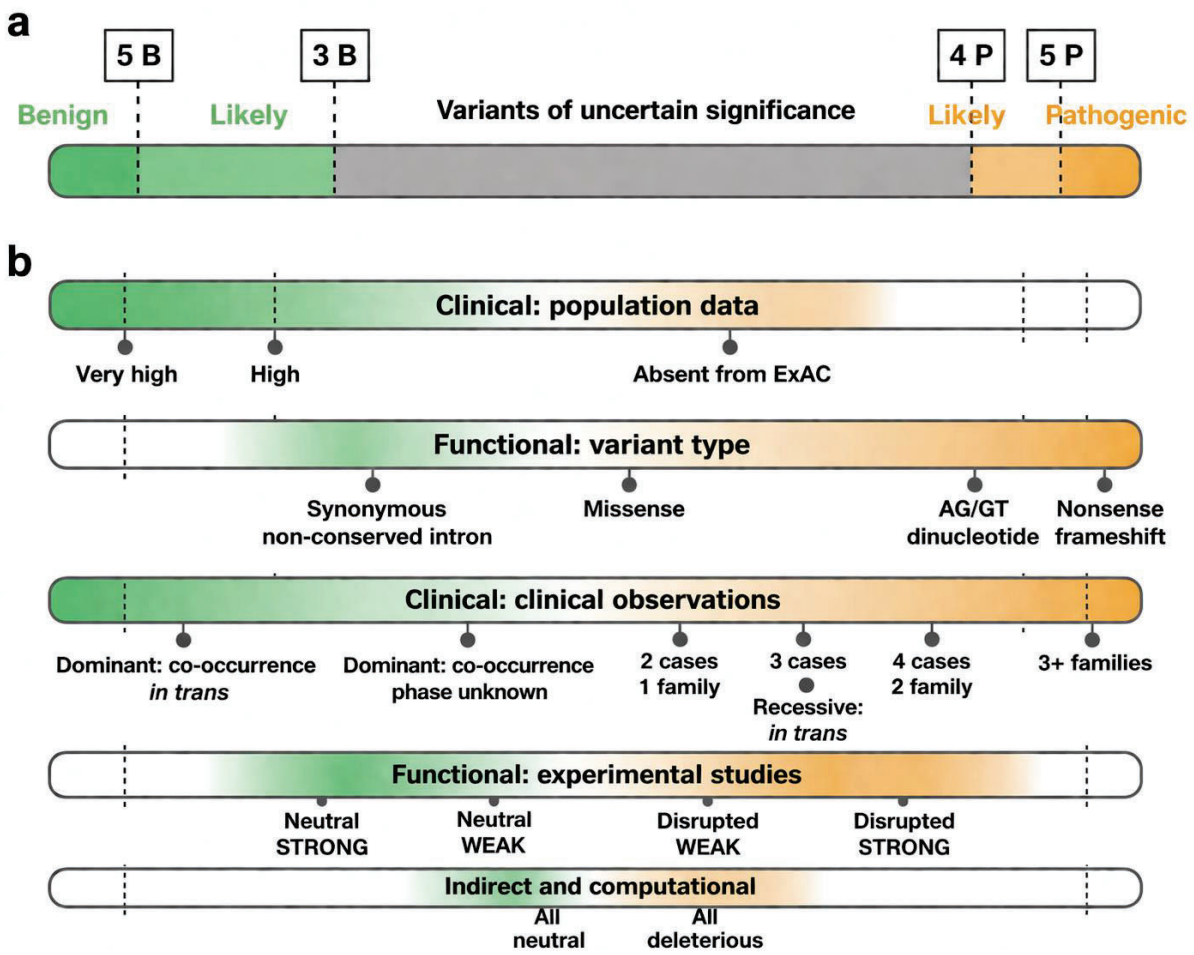


Figure 7 Classification scoring thresholds and evidence categories. (a) Point score thresholds for pathogenic (P), likely pathogenic, variant of uncertain significance, likely benign, and benign (B) classifications. Pathogenic and benign evidence is scored separately. Evidence in both directions can suggest a non-Mendelian variant. (b) Five evidence categories in the order in which they are evaluated, and with the point value of select criteria indicated. Clinical criteria include population data and clinical findings. Functional criteria include sequence observations, molecular studies, and indirect and computational data. ExAC, Exome Aggregation Consortium (extracted from Keith Nykamp, et al., Genetics in Medicine, 2017(42)).

2.3.2 Variant allele frequency and complex results

NGS assays are highly sensitive and have streamlined the simultaneous analysis of multiple genes. Laboratories utilizing NGS-based technologies can detect a broad spectrum of variant allele proportions, including those present at low allele variant frequencies (LAFVs)(46).

In germline testing, an inherited variant is typically found at ≈50% allele frequency (AF) in heterozygous individuals. When a variant is observed at levels significantly below this 50%, especially under 30% (classified as LAFVs)—a somatic origin becomes an important consideration(47-50). Identifying a somatic variant during testing intended to evaluate Mendelian risk may lead to misinterpretation of results, potentially impacting the clinical

management of both the patient and their at-risk relatives. Nonetheless, somatic findings can also carry distinct implications for patient monitoring and care.

When encountering LAFVs (<30%), it is essential to investigate their underlying cause, which may include the following categories(46-48, 51, 52):

- Somatic contamination: This refers to the unintentional admixture of tumor DNA or other somatic tissue DNA with the germline sample, potentially arising during sample collection or processing.
- Clonal hematopoiesis: Also known as clonal hematopoiesis of indeterminate potential (CHIP), this involves the age-related or therapy-induced expansion of hematopoietic clones carrying mutations, most frequently detected in peripheral blood DNA.
- Constitutional mosaicism (CM): This condition arises from postzygotic mutations leading to a pathogenic variant being present in a subset of the body's cells. The variant allele frequency can vary depending on the proportion of affected cells in the sampled tissue and may require analysis of additional tissues to clarify distribution.

Previous studies have estimated that the prevalence of somatic variants detected during germline testing is around 0.06%, with *TP53* being the most implicated gene (47). Slavin et al. evaluated over 348,000 individuals undergoing NGS hereditary pan-cancer panel testing and found that approximately 0.22% harbored likely-somatic variants, most commonly in *TP53*, *CHEK2*, and *ATM(48)*. These variants typically showed low allele frequencies (10–30%), suggesting origins consistent with clonal hematopoiesis, especially given associations with older age and personal cancer history—most notably ovarian cancer(53-55). Somatic findings can complicate germline interpretation, carrying implications for cancer surveillance, cardiovascular risks, and hematologic malignancies(56, 57). These results emphasize the necessity of precise laboratory methodologies and careful variant classification to ensure accurate clinical management and genetic counseling.

In the following sections, we will explore each of these potential origins beyond a classic germline variant (≈50% AF) in greater detail and discuss approaches to differentiate between these three groups.

- Somatic contamination(47-49):

Somatic contamination refers to the inadvertent admixture of non-germline DNA (tumor-derived DNA) in a germline testing sample. This can occur during biopsy or blood collection (for example, if circulating tumor DNA is abundant in plasma and contaminates the buffy coat during processing) or due to technical cross-contamination in the laboratory. Somatic contamination may produce misleading variant calls at LAFs that could appear in germline testing reports.

- Clonal hematopoiesis(53, 54, 57, 58):

CHIP refers to the expansion of hematopoietic stem cell clones harboring somatic mutations in the absence of a hematologic malignancy. It is most frequently observed in older adults (especially >60 years) and after exposure to cytotoxic chemotherapy or radiotherapy. Genes such as *TP53, DNMT3A, TET2*, and *ASXL1* are commonly involved. These variants are present in peripheral blood leukocytes at low to moderate variant allele frequencies and can be mistaken for germline alterations if detected during the germline testing.

Although CHIP itself does not indicate inherited cancer risk, it is associated with a higher probability of hematologic malignancies and cardiovascular disease and thus may require separate surveillance.

- Constitutional mosaicism(51, 59, 60):

Constitutional mosaicism results from a postzygotic mutation event occurring during embryonic development, leading to two or more genetically distinct cell populations within the same individual. In this context, the variant is present in only a subset of cells across different tissues, producing allele frequencies that can vary widely depending on the tissue tested and the proportion of affected cells **(see Figure 8)**.

Mosaicism may partially explain milder or atypical phenotypes in patients with variants typically associated with more severe hereditary syndromes, due to the tissue with the pathogenic variant present. Importantly, the presence of mosaicism can still confer risk to offspring, depending on whether germline cells are affected.

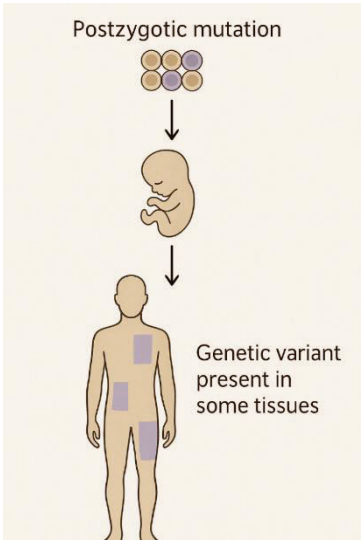


Figure 8. Illustration of constitutional mosaicism: a postzygotic mutation during embryogenesis results in a subset of cells carrying the genetic variant, leading to its presence in only some tissues (Adapted from: Batalini F, et al. Breast Cancer Research, 2019(51)).

To determine the origin of a somatic variant—whether due to contamination, CHIP, or CM—the first step is to confirm the variant by Sanger sequencing, particularly if the initial germline

analysis was performed using NGS(61, 62). Sanger sequencing can validate the presence of a LAFV or alternatively confirm a germline variant or a true negative result. Once the LAFV is confirmed, excluding a germline or wild-type finding, further investigations are warranted. These may include obtaining additional samples from the proband, such as cultured fibroblasts or independently extracted DNA, and, when possible, samples from family members. Testing these specimens for the pathogenic variant can help clarify the underlying etiology(63-65).

If the LAFV is detected in other tissues from the proband or in relatives, this supports a diagnosis of CM(60). In such cases, management should follow established guidelines for the corresponding hereditary cancer syndrome(20, 21, 28). Conversely, if the variant is not identified in supplementary testing, the differential shifts to somatic contamination—particularly if the patient has a known cancer diagnosis—where analysis of matched tumor tissue can help determine if the variant is present.

Alternatively, the finding may represent CHIP, commonly associated with aging, smoking, a history of cancer, or prior exposure to chemotherapy or radiotherapy. These patients require careful monitoring, including the exclusion of other hematological disease and yearly follow-up by experts, typically with annual complete blood counts and additional assessments as needed, given the increased risk of hematological and cardiovascular events(54, 57, 58, 66).

Importantly, when CHIP is suspected but CM cannot be entirely excluded—due to the impracticality of testing all tissues in the proband or cascade testing in the families—clinical variables such as patient age, cancer history, exposure to cytotoxic therapies, smoking status, and the gene involved *(e.g., ASXL1*, *DNMT3A*, *JAK2*, *PPM1D*, *SF3B1*, *SRFS2*, *TET2*, *TP53*) become critical in guiding differential diagnosis and subsequent management(51, 54, 55, 58-60, 63, 64). This distinction directly impacts recommendations for surveillance and, in the case of suspected CM, prompts consideration of genetic testing in family members(19, 28).

III. HEREDITARY BREAST AND OVARIAN CANCER SYNDROME (HBOC)

3.1 Epidemiology and genetic basis

HBOC is a hereditary cancer syndrome characterized by an increased risk of breast and/or ovarian cancer, involving multiple genes. Depending on the gene, there may also be a significantly elevated risk of other malignancies such as pancreatic, prostate, melanoma, uterine, gastric, or colorectal cancer(18).

Approximately 5–10% of breast cancers are attributable to gPVs, predominantly in *BRCA1* and *BRCA2*. In ovarian cancer, up to 20–25% of cases harbor such variants in *BRCA1, BRCA2,* or other susceptibility genes, most of which are involved in the homologous recombination repair (HRR) pathway(19, 67, 68).

Population-based studies have been essential for quantifying the odds ratios (ORs) and the magnitude of the lifetime breast cancer risk conferred by pathogenic variants in these genes.

Most of these estimates arise from large cohorts, such as the Breast Cancer Association Consortium (BCAC), CARRIERS, BRIDGE or population-based registries combined with multi-gene panel testing data. These studies allow for adjustment by age, family history, and competing mortality risks, providing more precise penetrance estimates. However, the estimates may vary by population ancestry, ascertainment methods (family-based vs unselected), and variant classification criteria. Importantly, penetrance figures reported for high-risk genes such as *BRCA1* and *BRCA2* typically stem from families with strong histories of breast or ovarian cancer, which might overestimate risks in broader populations. Conversely, studies like CARRIERS have helped refine the risk associated with moderate-penetrance genes by including unselected breast cancer cases and controls, offering a more accurate baseline for clinical decision-making(69, 70).

As an example, **Figure 9** shows the associations between gPVs in these genes and breast cancer risk. These data were published in 2021 and derived from the CARRIERS cohort, which included 32,247 women with breast cancer (cases) and 32,544 unaffected women (controls)(70).

Table 2. Associations between Pathogenic Variants in Established Breast Cancer–Predisposition Genes and Risk of Breast Cancer.*

Breast Cancer–Predisposition Gene[1,2,7]	Case Patients (N = 32,247)	Controls (N = 32,544)	Odds Ratio (95% CI)†	P Value
	no. with pathogenic variant (%)			
ATM	253 (0.78)	134 (0.41)	1.82 (1.46–2.27)	<0.001
BARD1	49 (0.15)	35 (0.11)	1.37 (0.87–2.16)	0.18
BRCA1	275 (0.85)	37 (0.11)	7.62 (5.33–11.27)	<0.001
BRCA2	417 (1.29)	78 (0.24)	5.23 (4.09–6.77)	<0.001
CDH1	17 (0.05)	6 (0.02)	2.50 (1.01–7.07)	0.06
CHEK2	349 (1.08)	138 (0.42)	2.47 (2.02–3.05)	<0.001
NF1‡	19 (0.06)	11 (0.03)	1.93 (0.91–4.31)	0.09
PALB2	148 (0.46)	38 (0.12)	3.83 (2.68–5.63)	<0.001
PTEN	8 (0.02)	3 (0.01)	NA	NA
RAD51C	41 (0.13)	35 (0.11)	1.20 (0.75–1.93)	0.44
RAD51D	26 (0.08)	14 (0.04)	1.72 (0.88–3.51)	0.12
TP53‡	19 (0.06)	2 (0.01)	NA	NA
Total	1621 (5.03)	531 (1.63)	—	—

* The studies in the CARRIERS consortium that were included in this population-based analysis were BWHS, CPSII, CPS3, CTS, MCBCS, MEC, MMHS, NHS, NHSII, WCHS, WHI, and WWHS. NA denotes not applicable (too few events [<5] to calculate a stable odds ratio).
† Odds ratio estimates for any breast cancer were adjusted for study, age, family history of breast cancer, and race or ethnic group.
‡ Pathogenic variants in *NF1* and *TP53* were restricted to those with an alternate allele fraction (calculated as the number of alternate allele reads divided by the total number of reads at a specific genomic position) between 0.3 and 0.7.

Figure 9. Associations between gPV in established breast cancer-predisposition genes and risk of breast cancer *(adapted from Hu, Chunling et al. NEJM, 2021(70)).*

Most studies report an increased relative risk (RR) of breast cancer associated with these genes. However, when counseling patients, it is more practical to discuss absolute lifetime risks (i.e., cumulative risk by age 80).

The thresholds at which an absolute risk is considered actionable—prompting more intensive breast cancer screening than that recommended for the general population—vary across guidelines and countries.

Several statistical models have been developed to estimate an individual's risk of developing breast cancer, incorporating family history, genetic factors (including the presence of gPV in breast and/or ovarian cancer genes, or a negative result), and a range of personal risk variables such as breast density, hormone replacement therapy, contraceptive use, age at menarche and menopause, parity, lifestyle factors, height, body mass index, among others. BOADICEA or Tyrer-Cuzick (IBIS) are the most widely used models, each differing slightly in the variables and algorithms employed. These models generate estimates of lifetime or age-specific absolute risk, which are then compared to the general population baseline (approximately 12-13% lifetime risk)(71-73). Risk predictions from these tools inform clinical decision-making, particularly regarding the initiation of intensified screening protocols such as annual MRI or criteria for risk-reduction surgeries. Different guidelines rely on these risk estimates to define actionable thresholds—for instance, the NCCN recommends additional MRI for the screening when lifetime risk exceeds 20%, while NICE and other European frameworks often reserve MRI for those exceeding 30%(18, 24).

These differences reflect regional approaches to balancing early detection with resource allocation and potential harms of over-screening.

3.2 HBOC-Associated genes and penetrance (High- and Moderate-risk)

Genes associated with increased breast cancer risk are classified by penetrance(12, 18, 19):

- **High-penetrance (≥4-fold increased lifetime risk):**
  BRCA1, BRCA2, PALB2, TP53, CDH1, PTEN, STK11
- **Moderate-penetrance (2–4-fold increased lifetime risk):**
  ATM, BARD1, CHEK2, NF1, RAD51C, RAD51D

Table 2 details the genes implicated in HBOC as well as the estimated risks of developing different neoplasms in carriers of gPV and **Figure 10** shows the absolute risk of breast cancer by age 80 for protein-truncating variants in selected genes(18).

Gen	Breast cancer	Tubo-ovarian cancers	Pancreatic cancer	Colon cancer	Other cancers
ATM	Yes (25%-30%)	Yes (~5%)	Yes (<5%)	No	Prostate 30%
BARD1	Yes (~20%)	No	No	No	No
BRCA1	Yes (>60%)	Yes (40%-60%)	Yes (<5%)	No	
BRCA2	Yes (>60%)	Yes (15%-30%)	Yes (<5%)	No	Prostate 33%
BRIP1	No	Yes (5%-10%)	No	No	No
CDH1	Yes (40%)	No	No	No	Diffuse gastric cancer 35-45%
CHEK2	Yes (25%-30%)	No	No	No	
PALB2	Yes (40%-60%)	Yes (3%-5%)	Yes (2%-3%)	No	No
PTEN	Yes (40%)	No	No	Yes (10%)	Thyroid 20%; endometrial 20%
RAD51C	Yes (20%)	Yes (10%)	No	No	No
RAD51D	Yes (10%)	Yes (10%)	No	No	No
STK11	Yes (40%)	No	Yes (10%-30%)	Yes (30%)	Gastric 30%; Sertoli-Leydig 10%-20%
TP53	Yes (40%)	Possibly	Possibly	Possibly	Sarcoma, brain, leukemia, adrenocortical carcinoma

Table 2. Absolute cancer risk by HBOC gene (adapted from ESMO guidelines)(18).

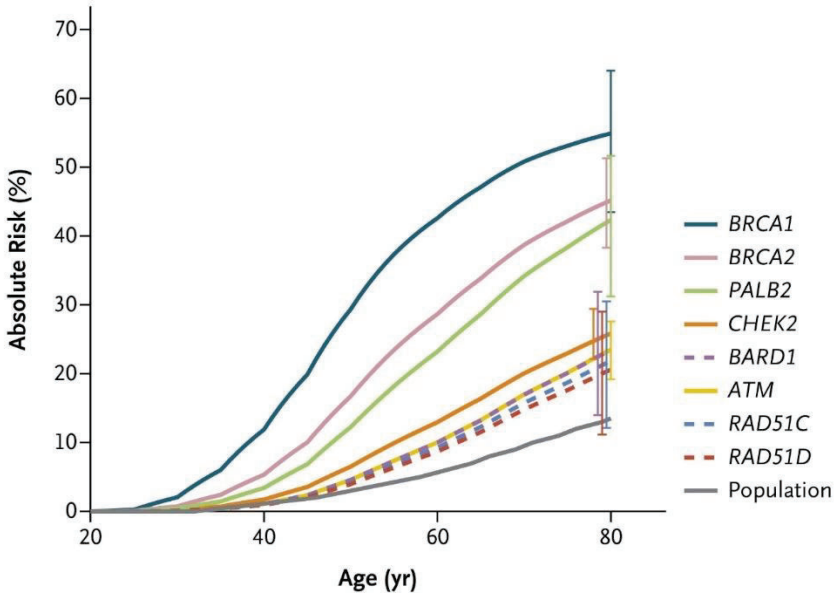


Figure 10. Estimated Absolute Risk of Breast Cancer by Age 80 for Protein-Truncating Variants in Selected Genes (Adapted from Dorling et al., New England Journal of Medicine (2021)) This figure displays the estimated lifetime risk (to age 80) of developing breast cancer for carriers of protein-truncating variants in eight genes with robust evidence of association, based on odds ratios derived from population-based studies. *TP53* was excluded from this analysis due to its wide confidence interval and its known association with early-onset cancers. Baseline risks were modeled using 2016 breast cancer incidence data from the UK. Error bars represent 95% confidence intervals(69).

3.3 Clinical testing criteria and guidelines

The most widely used clinical guidelines—those from the Spanish Society of Medical Oncology (SEOM), the European Society for Medical Oncology (ESMO), and the National Comprehensive Cancer Network (NCCN)—provide criteria for germline genetic testing in hereditary breast and ovarian cancer (HBOC) syndrome(18, 24). These criteria incorporate both traditional hereditary risk factors and indications related to treatment decisions, such as the choice of surgery or eligibility for PARP inhibitors (PARPi) in early or advanced disease(20, 74).

Over the past decade, the criteria for germline testing and the composition of gene panels have evolved substantially. Currently, testing is recommended for nearly all patients diagnosed with breast cancer before the age of 65, as well as for any patient who may be eligible for treatment with PARPi, regardless of age.

A recent analysis from the BRCA BCY Collaboration showed that patients diagnosed with breast cancer at age 40 or younger who underwent genetic testing prior to diagnosis had smaller tumors, lower nodal involvement, and improved DFS and OS compared with those tested at or after diagnosis(75). These findings underscore the potential impact of germline

testing in individuals with a family history or a diagnosis of breast cancer, both for themselves and for their relatives.

Several guidelines—including those from NCCN, ESMO, SEOM, and the American Society of Clinical Oncology (ASCO)—outline specific selection criteria(18, 19, 21, 28). Below is a summary of the most relevant testing indications and the genes most included across different panel recommendations:

The **Catalan Health Service (Cat Salut)** published updated recommendations in September 2023 based on SEOM and ESMO guidelines criteria(18, 28, 76), outlining clinical criteria for germline genetic testing in individuals and families with a history suggestive of HBOC syndrome, as well as the corresponding gene panels to be analyzed.

The clinical criteria are the following(76):

- Breast cancer diagnosed ≤40 years.
- Breast cancer diagnosed ≤50 years in non-informative families.
- Triple-negative breast cancer (TNBC) diagnosed ≤60 years.
- Male breast cancer.
- Three or more first- or second-degree relatives affected by breast cancer (at least one diagnosed ≤60 years).
- ≥3 cases among breast, pancreatic, ovarian, and/or metastatic prostate cancer (Gleason score ≥7).
- Two cases of breast cancer diagnosed ≤50 years.
- One case of bilateral breast cancer (first diagnosed ≤50 years).
- One case of bilateral breast cancer plus another case of breast cancer (one diagnosed ≤60 years).
- HER2-negative breast cancer for which treatment with PARPi is considered according to Cat Salut indications.
- Breast cancer with a family history of ovarian cancer.
- Invasive non-mucinous epithelial ovarian cancer (for low-grade tumors, individual assessment based on age, family history, and potential benefit to relatives is recommended).

Gene panel in breast and ovarian cancer
(Cases or families with breast cancer or both breast and ovarian cancer):

- *BRCA1, BRCA2, MLH1, MSH2, MSH6, TP53, BARD1, PALB2, CHEK2, ATM, BRIP1, RAD51C, RAD51D, PTEN, CDH1*.

- *TP53* testing only in cases of breast cancer <50 years or >50 years meeting CHOMPRET criteria.
- *CDH1* testing only with a suggestive phenotype or if histological confirmation of cases is not possible.

Gene panel in ovarian cancer
(Cases or families with ovarian cancer only):

- *BRCA1, BRCA2, MLH1, MSH2, MSH6, BRIP1, RAD51C, RAD51D, PALB2.*

The **ASCO-Society of Surgical Oncology Guideline published in 2024** provides the following recommendations(21). Below are the main differences in indications and the genes included in their panels:

- All patients newly diagnosed with breast cancer with stage I–III or de novo stage IV/metastatic disease who are 65 years or younger at diagnosis should be offered *BRCA1/2* testing.
- All patients newly diagnosed with breast cancer with stage I–III or de novo stage IV/metastatic disease who are older than 65 years should be offered *BRCA1/2* testing if:
    - They are candidates for PARPi therapy for early-stage or metastatic disease.
    - They have triple-negative breast cancer.
    - Their personal or family history suggests the possibility of a pathogenic variant
    - They were assigned male sex at birth
    - They are of Ashkenazi Jewish ancestry or members of a population with an increased prevalence of founder mutations.
- Patients undergoing *BRCA1/2* testing should also be offered testing for other cancer predisposition genes as suggested by their personal or family history.
- Testing for high-penetrance genes beyond *BRCA1/2*, including *PALB2, TP53, PTEN, STK11*, and *CDH1*, should be offered to appropriate patients.

Testing for moderate-penetrance breast cancer genes currently offers no benefit for treatment of the index breast cancer but may inform risks of second primary cancer or family risk assessment and thus may be offered to appropriate patients who are undergoing *BRCA1/2* testing.

Lynch syndrome genes (*MLH1, MSH2, MSH6*, and *PMS2*) are not associated with an increased risk of breast cancer, nor is *BRIP1*. However, all of them are linked to a potential increased risk of ovarian cancer. These genes are often included in breast cancer panels due to their relevance to ovarian cancer, as well as other malignancies such as colorectal or endometrial cancer.

In this section, we did not specify the NCCN genetic testing criteria(19). However, prior studies have demonstrated that expanding these criteria to include nearly all patients diagnosed with

breast cancer before the age of 65 significantly improves the sensitivity of germline pathogenic variant detection(2, 77-79). Specifically, detection rates increased from approximately 70% to nearly 90% across the nine breast cancer predisposition genes (ATM, *BRCA1*, *BRCA2*, *CDH1*, *CHEK2*, *NF1*, *PALB2*, *PTEN*, and *TP53*), and from 87% to over 98% for *BRCA1*/*BRCA2(2, 78)*. Additional data from the Mayo Clinic showed that, under a universal testing approach for all patients diagnosed before age 60, 9% of *BRCA1*/*BRCA2* carriers and 14% of those with germline pathogenic variants in other genes would have been missed if NCCN criteria alone had been applied(80).

In a US survey from 2017, they reported that just 29% of the patients who met the eligibility criteria for genetic testing were referred to the unit, and just 15% underwent germline testing(81).

In breast cancer care, the move toward mainstreaming genetic testing, by bringing it directly into routine oncology visits instead of relying solely on genetics clinics, is changing how we approach patient management. This approach allows oncology teams to handle pre-test counseling, consent, and ordering directly during regular visits, making the process faster and more accessible. As a result, more patients are getting tested, and results are available sooner. Since genetic testing is becoming a key part of treatment decisions, this approach helps make it more accessible and easier to scale, while freeing up genetics specialists to focus on post-test counseling and more complex situations.

Multigene panel testing offers several advantages in the evaluation of hereditary cancer risk. It increases the likelihood of identifying clinically actionable mutations, even in the absence of a strong family history, and often leads to changes in patient management(82). This approach is cost-effective, convenient, and aligns with patient preferences, as many favor broader testing. Panel testing also streamlines the diagnostic process by avoiding the need for stepwise gene selection. However, it has important limitations. Clinical validity and utility remain uncertain for many moderate-risk genes, and risk estimates are often imprecise. There is limited consensus on how to manage findings from these genes, and large panels yield VUS in over 30% of cases, requiring ongoing follow-up. Additionally, panel testing can lead to unnecessary screening or preventive surgeries and may negatively impact patient quality of life due to increased anxiety and uncertainty(83).

Recent ESMO Precision Oncology Working Group guidelines advocate for mainstreaming germline multigene panel testing in breast cancer, recommending a selected gene panel based on clinical utility and population health impact. The core panel includes *BRCA1, BRCA2, PALB2, RAD51C,* and *RAD51D*, with *TP53* recommended when breast cancer is diagnosed before age 40, and *BRIP1* considered a potential addition for the ovarian cancer risk association. In contrast, genes such as *CHEK2* and *ATM* were not included due to limited evidence of survival benefit, and syndromic genes like *STK11, PTEN, NF1,* and *CDH1* were specifically discouraged for routine use in this mainstreaming context(84).

Criteria for genetic testing, the composition of panels, and the methodologies applied—including mainstreaming approaches and models of pre-test counselling—are evolving rapidly. Prospective studies are needed to assess the effectiveness, efficiency, and psychological impact of different strategies for testing and surveillance.

3.4 Risk management and surveillance strategies

Current clinical guidelines provide gene-specific recommendations that guide surveillance strategies, risk-reducing surgeries, and treatment decisions. These guidelines are tailored to the cancer risks associated with each gene, helping healthcare providers offer more personalized care(18, 19, 28).

In recent years, several studies have explored why cancer risk (penetrance) varies among families with gPVs in different genes. Part of this variability is explained by the specific gene and the variant itself. Some variants, known as hypomorphic, lead to a partial loss of function and are linked to lower cancer risks compared to classic loss-of-function mutations—or even compared to other gPVs within the same gene. For example, certain *ATM* variants like *c.7271T>G* are associated with risks like those seen in *BRCA1/2* carriers(85). On the other hand, hypomorphic variants in *BRCA1* (e.g., *p.Arg1699Gln*)(86), *TP53* (e.g., *p.Arg181His*, *p.Arg337His*)(87-89), or *CHEK2* (e.g., *p.I157T*, *p.S428F*, *p.T476M*)(90, 91) are associated with more moderate risks.

Other tools under investigation to personalize surveillance strategies in carriers include polygenic risk scores (PRS). While not yet used in clinical practice to guide management in carriers of gPV, multiple studies suggest that PRS may help reclassify gPV carriers into higher or lower risk categories. However, PRS have important limitations, one of the most significant being the lack of prospective validation in mutation carriers. In addition, most PRS have been developed in non-Hispanic white populations, limiting their applicability across other race groups(92, 93).

3.4.1 Gene-specific recommendations

Current guidelines occasionally adapt management based on variant type—for instance, by recommending fewer intensive interventions for certain low-risk *CHEK2* variants(19). For other genes, like *TP53*, the evidence is not yet strong enough to support variant-specific recommendations. Still, emerging data are likely to shape future clinical guidance.

Surveillance protocols—such as the recommended starting age, screening intervals, and imaging modalities—may vary slightly between countries and healthcare systems. **Table 3** summarizes the ESMO consensus recommendations for follow-up and surveillance based on gene-specific risk(18).

This intensified screening for individuals with a gPV in a breast cancer related gene follows a different protocol than that used for the average-risk population. Its primary purpose is the potential for earlier detection of breast cancer, which can lead to improved clinical outcomes.

Several studies have demonstrated that diagnosis at an earlier stage is associated with better survival.

Table 3. ESMO Consensus Recommendations for Follow-Up and Surveillance According to Gene-Specific Breast and Ovarian Cancer Risk(18)

Gene(s)	Breast Surveillance	Ovarian Surveillance	Risk-Reducing Surgery
BRCA1	Annual MRI from age 30*; 30-39 MRI and mammo +/- US every 6 months; ≥40 MRI + mammo	Every 6 months TVUS + serum CA-125	RRM recommended; RRSO at 35–40 y.o
BRCA2	Annual MRI from age 30*; 30-39 MRI and mammo +/- US every 6 months; ≥40 MRI + mammo	Every 6 months TVUS + serum CA-125	RRM recommended; RRSO at 40–45 y.o
BARD1	Annual MRI from age >35**. Annual mammogram >40* y.o		
PALB2	Annual MRI from age 30*; 30-39 MRI and mammo +/- US every 6 months; ≥40 MRI + mammo	Not routinely recommended	RRM optional; RRSO postmenopausal
RAD51C/D	Annual MRI from age >35**. Annual mammogram >40* y.o	Not routinely recommended	RRSO around age 45–50
TP53	Annual MRI from age 20*	Not applicable	RRM recommended; avoid radiation when possible
CDH1	Annual MRI from age 30*	Not applicable	Discuss RRM in context of personal/family history
PTEN	Annual MRI from age 30*	Not applicable	Discuss RRM in context of personal/family history
STK11	Annual MRI from age 25*	Not applicable	Discuss RRM in context of personal/family history

ATM	Annual MRI from age 40*; add mammogram at 40	Not routinely recommended	Discuss RRM in context of personal/family history
CHEK2	Annual MRI from age 40*; add mammogram at 40	Not applicable	Discuss RRM in context of personal/family history
BRIP1	Not applicable	Not routinely recommended	RRSO around age 45–50

MRI = Magnetic Resonance Imaging; Mammo = Mammogram; RRM = Risk-Reducing Mastectomy; RRSO = Risk-Reducing Salpingo-Oophorectomy; US = Ultrasound; y.o: years old

*Or 5 years younger than the youngest diagnosis in the family ** If the lifetime absolute risk is >30% based on the models (BOADICEA or Tyrer-Cuzick)

In male *BRCA1/2* carriers, surveillance is generally limited to those with gynecomastia, with baseline mammography and annual follow-up starting at age 50. In both male and female carriers, surveillance is typically recommended up to age 75. The intensity and duration of follow-up should be individualized based on the specific gene involved, personal cancer history, and family history, ESMO guidelines recommend keeping screening while the carrier is in good health. In women who have undergone RRM, post-surgical breast MRI may be considered to assess residual breast tissue and inform further surveillance strategies. This approach may be important due to the variety of surgical techniques currently being used, such as nipple-sparing and skin-sparing mastectomies(12, 18).

Surveillance strategies are tailored to the specific gene involved and the associated spectrum of cancer risks. Recommendations vary depending on personal and family history and extend beyond breast and ovarian cancer in several syndromes. For instance, *BRCA1/2* and *PALB2* carriers with a family history of pancreatic cancer may benefit from pancreatic screening protocols, while *TP53* carriers require whole-body MRI, or *CDH1* carriers require gastroscopies and biopsies every 6-12 months(18, 19).

In Lynch syndrome–associated genes (*MLH1, MSH2, MSH6*), preventive strategies include consideration of total hysterectomy and RRSO, especially after age 40 (or after 50 for *MSH6*), in addition to colonoscopy screening(12, 18, 19).

There are some differences between the NCCN guidelines and other international recommendations for breast cancer surveillance strategies(19). As an example, for genes such as *ATM* and *CHEK2*, the NCCN recommends considering annual breast MRI starting at age 30–35. Additionally, in males with *BRCA1/2* gPV, the NCCN also advises annual mammography—particularly for those with a lifetime breast cancer risk exceeding 7%—beginning at age 50, or 10 years prior to the earliest diagnosis of male breast cancer in the family, regardless of the presence of gynecomastia.

In relation to ovarian cancer risk, the NCCN guidelines advise against using CA-125 and pelvic ultrasound for routine surveillance or screening in high-risk carriers. These tests are only recommended for RRSO preoperative assessment. This is supported by multiple studies showing no improvement in overall survival, such as the UKCTOCS trial in the general population(94), and the study lead by Evans et al., which demonstrated no benefit in *BRCA1/2* carriers compared to non-carriers(95).

Another notable difference involves pancreatic cancer screening. European and Spanish guidelines clearly recommend surveillance for *STK11* and *CDKN2A* carriers, given their high lifetime risk (>15%), with screening typically starting between ages 30 and 40 using alternating annual MRI and/or endoscopic ultrasound(18, 28).

In contrast, the most recent NCCN guidelines have expanded recommendations by advocating annual screening starting at age 50 for *ATM* and *BRCA2* carriers regardless of family history(19). This change reflects their estimated lifetime pancreatic cancer risk of 5–10%.

For other genes—such as *BRCA1*, *PALB2*, *TP53*, *MLH1*, *MSH2/EPCAM*, and *MSH6*—screening is sometimes considered, particularly when there is a positive family history of pancreatic cancer, but no major differences exist between international guidelines(19).

3.4.2 Risk-Reducing surgeries

Bilateral risk-reducing mastectomy (RRM), contralateral prophylactic mastectomy (CPM) and risk-reducing salpingo-oophorectomy (RRSO) are the main surgical strategies to lower cancer incidence in individuals with gPVs associated with hereditary breast and ovarian cancer. These surgeries impact in cancer risk, survival and quality of life(18, 96).

RRM significantly reduces the risk of developing breast cancer (by approximately 90%–95%) in unaffected carriers of high-risk genes such as *BRCA1* and *BRCA2(22, 97-100)*. Extrapolating these findings to carriers of other high-risk genes, such as *CDH1, PALB2, PTEN*, or *TP53*, a similar preventive benefit is expected. RRSO can reduce the risk of ovarian cancer by approximately 80% and is particularly important in *BRCA1/2* carriers, as it also confers a survival benefit(101-103).

Kurian et al. used a Monte Carlo simulation model to evaluate the preventive strategies alone and in combination in 25-year-old individuals with *BRCA1* or *BRCA2* gPV, including annual screening with mammography and MRI from ages 25 to 69, RRM at different ages, and RRSO at ages 40 or 50. Without any intervention, the estimated probability of survival by age 70 was 53% for *BRCA1* and 71% for *BRCA2* carriers. The combination of RRM and RRSO at age 40 yielded the largest survival advantage, improving overall survival by 24% for *BRCA1* and 11% for *BRCA2* carriers(104).

The decision to undergo risk-reducing surgery is influenced by multiple factors, including gene involved, age, reproductive plans, family history, and personal preferences. Guidelines differ

slightly in their age thresholds and gene-specific recommendations, particularly for moderate-penetrance genes where the benefit-risk balance is less clear.

Breast Cancer Risk Reduction with Risk-Reducing Mastectomy (RRM)

RRM has shown to reduce the risk of developing breast cancer by around 90%-95%, leaving carriers with a lower lifetime risk than the average-risk population. However, the benefit of RRM in terms of overall survival (OS) remains controversial, with different results across studies(105). In a large multicenter prospective cohort by Domchek et al., which included 2,482 women with *BRCA1* or *BRCA2* gPVs identified between 1974 and 2008, none of the 247 women who underwent RRM developed breast cancer, compared to 98 cases among the 1,372 women who opted for surveillance(101). Similarly, other studies have shown that RRM can prevent nearly all first breast cancers, with risk reductions approaching 100% in many cases(100-103).

The absolute benefit of RRM varies depending on the gene affected and age of the carrier. *BRCA1* carriers frequently develop early-onset (mean age at diagnosis: 39.9), high-grade tumors, often triple-negative breast cancer (TNBC), making interventions like RRM particularly impactful(106). In contrast, *BRCA2*-associated breast cancers tend to occur later (mean age: 42.2) and are more amenable to early detection through intensive screening, such as annual breast MRI, which may reduce the added survival benefit of RRM compared to enhanced surveillance in this group(107). In a Dutch multicenter cohort with a median follow-up of 10.3 years, 42% of *BRCA1* and 35% of *BRCA2* carriers underwent RRM. Among *BRCA1* carriers, the hazard ratios for overall survival and breast cancer–specific survival by age 65 were 0.40 and 0.06, respectively, favoring the RRM group. For *BRCA2* carriers, the hazard ratio for overall survival was 0.45 (95% CI: 0.15–1.36), although it did not reach statistical significance. Breast cancer–specific survival could not be formally calculated in this group, but the observed rates were 100% in the RRM group and 98% in the surveillance group(108). Despite gene-specific differences in timing and magnitude of benefit, RRM consistently provides a substantial reduction in breast cancer incidence for carriers of both *BRCA1* and *BRCA2*.

Currently, RRM is recommended for individuals with gPV in high-penetrance genes such as *BRCA1*, *BRCA2*, and in selected cases *CDH1, PALB2, PTEN, STK11*, and *TP53*, given their estimated lifetime breast cancer risk over 40–60%(18). In contrast, carriers of moderate penetrance gPV (e.g., *ATM, BARD1, CHEK2, RAD51C, RAD51D*) are generally managed with intensified surveillance (e.g., mammograms, MRI, breast ultrasounds), unless additional factors suggest significantly elevated risk, such as family or personal history of breast, ovarian, or other cancers related to HBOC(18, 19, 22, 99).

A recent economic analysis published in JAMA Oncology emphasized the potential cost-effectiveness of RRM to women who do not carry high penetrance gPV but have an elevated lifetime risk of breast cancer. The study found that RRM becomes cost-effective for women between the ages of 30 and 55 when their estimated lifetime breast cancer risk exceeds 35%. At the population level, implementing this approach could prevent approximately 11% of

annual breast cancer cases in the UK. These findings support the need for policymakers and health systems to incorporate individualized risk assessment(109).

While survival is a key endpoint in decision-making, it is equally important to consider patient preferences and other clinically relevant outcomes. RRM may allow carriers to avoid chemotherapy, radiation, hormone therapy, or additional surgeries in the event of a second breast cancer diagnosis. These treatments can significantly impact quality of life and long-term survivorship. Conversely, RRM can negatively affect body image, underscoring the importance of discussing surgical risks, potential complications, and aesthetic outcomes as part of shared decision-making(110).

Ovarian Cancer Risk Reduction with Risk-Reducing Salpingo-Oophorectomy (RRSO)

The estimated lifetime risk of ovarian cancer in the absence of RRSO is approximately 40–60% for *BRCA1* carriers and 15–30% for *BRCA2* carriers compared to the 1.1% of females in the general population(111, 112). At the time of diagnosis, around 95% of patients present with nonspecific symptoms, and approximately 80% are diagnosed at an advanced stage (stage III–IV)(113). Notably, the age at diagnosis in *BRCA1/2* carriers is, on average, 10 years younger than in non-carriers(19).

RRSO is an important preventive strategy in women carrying high-risk gPV such as *BRCA1* and *BRCA2*, as it lowers the risk of developing ovarian cancers. Evidence from prospective studies and meta-analyses suggests that RRSO can reduce the risk by approximately 80%(101, 103, 114, 115). Although, the remaining small risk is due to the possibility of primary peritoneal cancer (approximately 5%).

For *BRCA1* carriers, whose ovarian cancer risk increase in their early 40s, RRSO is generally recommended between ages 35 and 40, once childbearing is complete. In *BRCA2* carriers, whose risk increases later, the procedure is often advised between ages 40 and 45(18, 19, 28). Performing RRSO before menopause also seems to provide an added benefit by reducing breast cancer risk through the suppression of hormones production. Studies have shown that premenopausal RRSO is associated with a meaningful reduction in breast cancer incidence, particularly among *BRCA2* carriers, likely due to the stronger association of *BRCA2*-related tumors with estrogen receptor (ER)–positive disease. While earlier research suggested a strong protective effect in *BRCA1* as well, more recent analyses adjusting for surveillance and selection biases have reported mixed findings in this group. However, the overall body of evidence continues to support a moderate reduction in breast cancer risk when the procedure is performed before natural menopause(101, 103).

In **Table 4**, it is shown the risk reduction effect of RRSO against ovarian and breast cancer in *BRCA1/2* carriers without prior personal history of breast cancer.

Table 4. The risk reduction effect of RRSO against ovarian cancer and breast cancer(116).

Author	Year	Study Design	Ovarian Cancer	Breast Cancer	Total Mortality
			HR (95% CI)	HR (95% CI)	HR (95% CI)
Rebbeck et al. (103)	2009	Meta-analysis	0.21 (0.12–0.39)	0.49 (0.37–0.65)	NA
Marchetti et al. (117)	2014	Meta-analysis	0.19 (0.13–0.27)	NA	NA
Domchek et al. (101)	2010	Prospective cohort	0.28 (0.12–0.69) *	0.54 (0.37–0.79) *	0.40 (0.26–0.61)
			0.14 (0.04–0.59) †	1.00 (0.56–1.77) †	
Kauff et al. (118)	2008	Prospective cohort	0.15 (0.04–0.56) ‡	0.61 (0.30–1.22) ‡	NA
				0.28 (0.08–0.92) §	

Adapted from Sekine, M et al. Cancers 2021
* With no breast cancer history, NA: not applicable, with breast cancer history, *BRCA1*, § *BRCA2*. HR: Hazard Ratio; CI: confidence interval

In carriers of moderate-risk genes such as *BRIP1, RAD51C*, and *RAD51D*, who face a lower lifetime ovarian cancer risk (5–11%), the role of RRSO remains less clearly defined. Nevertheless, given the lack of effective screening tools for ovarian cancer and its typically poor prognosis, preventive surgery may still be considered in selected cases once childbearing is complete. Expert guidelines suggest that, in these individuals, it may be appropriate to discuss RRSO around age 45 or postmenopausal, or earlier in the presence of a strong family history. Prophylactic hysterectomy with RRSO should be considered as an option for Lynch syndrome gPV carriers, particularly in those who have completed childbearing or are postmenopausal. Cost-effectiveness models also support its use in postmenopausal women whose residual lifetime risk exceeds a certain threshold(18, 19).

Risk-Reducing Surgeries in Carriers with a Personal History of Breast Cancer

In patients with gPVs and a personal history of breast cancer, risk-reducing surgeries—namely contralateral prophylactic mastectomy (CPM) and RRSO—are essential components of long-term risk management. These interventions aim to lower the risk of contralateral breast cancer (CBC) and ovarian cancer, although their survival benefit varies depending on the gene involved, tumor characteristics, and timing of surgery.

Initial evidence supporting the role of RRSO in improving overall survival among *BRCA1*/*BRCA2* carriers was reported by Rebbeck et al. in a large international cohort study. This pivotal work

demonstrated that RRSO significantly reduced the risk of ovarian cancer in patients with prior history of breast cancer(103).

More recently, an international cohort study by Blondeaux et al. expanded on this evidence, showing that the combination of CPM and RRSO improved overall survival, disease-free survival, and breast cancer–free interval in *BRCA1*/*BRCA2* carriers diagnosed with early-stage breast cancer before the age of 40. RRM conferred benefit across *BRCA1* and *BRCA2* carriers regardless of age, tumor subtype, or clinical features. RRSO was protective in both subgroups but showed a particularly pronounced effect in *BRCA1* carriers with triple-negative breast cancer. The study also highlighted disparities in access: patients from high-income countries were more likely to undergo RRM, and those who received *BRCA* results before or at diagnosis had higher uptake of preventive surgery, reinforcing the importance of early genetic testing **(see Figure 11)**(119).

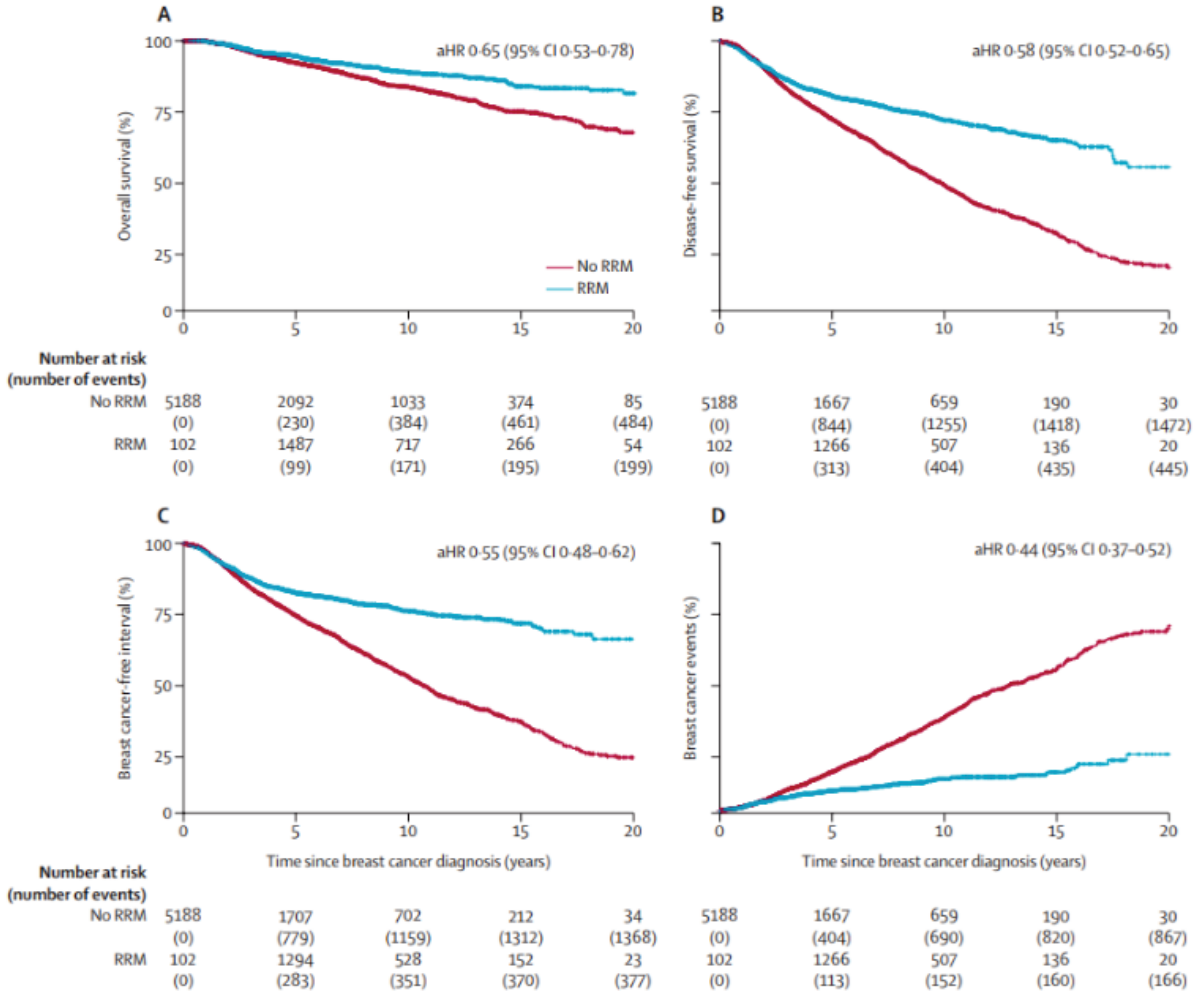
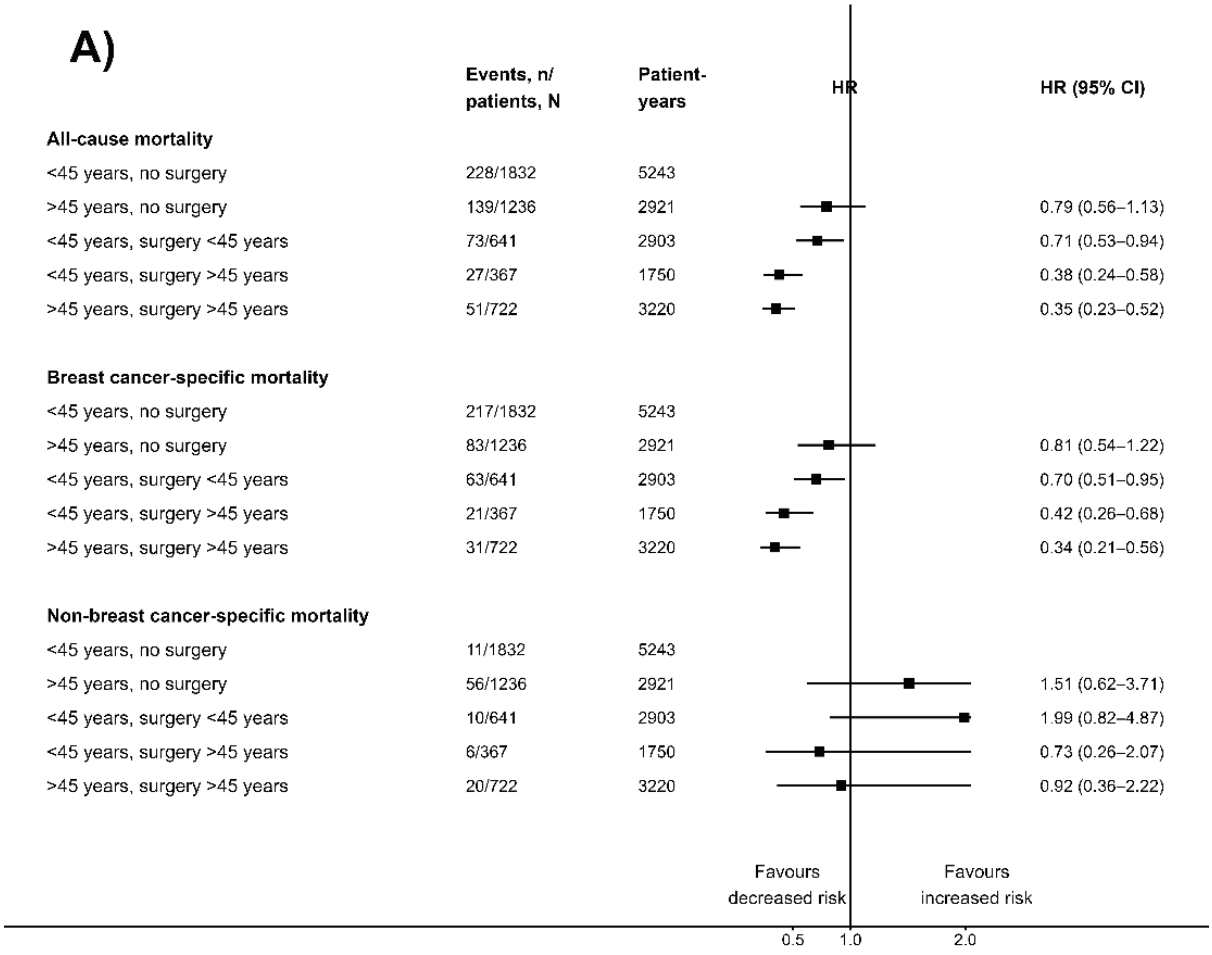


Figure 11. Kaplan–Meier curves illustrating the impact of risk-reducing mastectomy on overall survival (A), disease-free survival (B), time to second breast cancer (C), and cumulative breast cancer events (D) (Adapted from Blondeaux et al., *Lancet Oncology*, 2025(119)).

Similarly, Hassan et al. assessed long-term outcomes in *BRCA1*/*BRCA2* carriers with breast cancer using linked electronic health records. RRSO was associated with lower ovarian cancer

incidence and improved overall survival. Notably, it also contributed to reduced breast cancer–specific mortality, likely driven by estrogen deprivation in estrogen receptor–positive disease. However, uptake of RRSO was significantly lower among Black and Asian women and those in socioeconomically deprived regions of NHS England, underscoring persistent inequities in preventive care. The survival benefit was more pronounced in *BRCA2* carriers and in women who underwent RRSO after age 45, possibly due to tumor hormone receptor profile. Contrary to findings in the general population, RRSO in *BRCA1*/*BRCA2* carriers under age 50 was not associated with increased risk of cardiovascular disease, ischemic heart disease, or depression **(see Figure 12 A and B).** No association was found between RRSO and reduced CBC risk, although this may have been confounded by the high rate (>40%) of CPM in the same patients(115).



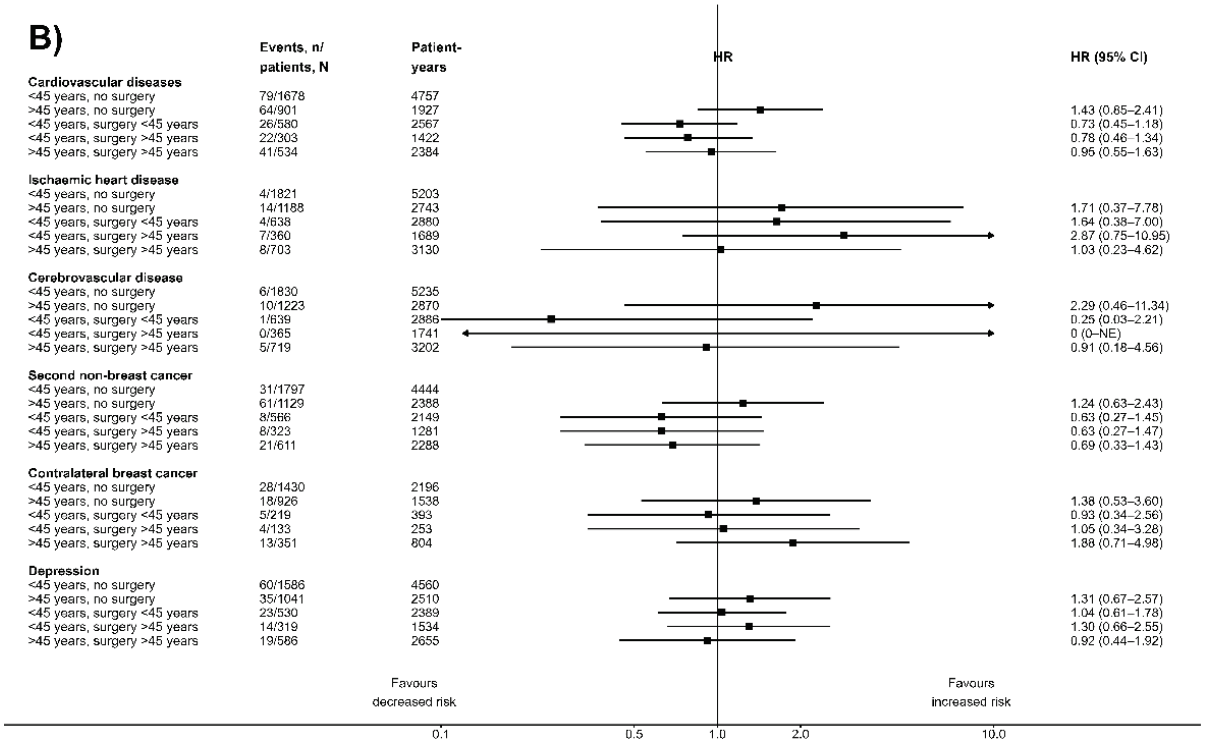


Figure 12. Association between bilateral RRSO and long-term outcomes, including overall and breast cancer–specific mortality (A), as well as non-oncologic outcomes such as cardiovascular disease and depression (B), stratified by age at breast cancer diagnosis and age at BSO (Adapted from Hassan et al., Lancet Oncology, 2025)(115).

While CPM clearly reduces CBC risk in *BRCA1*, *BRCA2* and *TP53* carriers, its impact on overall survival remains controversial. The benefit may depend on factors such as age at diagnosis, menopausal status, tumor subtype, and receipt of adjuvant therapy. For moderate-risk genes such as *ATM*, *CHEK2*, or *PALB2*, the evidence regarding elevated CBC risk is limited or variable, often depending on factors such as tumor subtype or menopausal status, for example, premenopausal patients with TNBC and a *PALB2* gPV. Moreover, data supporting a survival benefit from CPM in carriers of these genes, as well as in *RAD51C/D* or *BARD1*, are limited or lacking. In these cases, surgical decisions must be individualized, incorporating genetic risk, personal and family history, systemic treatment considerations, and patient preferences(120).

Overall, both CPM and RRSO remain important components of survivorship planning in patients with hereditary breast cancer syndromes and a personal history of disease. Their role should be evaluated not only in terms of oncologic benefit but also with respect to quality of life, reconstructive planning, surgical risks, and individual patient goals, particularly those related to reproductive health, body image, and long-term surveillance strategies(98, 121).

Survival outcomes are important in decision-making; however, it is also crucial to consider other endpoints and patient preferences. A RRM or CPM can allow a patient to avoid chemotherapy, additional breast cancer surgeries and/or hormone therapy if they have a

future second breast cancer diagnosis. Breast cancer treatments can adversely affect a patient's quality of life and survivorship experience. For premenopausal women, family planning considerations must be discussed as many may desire breastfeeding, and this may inform surgical decision-making. Patient satisfaction among young BC survivors who breastfeed is high. Additionally, CPM can negatively impact body image, making it essential to discuss the surgery, risks of complications, and aesthetic results with the patient(120, 122, 123).

It is important to acknowledge that patients who do not desire or have no indication of CPM and have a PV should be advised to undergo high-risk surveillance including annual MRI and mammograms. Notably, no differences in overall survival have been reported between CPM and high-risk surveillance(22).

3.4.3 Lifestyle factors, chemoprevention and hormonal contraception

Carriers of gPVs in genes associated with an increased risk of breast cancer should be assessed with respect to lifestyle recommendations. Key measures include encouraging regular physical activity, weight control, limiting alcohol intake, and other healthy lifestyle choices(124-126).

Chemoprevention refers to the use of pharmacologic agents to reduce, delay, or prevent the development of cancer in individuals at increased risk. In the context of hereditary breast cancer, chemoprevention strategies—particularly the use of selective estrogen receptor modulators (SERMs), such as tamoxifen and raloxifene or aromatase inhibitors (AI) like letrozole or anastrozole have shown efficacy in reducing the risk of ER–positive breast cancer in high-risk women(19, 127, 128). The NSABP-P1 trial, IBIS-I, and IBIS-II are good examples(2, 129-131); however, none of these included data specifics to *BRCA1/2* or other gene carriers. Consequently, in individuals carrying gPV in high- or moderate-risk genes, evidence regarding the preventive benefit of SERMs remains limited and somewhat inconsistent, particularly in the primary prevention setting.

Regarding hormonal contraception, the most used methods (such as oral contraceptive pills (OCPs)) remain controversial in this population. Although not contraindicated in unaffected *BRCA1/2* carriers, prolonged use of hormonal contraception (i.e., >5 years) should be minimized when feasible(18, 19, 124, 132).

Importantly, OCP use has been consistently associated with a substantial reduction in ovarian cancer risk among *BRCA1/2* carriers. Multiple studies and meta-analyses have shown that OCPs can lower this risk by approximately 40% to 60%, with the magnitude of benefit increasing with longer duration of use. This protective effect is particularly relevant for younger women who delay or decline risk-reducing salpingo-oophorectomy, and it may influence contraceptive decision-making in this group(133-135).

IV. BREAST CANCER

4.1 Epidemiology and screening programs

Breast cancer is the most common cancer diagnosis in women(136). According to the latest SEOM data (2024), 34,750 women were diagnosed with breast cancer in Spain in 2022 and for 2025 there are 37,682 new cases of breast cancer estimated. However, mortality was significantly lower, leading to a five-year prevalence of 144,233 cases by 2020(2, 137). This number is expected to keep growing due to ongoing therapeutic advances and consequently improving survival outcomes. Globally, breast cancer accounts for 6.9% of all cancer-related deaths(138).

As discussed earlier, there are multiple risk factors for cancer. In the case of breast cancer, around 5–10% of cases are hereditary, and gPV can be identified(18, 28, 136). Other known risk factors include alcohol consumption, obesity, and long-term hormone replacement therapy, especially when used for more than ten years(3).

Due to its high prevalence (1 in 8 women during their lifetime will have a breast cancer diagnosis) and the availability of new tools for early detection that can improve survival, breast cancer has become one of the main targets of population-based screening programs(2).

Currently breast cancer screening coverage is 100%. Ascunce et al., in 2007 showed a participation of the 67.0% with an adherence of 91.2%(139).

The population-based breast cancer screening program in Spain started in Navarra in 1990(140). Today, all women aged 50 to 69 in Spain are invited to undergo a mammogram every two years, unless earlier follow-up is needed due to suspicious findings. This is the recommendation by the European Commission Initiative on Breast Cancer (ECIBC). Some regions have extended this range; for example, Navarra includes women from age 45, and Galicia continues screening up to age 74(141). As mentioned in previous sections, individuals with a gPV in a breast cancer gene follow different surveillance protocols.

In Catalonia, Lee and Zelen's stochastic models were used to evaluate the effectiveness of different screening strategies. Biennial screening between ages 50 and 69 was linked to a 20% reduction in breast cancer mortality. Additionally, starting screening earlier, at age 40 or 45, resulted in more years of life gained than extending it beyond age 69(142).

4.2 Breast cancer classification

4.2.1 Immunohistochemical classification

Classical breast cancer classification is based on IHC assessment of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), tumor grade, and the proliferation marker Ki-67. ER and PR are considered positive when ≥1% of tumor cell nuclei show staining. These hormone receptors indicate potential benefit from endocrine therapy(24, 143).

HER2 is a membrane protein involved in cell proliferation; HER2 positivity is defined as IHC 3+ or IHC 2+ with ISH amplification. Ki-67 reflects the percentage of proliferating tumor cells; there is no universally accepted cutoff, but ≤20% is often considered low. Histological grade reflects how abnormal the tumor cells look and how quickly they are proliferating. Grade 1 tumors are well differentiated, grade 2 moderately differentiated, and grade 3 poorly differentiated or high grade(143).

Based on these parameters, breast cancer is classified into four main subtypes with distinct biology, prognosis, and treatment approaches (**see Figure 13**)(24, 143, 144):

- **Luminal A-like (~40–50%):** ER positive, PR ≥20%, HER2 negative, low Ki-67, and low to intermediate grade. These tumors are typically endocrine-sensitive and have a favorable prognosis.
- **Luminal B-like (~15–20%):** ER positive, and at least one of the following: PR <20%, high Ki-67, high grade, or HER2 positivity. Luminal B tumors are more proliferative and less hormone-sensitive than luminal A and may require chemotherapy in addition to endocrine therapy.
- **HER2-positive (~15–20%):** HER2 overexpressed or amplified (IHC 3+ or 2+ with ISH+), regardless of ER/PR status. These tumors are more aggressive but benefit from anti-HER2 targeted therapies.
- **Triple-negative breast cancer (TNBC) (~15%):** ER <1%, PR <1%, and HER2 negative. TNBCs are aggressive and lack targeted treatment options, so systemic therapy relies mainly on chemotherapy.

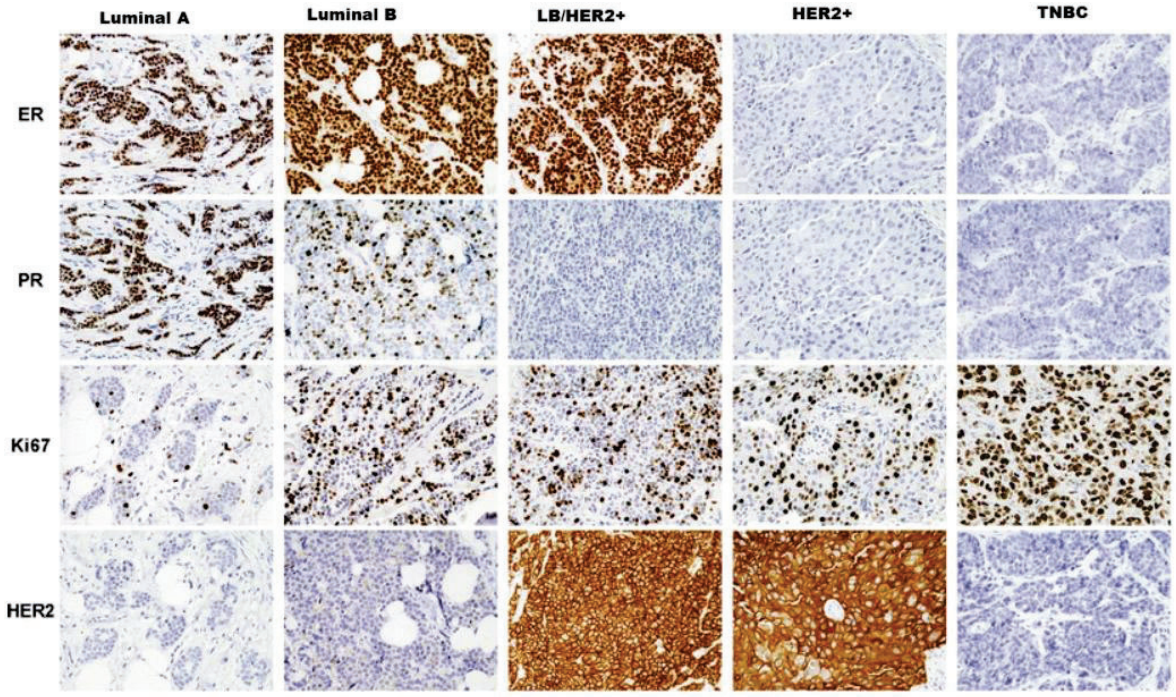


Figure 13. Immunohistochemical staining patterns for the main breast cancer subtypes (×20 magnification) (*Adapted from Gándara-Cortes M, et al. Virchows Arch. 2018(144)).*

4.2.2 Intrinsic molecular subtypes

Gene expression profiling can classify tumors into five intrinsic subtypes: Luminal A, Luminal B, HER2-enriched, Basal-like, and Normal-like. This molecular classification provides independent prognostic and predictive information that goes beyond conventional IHC(145).

As described by Prat et al., IHC-defined subtypes do not always match their molecular entities. For example, only about 70–80% of IHC-defined TNBC are truly Basal-like. Similarly, a high proportion of clinically HER2-positive or HR-positive tumors have a different intrinsic subtype when analyzed with PAM50(145).

As an example, Cejalvo et al. reported that a subset of HR-positive breast cancers are not luminal at the molecular level. Approximately 8–15% of HR+/HER2-negative tumors are classified as HER2-enriched or Basal-like, subtypes linked to endocrine resistance, poor prognosis, and potential sensitivity to chemotherapy **(see Figure 14).** These tumors may not benefit from CDK4/6 inhibition and could respond to alternative strategies, such as EGFR/HER2 blockade in the HER2-enriched group. Identifying non-luminal biology within HR-positive disease may help personalize treatment and improve clinical outcomes(146).

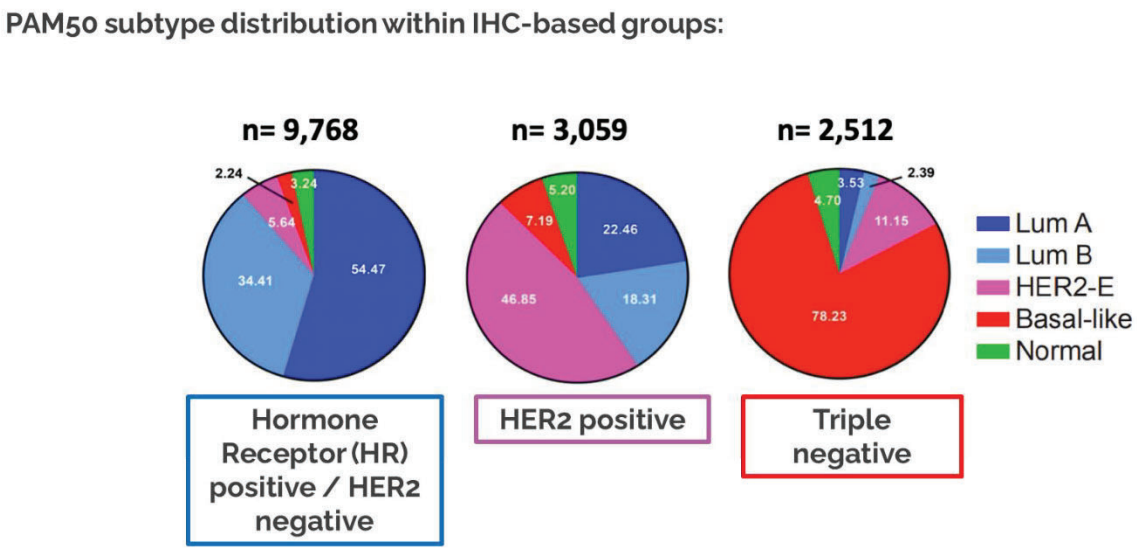


Figure 14. Intrinsic subtype distribution in HR-positive/HER2-negative breast cancer, HER2-positive and TNBC *(Adapted from Cejalvo J, et al. Cancer Treat Rev 2018(146)*).

Multiple studies have shown that intrinsic subtypes are associated with treatment response and prognosis more accurately than IHC alone **(see Figure 14**)(147).

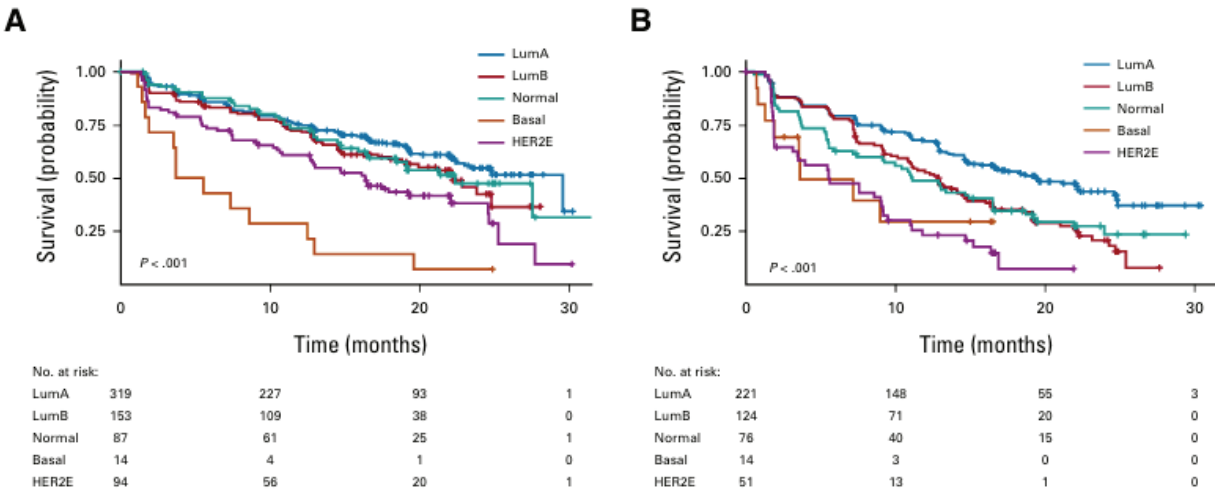


Figure 14. Progression-free survival (PFS) by intrinsic subtype in the combined MONALEESA dataset: (A) patients receiving ribociclib plus endocrine therapy; (B) patients receiving placebo plus endocrine therapy. Subtypes were determined using PAM50. Luminal A and B subtypes showed longer PFS with ribociclib, while HER2-enriched and Basal-like tumors had poorer outcomes, particularly in the placebo arm.
Basal = Basal-like; HER2E = HER2-enriched; LumA = Luminal A; LumB = Luminal B; Normal = Normal-like; RIB = ribociclib; PBO = placebo; NA = not achieved*(Adapted from: Prat A, et al. J Clin Oncol. 2021;39(24):2728–2738)(147).*

Several studies and treatment strategies have been developed based on intrinsic subtypes, as they offer a better understanding of tumor biology and help predict treatment response.

4.3 Clinical characteristics and outcomes according to germline status

Clinical features in carriers versus non-carriers

Patients with breast cancer who carry a gPV often present with distinct clinical features. These differences may be influenced by personal history—including factors such as obesity, prior cancer diagnoses, or a family history of breast cancer—as well as by the penetrance of the gene involved, the specific type of mutation, and other unknown modifiers(69, 148-150).

Some genes are associated with a higher risk of developing specific breast cancer subtypes. For example, 70–80% of patients with a *BRCA1* gPV develop TNBC, although ER-positive tumors can occur, and HER2-positive subtypes are rare(151-153). In contrast, *BRCA2* carriers are predominantly diagnosed with ER-positive/HER2-negative tumors (approximately 70-80%), though they also have an increased risk of TNBC(153). *PALB2* is associated with both ER-positive and ER-negative breast cancer, reflecting an intermediate-risk profile(2, 148). *TP53* gPV are most often linked to ER-positive and HER2-positive breast cancers(148, 154, 155). Other genes such as *BARD1, RAD51C*, and *RAD51D* are more strongly associated with TNBC, while their relationship with HR-positive disease remains less well defined(69, 148). *ATM* and *CHEK2* are typically associated with ER-positive/HER2-negative tumors(148, 156). However, some studies have also reported an increased prevalence of HER2-positive disease among

CHEK2 carriers(156). For other syndromic genes like *CDH1*, there is a well-established association with invasive lobular carcinoma. However, due to the rarity of carriers of *CDH1, PTEN*, or *STK11* mutations, the available data are often too limited to define clear associations with specific breast cancer subtypes **(see figure 15)**(69, 148, 149, 151, 152).

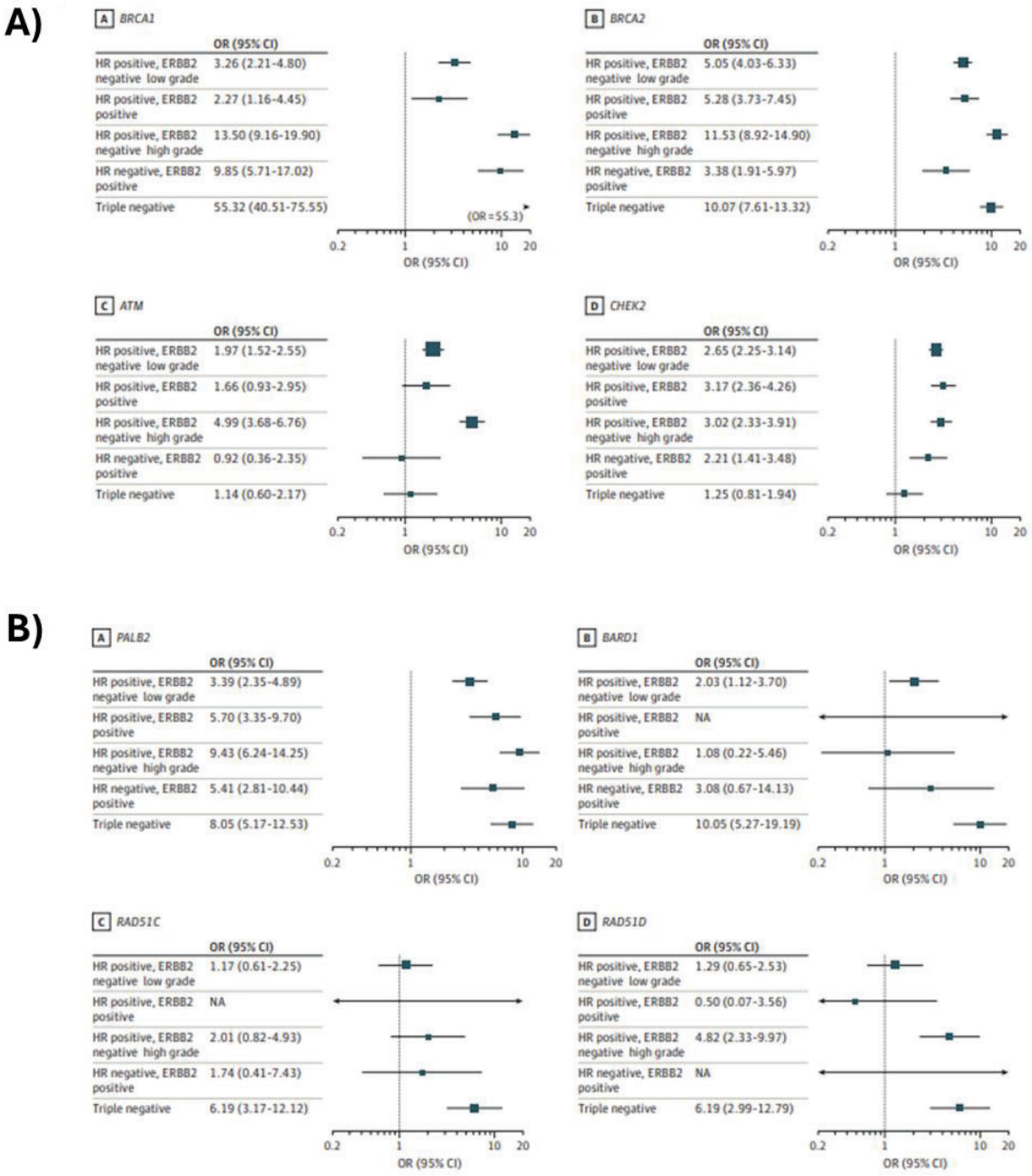


Figure 15. Associations Between gPV and Breast Cancer Subtypes. Forest plots showing the odds ratios (ORs) and 95% confidence intervals (CIs) for the association between germline gPVs in selected breast cancer susceptibility genes and specific breast cancer subtypes, based on Hormone Receptor (HR), and ERBB2 (HER2) status and tumor grade. Each plot represents a different gene: **A)** (A) *BRCA1,* (B) *BRCA2,* (C) *ATM*, (D) *CHEK2* and **B)** (A) *PALB2*, (B) *BARD1*, (C) *RAD51C*, and (D) *RAD51D*. The vertical dotted line represents an OR of 1. Squares indicate point estimates, and horizontal lines represent 95% CIs. Estimates are from multivariable

logistic regression models adjusted for age and ancestry. Data are derived from the BRIDGE study *(adapted from Breast Cancer Association Consortium, JAMA Oncol. 2022(148)).*

In the following table **(Table 5)**, we summarize the mean or median age at diagnosis and the predominant subtype associated with each gene.

Table 5. Associated Subtypes and Age at Breast Cancer Diagnosis by Germline Pathogenic Variant

Gene	Subtype	Age at diagnosis (years)
BRCA1	Overall, HR+/HER2-, TNBC	39.9
BRCA2	Overall, HR+/HER2-, TNBC	42.8
CDH1	Overall	53
PALB2	Overall, HR+/HER2-, TNBC	48
PTEN	Overall	38-50
STK11	Overall	40-60
TP53	Overall, HER2-positive	33-37
ATM	Overall, HR+/HER2-	46
BARD1	Overall, TNBC	41-42
CHEK2	Overall, HR+/HER2-, HER2-positive	47
NF1	Overall	40
RAD51C	Overall, TNBC	41-43
RAD51D	Overall, TNBC	38-41

Patients with a gPV in *BRCA1* or *BRCA2* often present with more aggressive tumor features(157-160). As previously noted, TNBC, higher histologic grade, and elevated Oncotype recurrence scores are more common in this group(159, 161). Some studies suggest that *BRCA2* carriers may have a higher likelihood of nodal involvement, and *BRCA1/2* mutations have been associated with a greater metastatic burden(162). For example, data from the PRAEGNANT registry showed a higher prevalence of brain metastases in *BRCA1/2* carriers compared to non-carriers(163). Invasive ductal carcinoma is the predominant histologic subtype among carriers of these mutations(153). However, *CDH1* mutations are specifically associated with invasive lobular carcinoma(164, 165).

For other genes, available clinical data are more limited. In a Chinese cohort of 11 women with breast cancer and a *BARD1* mutation, the median age at diagnosis was 43 years, and the proportion of TNBC was higher compared to non-carriers(166). Notably, no cases of contralateral breast cancer (CBC) were observed. Similarly, a Spanish study identified 10 *BARD1* carriers among 680 TNBC cases (carrier frequency: 0.9%), yielding an OR of 5.40 ($p = 0.001$). These findings were consistent with Shimelis et al., who reported BARD1 PVs in 0.61% of 4090 TNBC cases (OR = 5.92; $p = 2.20 \times 10^{-9}$)(167). Further studies in larger cohorts will be

necessary to refine risk estimates. In a prior study including 18 carriers of *RAD51C* or *RAD51D*, no clinical differences were found, though some trends emerged. The median age at diagnosis was 41 years, and carriers showed numerically higher rates of bilateral breast cancer (38.8% vs 17.7%, p = 0.0648) and TNBC (31.3% vs 13.9%, p = 0.0611)(168).

Sandoval et al. reported on a cohort of 227 women within situ or invasive breast cancer and a *TP53* gPV. Among those with invasive disease (186/227), the median age at diagnosis was 35 years (range: 21–71), and 11.9% had bilateral breast cancer. Most tumors were invasive ductal carcinoma (87.1%). Subtype distribution included hormone receptor positive/HER2 negative (40.9%), hormone receptor positive/HER2 positive (34.4%), hormone receptor negative/HER2 positive (12.4%), and TNBC (6.5%). At diagnosis, most patients had stage I (36.6%) or stage II (35.5%) disease(155).

Clinical outcomes: recurrence, and survival

Patients with gPV have been reported to experience different clinical outcomes compared to non-carriers(162, 169, 170). However, understanding the true impact of these variants on breast cancer outcomes has been challenging. Multiple sources of bias affect the analysis and interpretation of available data.

These include(48, 171, 172):

- Selection bias: only patients referred to genetic testing are included, often based on clinical criteria.
- Survival bias: patients must be alive at the time of testing.
- Most studies are retrospective, and the number of gPV carriers is often small.
- Some studies do not distinguish whether the mutation is germline or somatic, which limits interpretation.
- Many cohorts include patients diagnosed and treated over extended periods, during which treatment strategies have changed substantially, potentially affecting survival in ways not captured in earlier data.
- Additionally, many studies lack key clinical variables such as stage at diagnosis, ER status, or type of treatment received — all of which are known to significantly influence clinical outcomes.

The most extensively studied carriers are those with *BRCA1/BRCA2* mutations. One of the few prospective studies, the POSH study, enrolled 2,733 patients under 40 years of age from 127 hospitals in the UK, of whom 338 (12%) had *BRCA1/BRCA2* germline mutations. This study found no significant difference in OS between *BRCA* carriers and non-carriers overall. However, in the subgroup of patients with TNBC, *BRCA* carriers had better OS at 2 years compared to non-carriers (HR 0.59, p=0.047). This difference was not sustained at the 5- or 10-year follow-up(162). Other retrospective studies and meta-analyses have reported worse outcomes for *BRCA* carriers with HR-positive/HER2-negative or HER2-positive disease. In contrast, data suggest that *BRCA1/BRCA2* carriers with TNBC may fare better than non-carriers(170, 173). In

summary, current evidence remains inconclusive regarding the true impact of *BRCA1/2* mutations on survival and other clinical outcomes in breast cancer.

Data for other genes remain limited due to their lower detection rates and the inherent difficulty in assembling cohorts large enough for robust analyses. Sandoval et al. reported that patients with *TP53* gPVs and HR-positive/HER2-negative breast cancer had the highest recurrence free survival compared to those with HR-negative/HER2-negative or HER2-positive disease. However, the study lacked a control group of breast cancer patients with negative germline testing, limiting its overall impact(155). An analysis from the SEER database found no differences in survival among carriers of *ATM*, *CHEK2*, or *PALB2* gPVs compared to non-carriers(174). Among patients with *ATM* germline mutations and TNBC, a trend toward worse overall survival was observed but became non-significant after adjustment for clinical factors (**see Figure 16**)(174). Another smaller study evaluating carriers of *ATM, CHEK2, PALB2*, and *TP53* showed similar survival outcomes across gene groups.

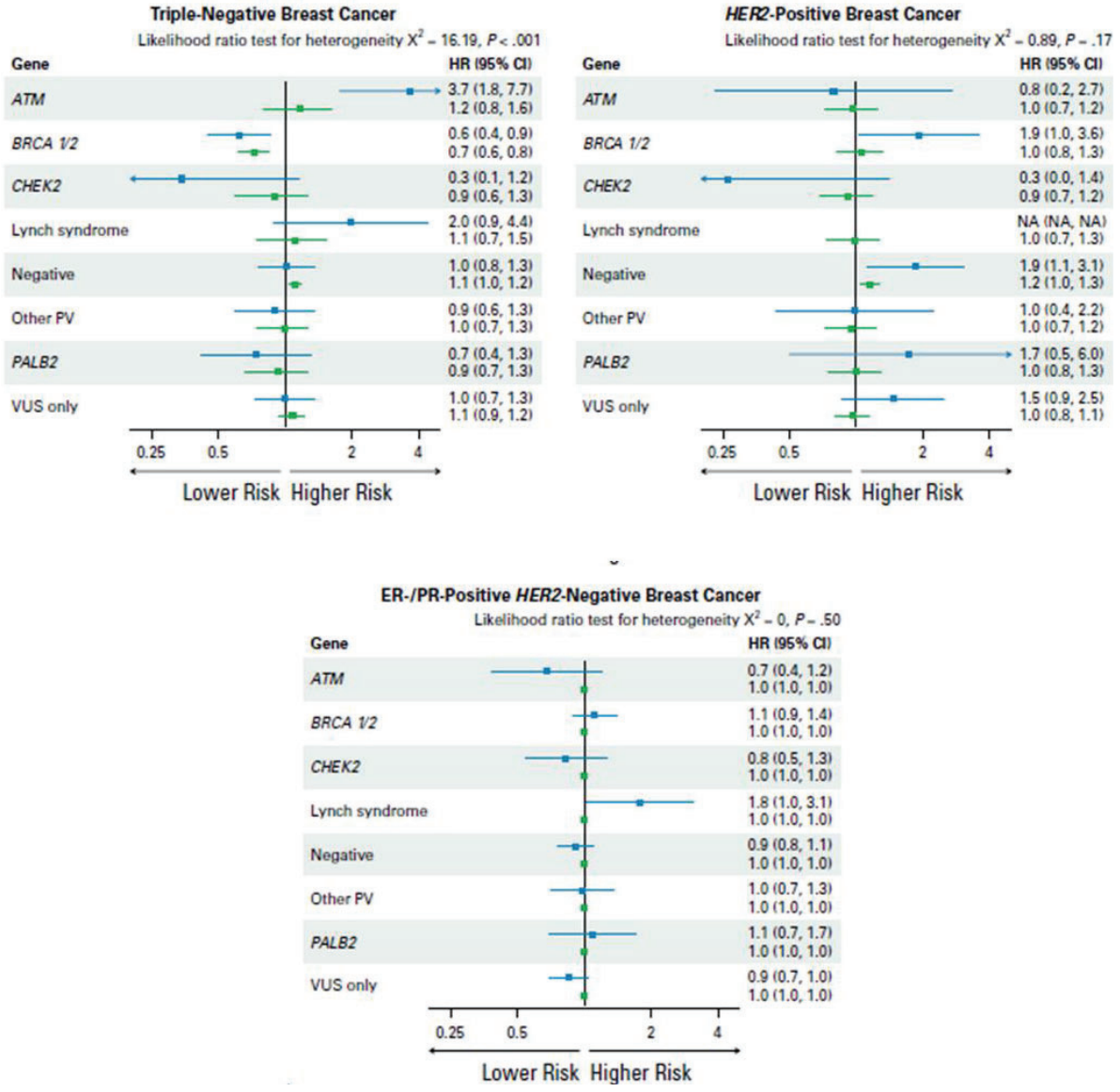


Figure 16. *Hazard ratios (HRs) for breast cancer–specific mortality by genetic testing results.* Separate gamma frailty models were run for each breast cancer subtype: triple-negative, HER2-positive, and ER and/or PR-positive, HER2-negative. Genetic testing results were categorized as: (1) PVs in *ATM, CHEK2, PALB2, BRCA1, BRCA2* (grouped as BRCA1/2), Lynch syndrome genes (*MLH1, MSH2, MSH6, PMS2, EPCAM*), or other genes; (2) VUS in any tested gene; or (3) no PV or VUS. HRs represent the relative risk of cancer-specific mortality compared to a hypothetical patient with the same subtype and the average hazard across all genetic results, adjusting for model covariates. *ER*, estrogen receptor; *HER2*, human epidermal growth factor receptor 2; *HR*, hazard ratio; *PV*, pathogenic variant; *VUS*, variant of uncertain significance (*adapted from Christine M. Veenstra et al., JCO 2025(174)*).

Prospective studies with larger, well-characterized cohorts and detailed clinical annotations are critically needed to validate these findings, clarify gene-specific associations with outcomes, and consequently inform personalized management strategies for patients with gPV.

V. DNA REPAIR PATHWAYS AND HOMOLOGOUS RECOMBINATION DEFICIENCY (HRD)

5.1 DNA repair pathways

Eukaryotic cells are constantly exposed to a wide range of DNA-damaging agents, both endogenous (e.g., reactive oxygen species, replication stress) and exogenous (e.g., ultraviolet radiation, alkylating agents, ionizing radiation). In addition, DNA damage can occur spontaneously during normal metabolic processes such as replication and transcription. If left unrepaired, this damage can compromise genomic integrity, leading to mutations, chromosomal rearrangements, or cell death. While mutagenesis plays a fundamental role in evolution and adaptation, it is also a key driver of carcinogenesis and various human diseases(175).

To maintain genomic stability, eukaryotic cells have evolved multiple specialized DNA repair pathways. These pathways are tailored to recognize and correct different types of DNA lesions. Broadly, DNA repair mechanisms are classified based on whether they resolve single-strand damage (SSD) or double-strand breaks (DSBs) **(see Figure 17)**.

- **Single-strand damage** is addressed by(175):
    - **Base Excision Repair (BER):** Repairs small, non-helix-distorting base lesions caused by oxidation, alkylation, or deamination.
    - **Nucleotide Excision Repair (NER):** Removes bulky, helix-distorting lesions such as pyrimidine dimers induced by UV light or DNA adducts from chemical exposure.
    - **Mismatch Repair (MMR):** Corrects base-base mismatches and small insertions/deletions that escape proofreading during DNA replication.

- **Double-strand breaks (DSBs)**, which are among the most cytotoxic types of DNA damage, are repaired by(175, 176):
    - **Homologous Recombination Repair (HRR):** HRR is a high-fidelity repair mechanism that restores the original DNA sequence by using a sister chromatid as a template. This pathway is predominantly active during the S and G2 phases of the cell cycle, when a sister chromatid is available. The repair process involves multiple proteins: initial sensing of double-strand breaks (DSBs) is mediated by factors such as γH2AX, ATM, and ATR, which trigger the recruitment and activation of key mediators including BRCA1, BRCA2, and PALB2(177). The final and critical step of HRR is the recruitment of RAD51, a small nuclear protein that binds to single-strand DNA, facilitates strand invasion, and stabilizes the replication fork to ensure accurate repair.
    - **Non-Homologous End Joining (NHEJ):** A quicker but error-prone mechanism that directly ligates the broken DNA ends without a homologous template. It is active throughout the cell cycle, including G1. This mechanism repairs DSB in a potentially inaccurate way.

Figure 17 illustrates the different types of DNA damage and the corresponding repair pathways. Defects in any of these pathways, particularly in HRR, MMR, or NER, can result in genomic instability and predispose individuals to cancer and other diseases(175, 176).

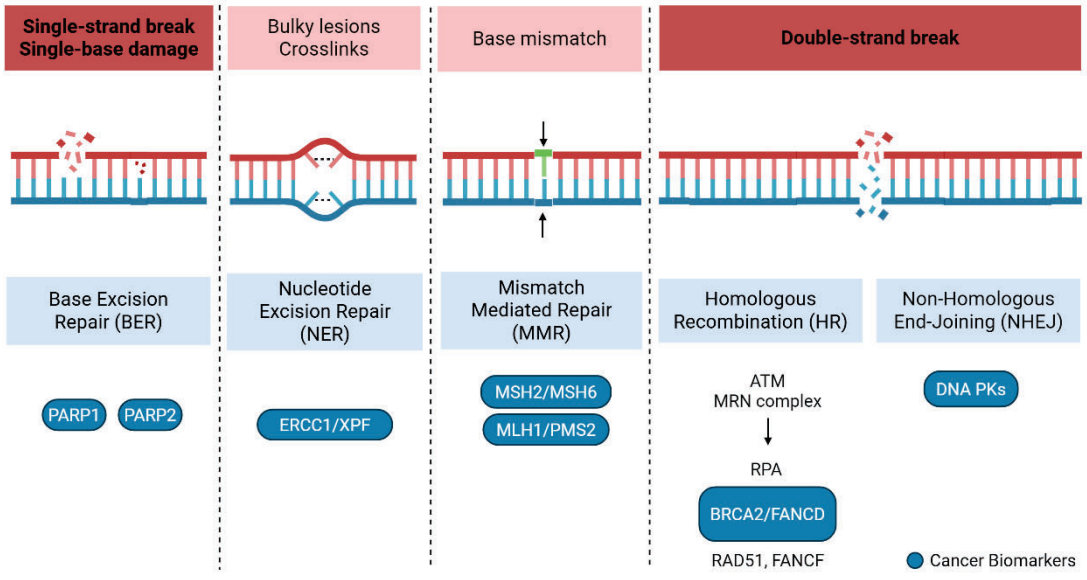


Figure 17. Overview of major DNA repair pathways and associated cancer biomarkers. Different types of DNA damage are repaired by specialized pathways. Single strand breaks and small base modifications are corrected by BER, which involves PARP1 and PARP2. Bulky DNA adducts and crosslinks are resolved by nucleotide excision repair (NER), involving proteins such as ERCC1 and XPF. Base mismatches from replication errors are corrected by mismatch

repair (MMR), primarily involving MSH2/MSH6 and MLH1/PMS2 complexes. DSBs are repaired through two distinct mechanisms: homologous recombination (HR), a high-fidelity pathway mediated by ATM, the MRN complex, RPA, BRCA2, FANCD, and RAD51; and NHEJ, an error-prone mechanism involving DNA-PK. Genes highlighted in blue are established cancer biomarkers commonly altered in hereditary or sporadic cancers *(figure created with Biorender)*.

5.2 Concept of homologous recombination deficiency (HRD)

HRD refers to the loss of functional HRR, resulting in impaired repair of DNA DSBs. This deficiency contributes to genomic instability, which can drive carcinogenesis. At the same time, it creates therapeutic vulnerabilities, making tumors more sensitive to DNA-damaging agents such as platinum-based chemotherapy and PARPi(178, 179).

As previously described, key proteins involved in the HRR pathway include *BRCA1, BRCA2*, and *PALB2*. Germline pathogenic variants in these genes—or in other HRR-related genes such as *ATM, CHEK2, BARD1, RAD51C,* or *RAD51D*—can lead to HRD. In normal cells with intact *BRCA1/2* (*BRCA1/2*$^{+}$), single-strand DNA breaks are repaired by BER. When PARP is inhibited, unrepaired single-strand breaks can lead to double-strand breaks, which are effectively repaired by the HRR pathway through active *BRCA1/2*, allowing cell survival. In contrast, tumor cells with HRD due to loss of *BRCA1/2* function (*BRCA1/2*$^{-}$) cannot repair DSB via HRR. When PARP is inhibited in these cells, the accumulation of unrepaired DNA damage leads to synthetic lethality and cell death(180) **(see Figure 18).**

This is the mechanistic basis for the heightened sensitivity of *BRCA1/2*-mutated tumors to PARPi.

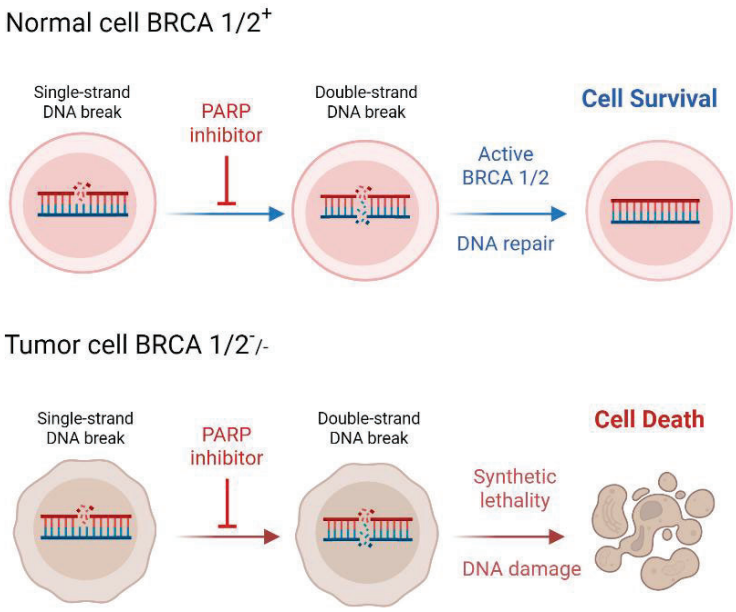


Figure 18. Mechanism of synthetic lethality with PARPi in cells with and without functional *BRCA1/2 (figure created with Biorender)*.

5.3 Methods and clinical implications of HRD detection

Classically, the concept of HRD has been associated with tumors harboring *BRCA1* and *BRCA2* mutations. However, it is now recognized that alterations in other HRR genes, as well as currently unknown mechanisms, can also lead to tumors with an HRD or "*BRCA*ness" phenotype. Approximately 50% of ovarian cancers and 30%–50% of TNBC exhibit this phenotype, although only 10%–20% of TNBC cases are attributable to *BRCA1/2* mutations and around of 20% of ovarian cancer cases(178, 181, 182). As previously discussed, the identification of HRD-positive tumors is clinically relevant due to their potential sensitivity to HRD-targeted therapies, PARPi, platinum-based chemotherapy, or combinations of PARPi with immunotherapy or antibody-drug conjugates (ADCs).

Tumors with an HRD phenotype may arise through germline or somatic mutations in HRR genes or through dysregulation of HRR gene expression via alternative mechanisms. This functional HRD leads to characteristic genomic instability, often referred to as "genomic scars," which can be detected using various molecular approaches(178, 181-183) **(see Figure 19).**

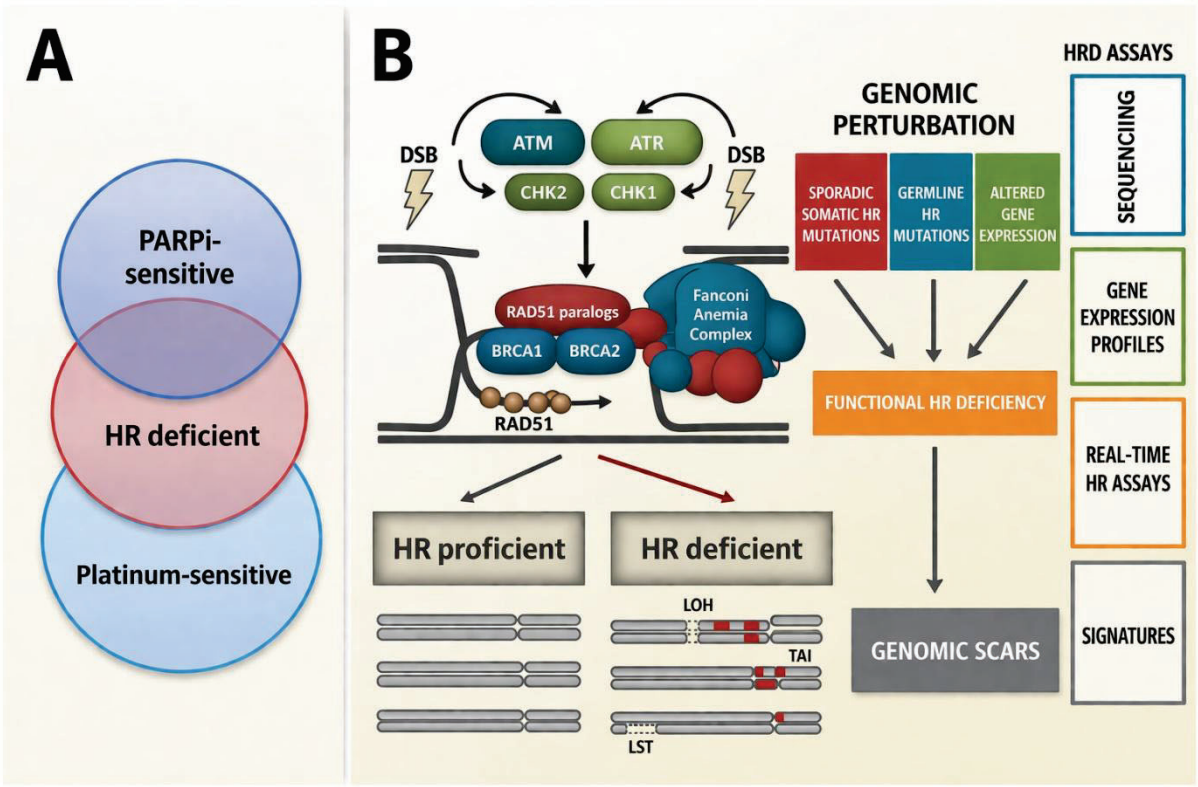


Figure 19. Overview of homologous recombination deficiency (HRD) in cancer and methods used to assess it. (A) Interaction between HRD status and sensitivity to platinum agents and PARPi. (B) Schematic summary of assays used to detect HRD and their biological basis. These alterations contribute to HRD, which can be detected through functional or genomic scar-

based assays that reflect accumulated DNA damage patterns (*Adapted from Hoppe MM, et al., J Natl Cancer Inst. 2018(183)).*

Several assays have been developed or are under development to identify HRD-positive tumors. The most widely validated is the Myriad myChoice® HRD test, which assesses three genomic instability metrics: telomeric allelic imbalance (TAI), large-scale state transitions (LST), and loss of heterozygosity (LOH). A composite HRD score ≥42 is considered positive, suggesting increased likelihood of benefit from HRD-targeted therapies. Other commercial assays focus primarily on LOH, typically using a threshold of >16% to define HRD(178, 181, 182).

In addition, specific mutational signatures, particularly signature 3, have been correlated with *BRCA1/2* mutations and with epigenetic silencing of *BRCA1* or *RAD51C* and are interpreted as indicators of HRD. Emerging techniques, such as gene expression profiling (RNA-based) and real-time functional assays, are also being explored to refine the identification of HRD-positive tumors(178, 181, 182).

A key limitation of genomic scar assays and mutational signatures is their static nature; they reflect historical defects in DNA repair rather than the current functional status of the tumor. As a result, they may fail to capture dynamic changes in HRR capacity, such as those resulting from acquired resistance mechanisms. Functional assays performed immediately prior to HRD-targeted therapies (e.g., PARPi or platinum agents) may better predict treatment response(154, 176, 184, 185).

Among the functional assays developed to evaluate HRR capacity, the RAD51-based immunofluorescence assay described by Violeta Serra and colleagues stands out for its compatibility with formalin-fixed paraffin-embedded (FFPE) tumor samples. This method assesses the ability of tumor cells to form RAD51 nuclear foci in response to endogenous DNA damage. RAD51 and γH2AX foci (a marker of double-stranded DNA breaks) are visualized by immunofluorescence and counterstained with geminin (a marker of the S/G2 phase, during which HRR is active) and 4’,6-diamidino-2-phenylindole (DAPI) (stain cell nuclei and allows to identify cells in a sample)(184, 186, 187). The assay applies a predefined threshold: tumors in which ≤10% of geminin-positive cells display ≥5 RAD51 foci are considered HR-deficient **(see Figure 20)**(184).

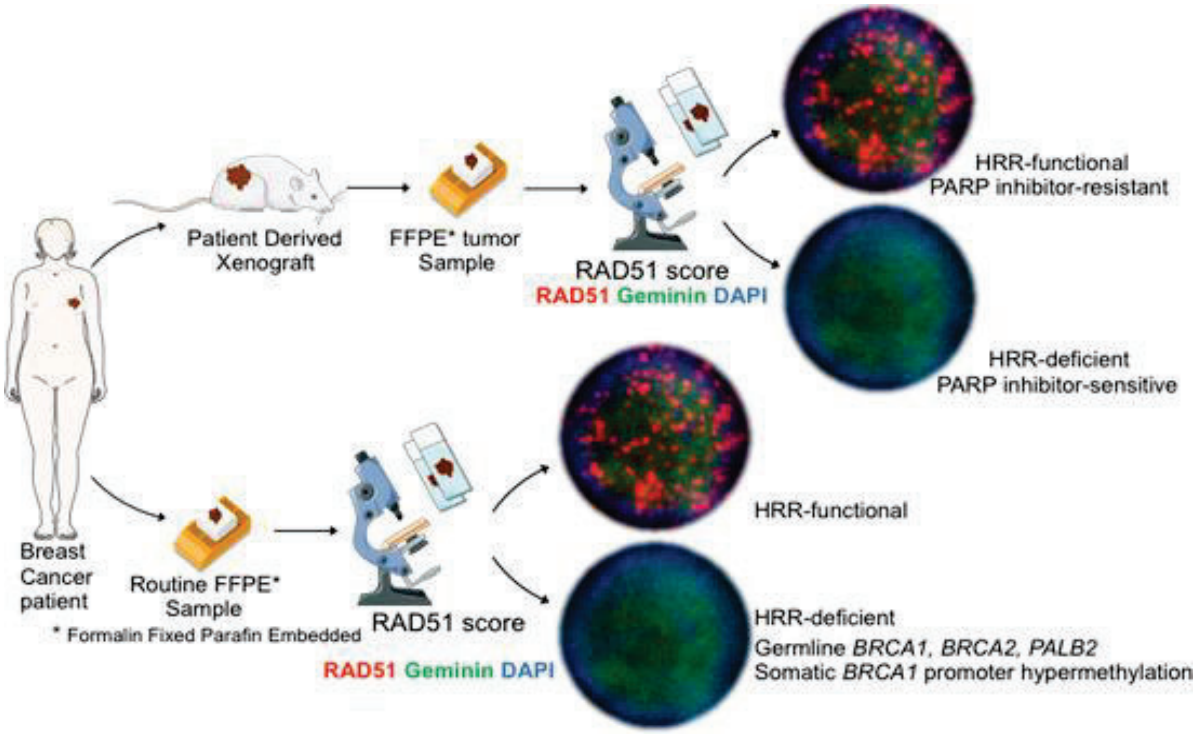


Figure 20. Evaluation of homologous recombination repair (HRR) function using the RAD51 assay in formalin-fixed paraffin-embedded (FFPE) tumor samples *(Adapted from Marta Castroviejo-Bermejo, et al., EMBO Molecular Medicine 2018(184)).*

This assay has been validated in breast, ovarian and prostate cancer samples, showing strong concordance with *BRCA1/2* mutation status and clinical outcomes, including response to PARPi and platinum chemotherapy(184, 186, 188). Notably, it can identify HRD due to non-genetic mechanisms, such as promoter methylation or protein degradation, which are not captured by DNA-based tests. As such, the RAD51 foci assay offers a real-time, functional readout of HRR proficiency and may add value in selecting patients likely to benefit from HRD-targeted therapies—beyond what is possible with genomic or epigenetic profiling alone(184, 186).

ESMO recommendations on predictive biomarker testing for HRD in ovarian cancer are as follows(189):

In the first-line maintenance setting, germline and somatic *BRCA* mutation testing is routinely recommended to identify patients with high-grade serous carcinoma (HGSC) who may benefit from PARPi therapy. Additionally, in *BRCA* wild-type HGSC, the use of a validated scar-based HRD assay is considered reasonable to determine both the magnitude of benefit from PARPi treatment and to identify patients who are unlikely to derive meaningful benefit(18, 19, 189).

In contrast, in breast cancer, the identification of HRD for the use of approved targeted therapies (PARPi) is currently limited to the detection of germline pathogenic variants in *BRCA1/2(20, 21, 24, 28)*. No other genomic or functional assays have been approved to guide

PARPi use in this setting. Prospective studies and additional clinical trials are needed to identify more breast cancer patients who may benefit from these treatments(181).

VI. THERAPEUTIC IMPLICATIONS IN BREAST CANCER WITH GERMLINE PATHOGENIC VARIANTS

Therapeutic decisions and treatment goals vary according to multiple factors. To better illustrate how the detection of these mutations may influence patient care, we have structured this section by disease stage and treatment intent. Accordingly, the following chapter is divided into early-stage and advanced diseases.

6.1 Early-stage Disease

Key clinical areas impacted by the identification of gPVs include:

6.1.1 Neoadjuvant treatment selection:

The presence of gPVs can shape neoadjuvant treatment strategies. Tumors with *BRCA1/2* mutations tend to have a high sensitivity to DNA-damaging chemotherapy (such as platinum-agents) and achieve higher rates of pathologic complete response (pCR), especially TNBC(185). Platinum-based regimens have been evaluated to exploit this sensitivity, with multiple TNBC trials showing that adding carboplatin to standard anthracycline- and taxane-based therapy significantly improves pCR, often exceeding 50%. These results support a role for platinum in TNBC, although its specific added benefit in *BRCA*-mutated tumors remains uncertain. While early cisplatin monotherapy studies reported impressive pCR, subsequent randomized trials have not consistently confirmed a clear advantage over standard chemotherapy(190-194).

Beyond chemotherapy, targeted strategies are under investigation. PARPi, alone or combined with immunotherapy, are tested in the neoadjuvant setting. The NEOTALA(195) phase II trial of neoadjuvant talazoparib did not achieve its predefined pCR goal, and the BrighTNess(196) study found no additional benefit with veliparib added to carboplatin plus paclitaxel. Ongoing studies, such as TBCRC-056 (NCT04584255), are evaluating combinations like niraparib with dostarlimab in patients with *BRCA1/2* or *PALB2* mutations, with early data showing modest pCR in HR-positive, HER2-negative tumors and immune correlates in TNBC(197).

Evidence for genes beyond *BRCA1/2* is limited. *CHEK2* carriers, for example, generally show lower pCR rates(198), though certain variants may respond better(199). At present, robust conclusions for these subgroups are lacking.

6.1.2 Surgical planning

Current guidelines recommend discussing bilateral mastectomy or contralateral prophylactic mastectomy (CPM) in patients with breast cancer carrying a gPV in *BRCA1, BRCA2*, or *PALB2*. Additionally, knowing the genetic testing results before deciding the type of surgery, increase the number of CPM and optimize surgery decisions(200). CPM may also be considered in

carriers of *CDH1, PTEN, STK11*, and *TP53* due to the potential risk of contralateral breast cancer (CBC). However, this strong recommendation is less clear for other genes such as *ATM, BARD1, CHEK2* or *RAD51C/D* **(see figure 21)**(18, 19, 22).

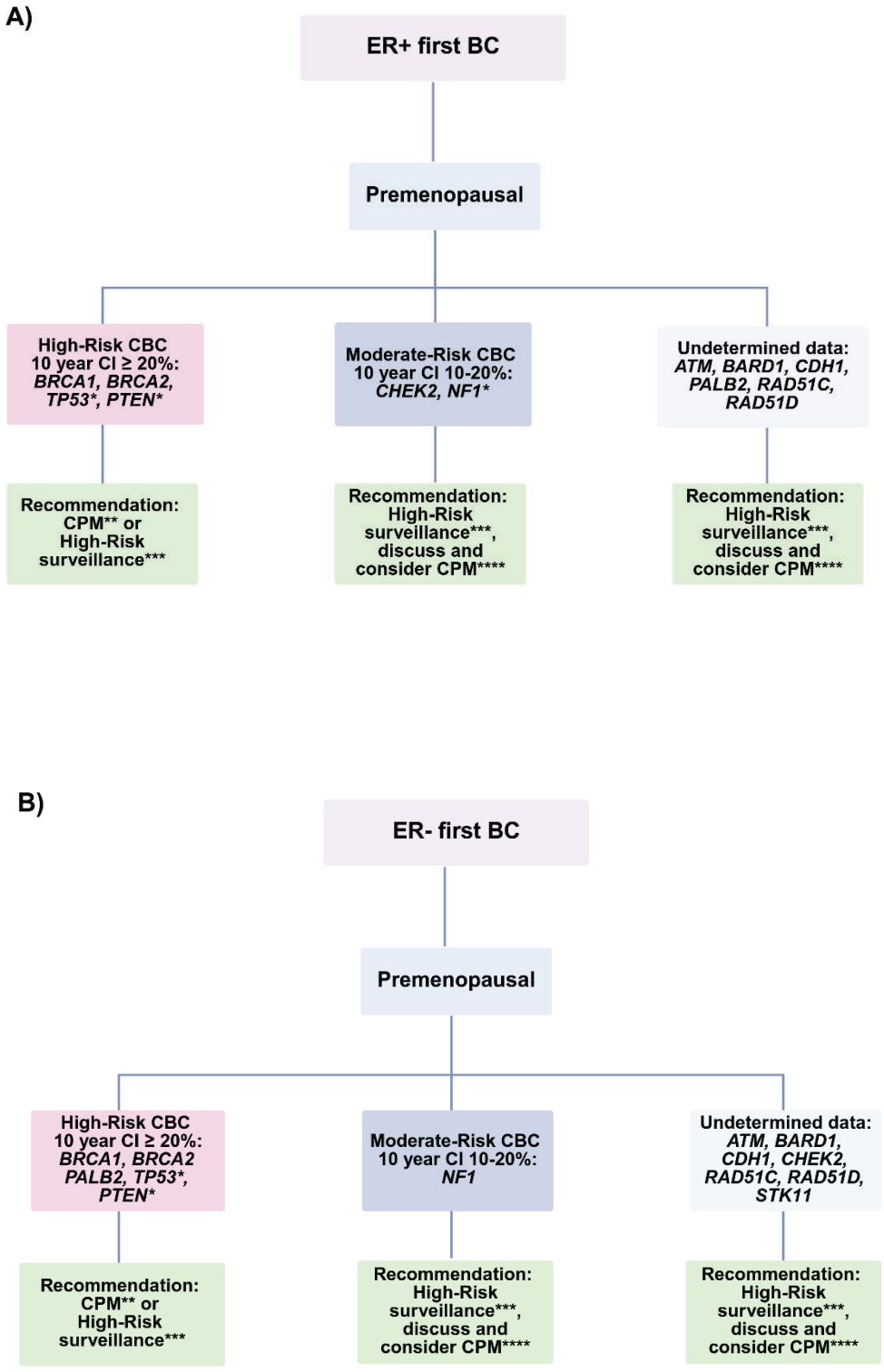


Figure 21*:* A) CBC risk by gene in premenopausal patients with primary ER-positive (ER+) BC. **B)** CBC risk by gene in premenopausal patients with primary ER-negative (ER-) BC. ER+: Estrogen Receptor-positive, CBC: Contralateral Breast Cancer, CI: Cumulative Incidence, CPM:

Contralateral Prophylactic Mastectomy. *Unknown differences between ER status and menopausal status. **CPM should be considered for eligible candidates. Breast conservation is a reasonable option after counseling on CBC risk. For patients prioritizing lactation, mastectomy for risk reduction may be deferred until after family planning. ***High-risk surveillance should consist of alternating MRI and mammogram every six months for individuals who are not candidates for CPM or choose breast conservation***CPM is a viable option, considering personal and familial risk factors, age at initial breast cancer diagnosis, and patient preferences *(figure created with Biorender)*.

Evidence suggests that CPM is performed more often than expected, both in PV carriers and non-carriers. This may reflect patients' fear of developing CBC as well as the difficulty providers face when counseling on absolute CBC risk and explaining the distinction between CBC risk reduction through mastectomy and the lack of clear evidence for a survival benefit from CPM(91, 102, 201). Recent studies have shown worse survival in patients with CBC(202). Nonetheless, although CPM reduces CBC risk by up to 90%, it has not been associated with improved overall survival compared with breast-conserving surgery (BCS) or unilateral mastectomy(202). Notably, a recent retrospective cohort study suggested an overall survival benefit from CPM and the RRSO in patients with early breast cancer diagnosed before age 40 and carrying a *BRCA1/2* gPV(119). These apparently contradictory findings likely reflect the effectiveness and safety of current breast cancer treatments if a woman develops CBC.

For patients with a gPV who do not wish to undergo or do not meet criteria for CPM, high-risk surveillance should be recommended, including annual MRI and mammography up to age 75(18, 19). Importantly, no differences in overall survival have been observed between CPM and high-risk surveillance(98).

6.1.3 Radiotherapy indications and modalities

Treatment with radiotherapy is not contraindicated in patients with a gPV, although certain special considerations apply. Patients with a gPV in *TP53* have an increased risk of radiation-induced sarcoma(203, 204). Sandoval et al. reported a 5-year incidence of 4.8% among breast cancer patients(155). Consequently, BCS is not advisable if adjuvant radiotherapy is required(203, 204). In this setting, mastectomy is preferable to avoid radiotherapy and the associated risk of secondary malignancies. Accordingly, mastectomy with CPM is often recommended to both avoid radiation exposure and reduce the risk of CBC. High-risk surveillance with MRI and mammography remains a viable alternative for patients who do not undergo, or prefer not to pursue, CPM(205).

For *ATM* carriers, earlier studies suggested an increased risk of CBC after radiotherapy. However, more recent evidence does not support this association, instead indicating only a potential increase in radiosensitivity(206, 207)

In summary, radiotherapy remains a safe option for most gPV carriers, with a special precaution with patients with a gPV in *TP53*, where mastectomy and CPM should be

prioritized; individualized counseling is essential to balance risks, benefits, and patient preferences.

6.1.4 Adjuvant treatment choices

The pivotal trial that has changed adjuvant treatment options for patients with early breast cancer carrying a gPV in *BRCA1/2* is the OlympiA trial(208, 209). This study enrolled patients with HER2-negative disease who had received neoadjuvant or adjuvant chemotherapy, thereby selecting a high-risk population. Eligibility criteria included residual disease after neoadjuvant chemotherapy in TNBC and at least four positive lymph nodes at surgery in HR-positive disease. The trial demonstrated a significant benefit of adjuvant Olaparib over placebo, with an absolute improvement in invasive disease–free survival (IDFS) of 9.4% at 6 years and an OS benefit of 4.4%, independent of IHC subtype, prior platinum exposure, or mutation type (**see figure 22**)(210).

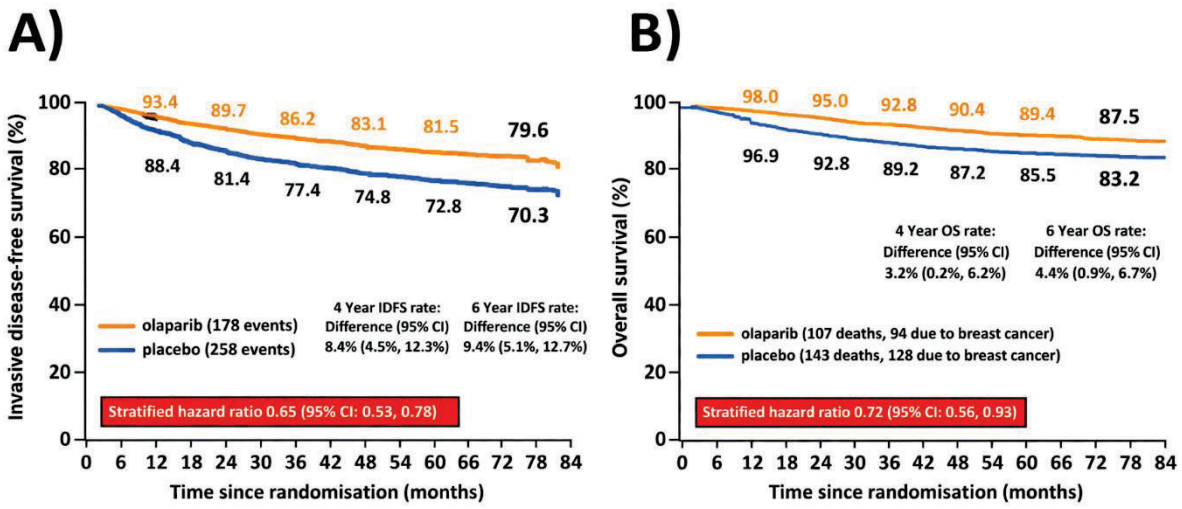


Figure 22: A) Invasive disease-free survival (IDFS) in the intention-to-treat (ITT) population of the OlympiA trial: HR 0.65 (95% CI, 0.53–0.78) in favor of olaparib versus placebo. B) Overall survival (OS) in the ITT population of the OlympiA trial: HR 0.72 (95% CI, 0.56–0.93) in favor of olaparib *(Adapted from Judy E.Garber, SABCS 2024)(210).*

In the metastatic setting, PARPi have also shown activity in patients with a gPV in *PALB2*, although no dedicated trials have specifically evaluated this subgroup in the advanced or early setting(211). Importantly, OlympiA(209) will provide not only mature data on IDFS and OS, already established, but may also clarify whether the incidence of secondary cancers associated with these genes is reduced in the adjuvant olaparib-treated group.

Based on OlympiA, adjuvant olaparib is approved for patients with HER2-negative breast cancer, a gPV in *BRCA1/2*, and high-risk clinical features(24). This approval has broadened the indications for germline testing in breast cancer, underscoring its importance for identifying patients who may benefit from targeted therapies.

6.2 Advanced Disease

6.2.1 PARPi and platinum-chemotherapy

Platinum-based chemotherapy has demonstrated increased activity in patients with breast cancer harboring alterations that cause HRD(212). However, trial results regarding superior response rates, PFS, and OS compared to other chemotherapies have been inconsistent. More consistently, patients with gPV in HRR genes have shown higher sensitivity to PARPi, including olaparib, niraparib, talazoparib, and veliparib(213, 214).

The OlympiAD(215) and EMBRACA(216) trials demonstrated that olaparib and talazoparib, respectively, improved PFS in patients with *gBRCA1/2* and HER2-negative advanced breast cancer compared with standard chemotherapy (e.g., eribulin, capecitabine). However, neither trial showed a significant OS benefit. These findings led to FDA approval of both agents for patients with metastatic breast cancer carrying g*BRCA* mutations, previously treated with chemotherapy in the neoadjuvant, adjuvant, or metastatic setting(19).

Other trials, such as TBCRC-048(211), have evaluated PARPi activity in tumors with mutations in *CHEK2, ATM, RAD51C, RAD51D*, and *PALB2*, considering both germline and somatic origins. Among these, only germline *PALB2* carriers and patients with somatic mutations in *BRCA1/2* demonstrated clear clinical benefit from PARPi. For the other genes, activity did not exceed that of standard chemotherapy. While PARPi improve PFS, the median benefit remains modestly around 7 months in TNBC and 10 months in HR-positive disease(217).

To extend PFS and translate this into an OS benefit, combination strategies are under investigation. PARPi are known to increase PD-L1 expression and release of neoantigens, but trials such as MEDIOLA(218) have not shown substantially better outcomes compared to PARPi monotherapy, though additional phase III data are pending.

Given these limitations, newer therapeutic strategies are being investigated. These include next-generation PARPi, such as selective PARP1 inhibitors (e.g., suraparib), and novel combination approaches with targeted agents like axatilimab (humanized monoclonal antibody that targets the colony stimulating factor-1 receptor (CSF-1R)) (NCT06488378). Additional strategies involve targeting DNA damage response pathways with POLθ and ATR inhibitors, which may show activity beyond *BRCA1/2*-mutated disease and help overcome resistance mechanisms to PARPi.

6.2.2 CDK4/6 Inhibitors + endocrine therapy

In HR+/HER2− breast cancer, CDK4/6i in combination with ET represent the current standard of care in the advanced setting, with palbociclib, ribociclib, and abemaciclib consistently improving PFS compared with ET alone(219-222). In the adjuvant setting, ribociclib and abemaciclib have also demonstrated significant benefits in iDFS(223-227) and abemaciclib in OS(227). However, patients carrying germline or somatic PVs in HRR genes have not been

specifically studied in the pivotal trials that led to these approvals. Emerging retrospective evidence and meta-analyses suggest that patients with gPVs in *BRCA2* or other HRR-related genes may derive less benefit from CDK4/6i compared with non-carriers, with shorter PFS and OS observed in multiple cohorts(228-232). One proposed explanation is the higher frequency of *RB1* biallelic loss in *BRCA2*-mutated tumors, a mechanism that may underline intrinsic or acquired resistance to CDK4/6i(233). These observations raise questions about the optimal sequencing of therapies in this subgroup, including the potential role of PARPi in earlier lines of treatment for HR+/HER2− disease with *BRCA2* PVs or other HRR genes where the numbers don't allow to reach conclusions regarding responses to different treatments. Currently, the phase III EvoPAR-Breast 01 trial (NCT06380751) is addressing this question by comparing CDK4/6 inhibitor plus ET with suraparib (a PARP1 inhibitor) plus camizestrant (ET) in patients with germline and/or somatic PVs in *BRCA1, BRCA2,* and *PALB2*.

VII. KEY AREAS REQUIRING ADVANCEMENT

The rapid progress in sequencing technologies, the wider availability of genetic testing, and the growing clinical use of these results have created new opportunities for patients and their families. At the same time, these advances have introduced new challenges and uncertainties that require attention.

The increasing use of multi-gene panel testing has brought important difficulties, particularly regarding the management of pathogenic variants detected at low allele frequencies—referred to in this work as LAFVs. These findings are now more frequently identified and often raise uncertainty in both diagnostic interpretation and clinical decision-making. Refining diagnostic algorithms to evaluate LAFVs and establishing clear guidance for clinical management are essential. The implications for the individual and their family can differ substantially depending on how these variants are interpreted. Equally important is the need to communicate these results clearly across specialties so that the complexity of variant interpretation is translated into practical, actionable strategies.

The psychosocial implications of uncertain results cannot be underestimated. Informing a patient that they carry a VUS or a LAFV without clear clinical implications may lead to significant distress, anxiety, and inappropriate clinical decisions(83). Establishing standardized counseling frameworks and educational resources for both patients and providers will be vital to mitigate these risks.

One of the main limitations lies in the interpretation of genetic testing results and the classification of germline variants. As discussed earlier, additional clinical evidence and robust datasets are needed to better characterize variants in moderate-risk genes such as *ATM, CHEK2* or *TP53*, as well as in more widely studied genes such as *BRCA1* and *BRCA2*. Improved understanding will enable more accurate estimation of cancer risks, refine penetrance models, and support the development of tailored prevention strategies, including high-risk surveillance and risk-reducing interventions(90, 234, 235).

Accessibility and equity also remain major challenges. Although the cost of genetic testing has decreased considerably in recent years, affordability continues to be a barrier in many low- and middle-income countries. In these contexts, strategies to prioritize testing for individuals at highest risk—based on family history, clinical features, and tumor characteristics—are particularly valuable. Prioritization should also focus on genes with the greatest impact on treatment decisions, surveillance strategies, and familial risk assessment.

Finally, clinical trials to date have rarely been designed to evaluate treatment outcomes specifically in carriers of germline or somatic pathogenic variants and breast cancer, largely due to the relative rarity of these patients and the challenges of accrual. As a result, the differential responses of carriers to established or emerging therapies remain poorly characterized. Expanding dedicated trials, as well as integrating germline variant data into ongoing large-scale studies, will be critical to advance precision oncology in this setting.

For all these reasons, the present work aims to contribute in three major ways: first, by proposing an improved algorithm to guide the detection and interpretation of complex results such as LAFVs; second, by providing a more comprehensive clinical characterization of breast cancer patients with mutations in HRR genes, to support better prioritization of testing and individualized care; and third, by exploring in the metastatic setting the patterns of treatment sensitivity and the optimal sequencing of therapies for patients carrying germline or somatic variants in HRR genes. Advancing our understanding of germline variation and treatment response will shape the future of precision oncology, ensuring that genetic testing evolves to actionable knowledge.

HYPOTHESIS

Based on the above considerations, the hypotheses of this thesis propose that:

In breast cancer, accurate interpretation of germline alterations in DNA-repair pathways, including those that require complex interpretation, such as low-allele-frequency variants, has direct implications for risk assessment and may require adaptation of current management strategies and treatment selection.

More specifically, this thesis is based on the following 3 sub-hypotheses:

1. Low-allele-frequency variants identified through germline testing constitute a heterogeneous group that includes technical artifacts, clonal hematopoiesis, and constitutional mosaicism, and must be distinguished from true heterozygous variants. Accurate classification is clinically critical, as misinterpretation may result in inappropriate management of both patients and their families.
2. Patients with breast cancer who carry germline pathogenic or likely pathogenic variants in genes involved in homologous recombination repair, present distinct clinical and molecular characteristics that influence tumor biology and clinical presentation. Improved characterization of these patients may enhance prioritization of genetic testing and individualized care.
3. Patients with germline pathogenic or likely pathogenic variants in homologous recombination repair genes exhibit differential treatment response and distinct resistance profiles to cyclin-dependent kinase 4 and 6 inhibitors and other targeted therapies in advanced hormone receptor–positive, human epidermal growth factor receptor 2–negative breast cancer compared with non-carriers, potentially impacting therapeutic efficacy and optimal sequencing strategies.

To validate this hypothesis, the following objectives were defined:

OBJECTIVES

GENERAL OBJECTIVE:

To evaluate the clinical utility, interpretative challenges, and therapeutic implications of germline variants involved in DNA-repair pathways, with particular focus on low-allele-frequency variants and pathogenic alterations in genes related to homologous recombination repair in breast cancer.

More specifically, this thesis consists of nine sub-objectives, structured according to the following specific study aims:

1. To describe the frequency and technical characteristics of low-allele-frequency variants identified through multigene panel testing, in comparison with conventional heterozygous germline variants.
2. To determine the potential biological origin of low-allele-frequency variants, including somatic mosaicism, clonal hematopoiesis, or true germline events.
3. To assess the clinical and interpretative implications of low-allele-frequency variants, particularly the risks associated with variant misclassification in oncology.
4. To characterize the prevalence of germline pathogenic variants in genes involved in homologous recombination repair in a cohort of patients with breast cancer tested according to institutional genetic testing criteria.
5. To compare relevant clinical and tumor-related characteristics, such as age at diagnosis, tumor subtype, bilateral disease, and stage, between carriers and non-carriers of germline pathogenic variants in homologous recombination repair genes.
6. To determine the impact of germline pathogenic variants in homologous recombination repair genes on clinical management, including surgical decision-making, preventive interventions, and access to targeted therapies.
7. To investigate the association between germline pathogenic variants in homologous recombination repair genes and response to cyclin-dependent kinase 4 and 6 inhibitors in advanced hormone receptor–positive and human epidermal growth factor receptor 2-negative breast cancer.
8. To explore potential mechanisms of therapeutic resistance to cyclin-dependent kinase 4 and 6 inhibitors related to impaired DNA-repair pathways.
9. To inform optimal therapeutic sequencing and precision-medicine strategies for patients with germline or somatic alterations in homologous recombination repair genes in the metastatic setting.

MATERIAL METHODS AND RESULTS

First publication: ***"Low allele frequency variants identified on germline multi-gene panel testing for cancer predisposition can suggest the presence of constitutional mosaicism"***

Abstract:

Purpose: Enabled by advancements in next-generation sequencing for hereditary cancer conditions, low allele frequency variants (LAFV) are detected by testing laboratories. This study describes the frequency and clinical factors associated with LAFVs and reports results of follow-up testing when available.

Experimental Design: This retrospective study analyzed LAFV cases identified through multi-gene panel testing (MGPT) at a single high-volume germline genetic testing laboratory. LAFVs were defined as variant allele frequencies (VAF) between 10% and 30% as confirmed by Sanger sequencing. Comparative analyses were conducted between pathogenic variants with a VAF of 30% to 60% (inferred heterozygous) and LAFV cohorts. Clinical characteristics were analyzed using descriptive statistics and logistic regression models. Ancillary testing was performed on alternative specimens or family members to determine whether LAFVs were due to constitutional mosaicism.

Results: Among 363,405 individuals undergoing MGPT, 965 (1.8%) had variants with a VAF between 10% and 30%. Sanger sequencing confirmed 463 (0.1%) as LAFVs. Among the confirmed LAFVs, 262 (57.6%) were classified as pathogenic variants. The LAFV cohort compared with the control heterozygous cohort was significantly older, with a higher proportion of individuals >50 years (84.1% vs. 54.9%; $P < 0.001$). LAFVs were present in the following genes: *TP53* (110; 64.7%), *NF1* (23; 13.5%), *CHEK2* (13; 7.6%), *ATM* (12; 7.1%), and *BRCA1* (4; 2.4%). Ancillary testing performed in 62 cases with LAFVs confirmed 17.7% (11/62) as constitutional mosaicism.

Conclusions: LAFVs were infrequently detected in MGPT, representing 0.8% of the total variants and 0.1% of total tested. Ancillary testing is needed to understand the origins and clinical implications of LAFVs in patients and families.

Low Allele Frequency Variants Identified on Germline Multi-Gene Panel Testing for Cancer Predisposition Can Suggest the Presence of Constitutional Mosaicism

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Conclusions: LAFVs were infrequently detected in MGPT, representing 0.8% of the total variants and 0.1% of total tested. Ancillary testing is needed to understand the origins and clinical implications of LAFVs in patients and families.

Introduction

Cancer predisposition genetic testing uses peripheral blood or saliva as sources of germline tissue because of their accessibility (1). Next-generation sequencing (NGS) assays are highly sensitive and have simplified simultaneous testing of multiple genes (1, 2). In some cases, this high sensitivity has led to the detection of low allele frequency variants (LAFV; refs. 3, 4). We defined LAFVs as measurable variants with frequencies between 10% and 30% in NGS and Sanger sequencing. This contrasts with inherited germline variants with an approximately 50% allele frequency (heterozygous; refs. 3, 5). When LAFVs are identified, it is imperative to determine whether they are attributable to constitutional mosaicism (CM; post-zygotic mosaicism) or acquired through either somatic contamination or clonal hematopoiesis (CH; refs. 2, 3, 5). LAFVs are commonly detected in genes associated with hematologic malignancies (6). The use of multi-gene panel testing (MGPT) for patients with solid tumors and their families has led to the increased identification of LAFVs in other cancer predisposition genes, such as *ATM*, *BRCA1*, *BRCA2, NF1*, or *TP53* (3, 7). However, their frequency, interpretation, and clinical implications remain unclear.

The primary challenge in interpreting LAFVs is distinguishing between CM and CH, which is an expansion of clonal blood cell subpopulations. CH indicates a higher risk of a hematologic malignancy with or without cytopenia (8–11). Factors such as aging, smoking habits, personal cancer history, and prior chemotherapy or radiotherapy have been linked to CH (2, 12–15). One study reported a 25% prevalence of CH among patients with cancer across different cancer types (12).

CM refers to the presence of a discernible clonal group of cells that contains a somatic mutation that is different from inherited germline DNA (16). In a prior study, among more than one million unrelated individuals who underwent germline testing, mosaic variants were identified in 5,695 subjects, representing 0.6% of the overall cohort, accounting for 2% of the molecular diagnoses in the cohort. Mosaic variants were primarily found in genes associated with hereditary cancer predisposition, including *TP53*, *ATM*, *CHEK2*, and *NF1* (17). To determine whether a variant is due to CM, somatic contamination, or CH, evaluation of additional tissues that do not contain leukocytes, such as cultured skin fibroblasts or testing family members, is required (18, 19).

This study aimed to delineate the frequency, distribution, and clinical factors associated with LAFV detection in individuals undergoing MGPT for cancer-related genes. We compared the clinical factors associated with LAFVs with those with heterozygous variants

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Translational Relevance

This study investigated variants with allele frequencies between 10% and 30% identified through next-generation sequencing multi-gene panel testing of a large cohort of consecutively tested individuals. Low allele frequency variants (LAFV) create challenges in test interpretation and clinical management, as the frequency and impact of LAFVs in genetic testing, particularly in cancer-related genes, are not yet well established. We aimed to address these knowledge gaps and to evaluate the characteristics that may distinguish LAFVs from heterozygous variants. Additionally, we highlight the necessity of ancillary tissue or family member studies to clarify the variant origins and guide clinical management.

that have an allele frequency of more than 30%. Additionally, we reported the results of ancillary tissue testing and family members when available.

Materials and Methods

Study design and population

In this retrospective cohort study, we analyzed consecutive cases of LAFVs identified through MGPT for hereditary cancer indications, ordered between January 2019 and January 2021 at a single diagnostic testing laboratory (Ambry Genetics). The WGC Institutional Review Board (formerly the Western Institutional Review Board) determined that the study was exempt from the Office for Human Research Protection Regulations for the Protection of Human Subjects (45 CFR 46) as (i) no additional patient information was acquired for this study beyond already provided information via clinical testing and (ii) investigators could not readily ascertain the identity of subjects included in the coded de-identified dataset.

Clinical characteristics were obtained from clinician-completed test requisition forms supplemented with clinical documentation, including pedigrees and clinical notes, when available. The clinical characteristics included age at testing, sex, and self-reported ethnicity. For cancer diagnoses, International Classification of Diseases tenth revision (ICD10) diagnosis codes of patients were collected from the XIFIN Revenue Cycle Management software via a patient client portal. The portal interfaces with ordering physicians and directly feeds into the Ambry databases. To determine the accuracy of ICD10 diagnosis for cancer type, we compared human curated disease characteristics and risk factors among Ambry patients (PMID: 36008414) with ICD10 codes aggregated to define breast, ovarian, prostate, colorectal, and/or pancreatic cancer cases (RRID: SCR_010351; Supplementary Table S1). All other cancers were combined into one category denoted as "other," whereas multiple cancers were defined as having more than one of the five types previously specified (breast, ovarian, prostate, colorectal, and/or pancreatic cancer).

Germline testing panels and variants classification

The variants were classified as variants of uncertain significance (VUS) or pathogenic/likely pathogenic (PV). Variant interpretation was based on the guidelines of the American College of Medical Genetics and Genomics (RRID: SCR_005769) and the Association for Molecular Pathology (refs. 20, 21). In this study, pathogenic and likely pathogenic variants were grouped and termed as PVs.

MGPT was performed using bait-capture methodology followed by PCR and NGS for the entire cohort ($n = 363{,}405$). Additional Sanger sequencing for confirmation was conducted on variants with 10% to 30% variant allele frequency (VAF; $n = 965$). Variants with a VAF of 10% to 30% on NGS and Sanger sequencing (concordant) were classified as LAFVs ($n = 463$). Variants with a VAF of 30% to 60% on MGPT ($n = 54{,}076$) and/or Sanger sequencing ($n = 362$) were inferred as heterozygous variants (**Fig. 1**). We used a cutoff of >30% VAF to infer heterozygosity; this is an operational threshold rather than a strict biological distinction.

The cases with a VAF of 10% to 30% ($n = 965$) underwent testing through a targeted cancer panel (i.e., breast, ovarian, and colon; 8–25 genes; $n = 157$), a pan-cancer panel (34–77 genes; $n = 592$), or with a customizable panel of 1 to 75 genes (1–91 genes; mean number of genes = 59; $n = 216$). The wild-type cohort included 312,726 subjects without any PVs on a pan-cancer panel (49–67 genes). Supplementary Table S2 lists the different panels performed and the number of individuals tested with each panel, specified by group (heterozygous variants and LAFVs).

Assessment of CM

To assess whether these variants were CM ($n = 262$), additional tests were performed using either an alternative sample type (cultured fibroblasts or isolated DNA collected separately) from the proband ($n = 44$) or, when possible, through familial testing ($n = 23$; **Fig. 1**). Although, the possibility of a technical issue involving the initial specimen or analysis cannot be entirely ruled out, the results of additional testing can help determine whether LAFVs represent CM. If the LAFV was detected in the proband or their family, it was classified as CM. Conversely, if the LAFV was not detected, it was classified as an indeterminate LAFV on complementary testing. Because of the limited availability of alternative tissue testing and familial samples, it was not feasible to exclude CM in these cases, and a definitive classification of CH could not be established.

Statistical analysis

For comparative analyses, we restricted the evaluation to only overlapping variants present in both the inferred heterozygous and LAFV cohorts, with unique protein and coding sequence notations and evaluable ICD10 clinical data to create an OR measure for LAFV. After excluding VUS and counting each unique PV only once per individual, the final cohort used for the clinical comparative analysis and OR calculations consisted of 1,197 total individuals with 1,027 individuals with inferred heterozygous variants (30%–60% VAF) and 170 individuals with LAFVs (10%–30% VAF on both MGPT and Sanger; **Fig. 1**).

Descriptive statistics for individuals stratified by variant categories were summarized as proportions for categorical characteristics. The frequencies of specific tumor types and ORs with 95% confidence intervals (CI) were summarized by genotype. The median and IQR of the age distribution at testing were used to describe dispersion. The Mann–Whitney U non-parametric test was used to test the median age differences by LAFV status with bootstrapped CIs. Otherwise, χ^2 tests, Fisher exact test, or unadjusted logistic regression were performed to identify individual factors associated with LAFVs versus heterozygous variants. Separate logistic regression models were used to compare cancer outcomes to the LAFV

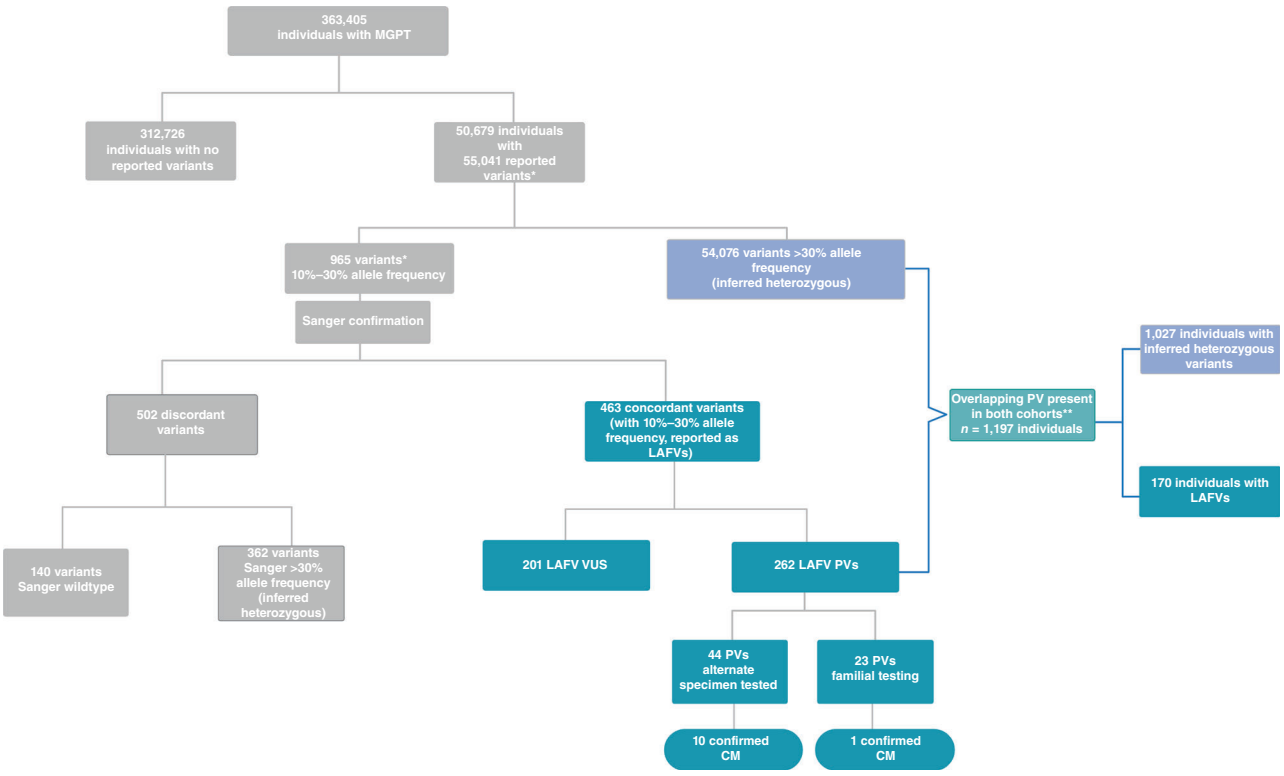

Figure 1.

CONSORT diagram of patients with MGPT. *, Variants: PVs. **, For comparative analyses, we used an inferred heterozygous (30%–60% VAF) cohort of PVs (1,027 individuals), excluding LAFVs (10%–30% VAF) based on MGPT confirmed after Sanger sequencing, and a separate LAFV cohort of PVs (170 individuals) excluding heterozygous variants. VUS were excluded from both cohorts. We enumerated only overlapping variants in both cohorts, with unique protein and coding sequence notations and evaluable ICD10 clinical data to create an OR measure for LAFV.

category (yes/no), while adjusting for continuous age at testing, sex (treated as a categorical variable: male/female or restricted to specific categories for breast, ovarian, or prostate cancer), and self-reported ethnicity. For each model, the dependent variable represented the presence of a specific cancer type (e.g., colorectal cancer: yes/no). A "yes" response indicates the presence of colorectal cancer, whereas a "no" response includes individuals without colorectal cancer, irrespective of whether they have other cancers or are cancer-free. By structuring the analysis in this way, we aimed to first describe the demographic factors associated with LAFV status independently and then explore clinical associations in a way that allows for both unadjusted and adjusted analyses (age, sex, and ethnicity). This approach allowed us to assess potential confounders explicitly, while maintaining interpretability of results. All statistical tests were two-sided, and a P value of less than 0.05 was considered statistically significant. All analyses were conducted using R v.4.3.0 (RRID: SCR_001905).

Data availability

The data generated in this study are not publicly available because of patient privacy requirements and were made available under approval for this study from Ambry Genetics. Data are available from the authors upon request and on permission from Ambry Genetics, provided that they remain de-identifiable. All relevant data are presented in the article and its supporting information files.

Results

Prevalence of variants with 10% to 30% VAF in individuals undergoing MGPT

Between January 2019 and January 2021, 363,405 individuals underwent MGPT. In 50,679 of 363,405 (13.9%) individuals, a PV or VUS was detected, including 55,041 PVs and VUSes. Of these variants, 965 (1.8%) had 10% to 30% VAF and 54,076 (98.2%) were inferred as heterozygous (based on 30%–60% VAF). After Sanger sequencing of the variants with 10% to 30% VAF, 140/965 (14.5%) were classified as wild type, 362/965 (37.5%) as heterozygous variants (30%–60% VAF), and 463/965 (48.0%) as LAFVs (10%–30% VAF). Of the 463/55,041 (0.8%) LAFV variants, 201 (43.4%) were classified as VUS and 262 (57.6%) as PVs (**Fig. 1**).

Clinical characteristics of individuals with PV LAFVs compared with heterozygous PV

Demographic characteristics of the 1,197 individuals used for comparative analysis are shown (**Table 1**). Notably, the LAFV cohort was older than the heterozygous cohort, with a significantly greater proportion of individuals over 50 years of age (84.1% vs. 54.9%; OR = 4.35; 95% CI, 2.83–6.68; $P < 0.001$). Additionally, the age covariate association remained significant in the adjusted analysis for ethnicity and sex (OR = 1.06; 95% CI, 1.05–1.08; $P < 0.001$; **Table 2**).

Table 1. Cohort characteristics stratified by LAFVs versus heterozygous variants.

	N (%)			
Characteristic	**Het variants**	**LAFVs**	**Total**	***P* value**
Sex				
Female	865 (84.2)	142 (83.5)	1,007 (84.1)	0.818
Male	162 (15.8)	28 (16.5)	190 (15.9)	
Individuals				
Cancer diagnosis	473 (46.1)	112 (65.9)	585 (48.9)	0.036
No cancer diagnosis	554 (53.9)	58 (34.1)	612 (51.1)	
Ethnicity				
African American/Black	69 (6.7)	8 (4.7)	77 (6.4)	0.269
AJ	30 (2.9)	11 (6.5)	41 (3.4)	0.048
Asian	35 (3.4)	7 (4.1)	42 (3.5)	0.783
Non-AJ Caucasian (referent)	658 (64.1)	117 (68.8)	775 (64.7)	
Hispanic	65 (6.3)	7 (4.1)	72 (6.0)	0.221
Other	170 (16.6)	20 (11.8)	190 (15.9)	0.108
Age				
<50 years	463 (45.1)	27 (15.9)	490 (40.9)	1.36E−12
≥50 years	564 (54.9)	143 (84.1)	707 (59.1)	
Classification				
PV	885 (86.2)	155 (91.2)	1,040 (86.9)	0.0955
LPV	142 (13.8)	15 (8.8)	157 (13.1)	
Gene[a]				
TP53	117 (11.4)	110 (64.7)	227 (19.0)	
NF1	30 (2.9)	23 (13.5)	53 (4.4)	0.5440
CHEK2	503 (49.9)	13 (7.6)	516 (43.1)	1.067E−51
ATM	101 (9.8)	12 (7.1)	113 (9.4)	9.618E−13
BRCA1	155 (15.1)	4 (2.4)	159 (13.3)	1.458E−26
PTEN	6 (0.6)	2 (1.2)	8 (0.7)	0.2850
MSH6	87 (8.5)	1 (0.6)	88 (7.4)	4.244E−19
APC	7 (0.7)	1 (0.6)	8 (0.7)	0.0690
NBN	7 (0.7)	1 (0.6)	8 (0.7)	0.0690
BRCA2	6 (0.6)	1 (0.6)	7 (0.6)	0.1228
CDH1	6 (0.6)	1 (0.6)	7 (0.6)	0.1228
MLH1	2 (0.2)	1 (0.6)	3 (0.3)	1.0000

Abbreviations: Het, heterozygous; LPV, likely pathogenic variant.
[a]*TP53* compared with each gene separately.

The most frequent genes associated with hereditary cancer detected in the LAFV group were *TP53* (110/170; 64.7%), *NF1* (23/170; 13.5%), *CHEK2* (13/170; 7.6%), *ATM* (12/170; 7.1%), and *BRCA1* (4/170; 2.4%; **Table 1**). No significant differences in female and male distributions were observed (OR = 1.05; 95% CI, 0.68–1.63; $P = 0.818$) compared with the heterozygous group. Non–Ashkenazi Jewish (AJ) Caucasians were the most prevalent in both the groups. Adjusted for continuous age and sex, individuals of AJ ethnicity compared with non-AJ Caucasians were over two times more likely to have LAFVs relative to heterozygous variants within the group of individuals with LAFVs (OR = 2.26; 95% CI, 1.03–4.94; $P = 0.041$; **Table 2**). This finding indicates that among individuals with LAFVs, AJ individuals are more than twice as likely to have LAFVs compared with non-AJ Caucasians. No statistical differences were detected among other ethnicities (**Table 2**).

Table 2. LAFVs versus heterozygous logistic regression adjusted by age and sex associations with ethnicity.

Ethnicity	**OR (95% CI)**	***P* value**
Non-AJ Caucasian (referent)		<0.001***
African American/Black	0.71 (0.32–1.58)	0.405
AJ	2.26 (1.03–4.94)	0.041*
Asian	1.24 (0.51–3.03)	0.633
Hispanic	0.86 (0.36–2.01)	0.721
Other	0.82 (0.49–1.39)	0.471
Age	1.06 (1.05–1.08)	<0.001***
Sex	0.84 (0.53–1.34)	0.462

Logistic regression model comparing the likelihood of LAFVs versus heterozygous variants, adjusted for continuous age and sex (categorical variable: male/female). ORs and 95% CIs are presented. Non-AJ Caucasian ethnicity was used as the referent category. *, $P < 0.05$; ***, $P < 0.001$.

Individuals without a cancer diagnosis (612/1,197, 51.1%) had a lower proportion of LAFVs (34.1% vs. 53.9%; OR = 0.68; 95% CI, 0.47–0.97; $P = 0.036$) compared with individuals with heterozygous variants. Among patients with a cancer diagnosis (585/1,197; 48.9%), an LAFV was detected in 19.1% (112/585) individuals. In females, breast cancer adjusted for continuous age was associated with the presence of LAFVs (OR = 1.52; 95% CI, 1.04–2.21; $P = 0.030$). No association was found between LAFVs and any other specific cancer diagnoses (**Table 3**), with adjustments for sex and age made as appropriate for each cancer type.

Table 3. Cancer associations stratified by LAFVs.

	N (%)			LAFVs vs. Het variants	
Characteristic	**Het variants**	**LAFVs**	**Total**	**OR (95% CI)**	***P* value**
Breast[a]					
No (ref)	529 (61.2)	62 (44.0)	591	1.52 (1.04–2.21)	0.030[a]
Yes	336 (38.8)	80 (56.0)	416		
Colorectal[b]					
No (ref)	984 (95.8)	158 (92.9)	1,142	1.42 (0.69–2.91)	0.344
Yes	43 (4.2)	12 (7.1)	55		
Pancreatic[b]					
No	1,010 (98.3)	165 (97.1)	1,175	0.78 (0.27–2.27)	0.653
Yes	17 (1.7)	5 (2.9)	22		
Prostate[c]					
No (ref)	134 (82.7)	16 (57.1)	150	1.10 (0.42–2.92)	0.849
Yes	28 (17.3)	12 (42.9)	40		
Ovarian[a]					
No (ref)	818 (94.6)	134 (94.4)	952	0.78 (0.35–1.75)	0.553
Yes	47 (5.4)	8 (5.6)	55		
Other cancers[b]					
No (ref)	1,004 (97.8)	169 (99.4)	1,173	0.24 (0.03–1.88)	0.174
Yes	23 (2.2)	1 (0.6)	24		
Multiple cancers[b]					
None (ref)[d]	554 (96.0)	58 (89.2)	612	0.99 (0.37–2.65)	0.986
Yes (>1–5)	23 (4.0)	7 (10.8)	30		
No cancer diagnosis[b]					
Any (ref)	473 (46.1)	112 (65.9)	585	0.68 (0.47–0.97)	0.036[a]
None[d]	554 (53.9)	58 (34.1)	612		

Logistic regression models assessing cancer associations with LAFVs versus heterozygous variants. Models are adjusted for continuous age and sex (categorical variable: male/female) or age alone for sex-specific cancers. ORs and 95% CIs are reported.
Abbreviations: Het, heterozygous; ref, reference.
[a]Among females only adjusted for continuous age.
[b]Adjusted for sex (categorical variable: male/female) and continuous age.
[c]Among males only adjusted for continuous age.
[d]None: absence of any diagnosis of the five types previously specified (breast, ovarian, prostate, colorectal, and/or pancreatic cancer) or other cancers (not part of the prespecified five). For the multiple versus none comparison, a patient denoted as none had no diagnosed cancer of any kind (i.e., multiple, other, among the top five, or otherwise). Individuals not meeting the none or multiple (prespecified five) criteria were set to missing for this comparison.

The LAFV cohort had a significantly older median age than the heterozygous variant group. This age difference between the LAFVs and heterozygous groups was observed irrespective of cancer diagnosis: individuals with no cancer diagnosis [65.5 years (57.0–72.75) vs. 48.0 years (36.0–59.0); $P < 0.001$], with breast cancer [64.0 years (52.5–74.0) vs. 55.5 years (44.0–66.0); $P < 0.001$], with ovarian cancer [72.5 years (68.25–80.5) vs. 55.0 (47.0–63.0); $P < 0.001$], and with prostate cancer [80.0 years (76.0–82.0) vs. 67.0 years (62.5–75.5); $P = 0.006$; **Fig. 2A**; Supplementary Table S3].

TP53 exhibited the largest number of LAFVs across all cancer diagnoses, with the distribution as follows: 48.2% (53/110) of patients with a *TP53* LAFV had breast cancer, 3.6% (4/110) had colorectal cancer, 2.7% (3/110) had pancreatic cancer, 6.4% (7/110) had prostate cancer, 5.5% (6/110) had ovarian cancer, and 4.5% (5/110) had multiple cancer diagnoses. Additionally, 32.0% (32/110) of individuals with a *TP53* LAFV did not have a cancer diagnosis. The distribution of genes by cancer type among LAFVs is illustrated in **Fig. 2B** (Supplementary Table S4).

Ancillary tissue and family member testing

Of the 262 total pathogenic LAFVs (identified through MGPT and confirmed by Sanger sequencing), 62 cases underwent ancillary testing. Testing was conducted to determine whether these variants represented CM and involved either an alternative specimen type (e.g., cultured fibroblasts or isolated DNA) collected separately from the proband ($n = 44$) or familial testing (40 individuals from 23 families). Among these 23 families, five included relatives of a proband whose sample was collected (Supplementary Table S5). Site-specific genetic testing was performed in 77.3% (65/84) of cases, and MGPT was conducted in 22.6% (19/84).

Cultured fibroblasts from 44 individuals were analyzed, confirming that 10 PVs (10/44, 22.7%) were CM, and 34 PVs (34/44, 77.3%) were absent from the alternative tissue. Among the 10 CM PVs, three of 10 were detected in *TP53*, two of 10 in *PTEN*, and one each in *BRCA1*, ATM, *BRCA2*, *APC*, and *CHEK2* (Supplementary Table S5). The difference in the median VAF between CM PVs and the indeterminate LAFVs was not statistically significant (15.9% vs. 15.4%; $P = 0.529$). Of the 40 individuals tested from 23 different families, only one extra PV in *ATM* (4.4%; 1/23) was detected in the offspring and siblings, confirming CM in the proband. No other mosaic variants were detected among the family members tested for LAFVs in *BRCA1* (0/1), *CHEK2* (0/4), *NF1* (0/2), or *TP53* (0/15). Among the 62 cases studied, 11 (17.7%) were confirmed to have CM, and seven (63.6%) had a personal history of cancer. In this group, five had breast cancer (one *ATM*, one *BRCA1*, one *PTEN*, and two *TP53*), one had prostate cancer (*BRCA2*), and one had another type of cancer (*APC*; Supplementary Table S5).

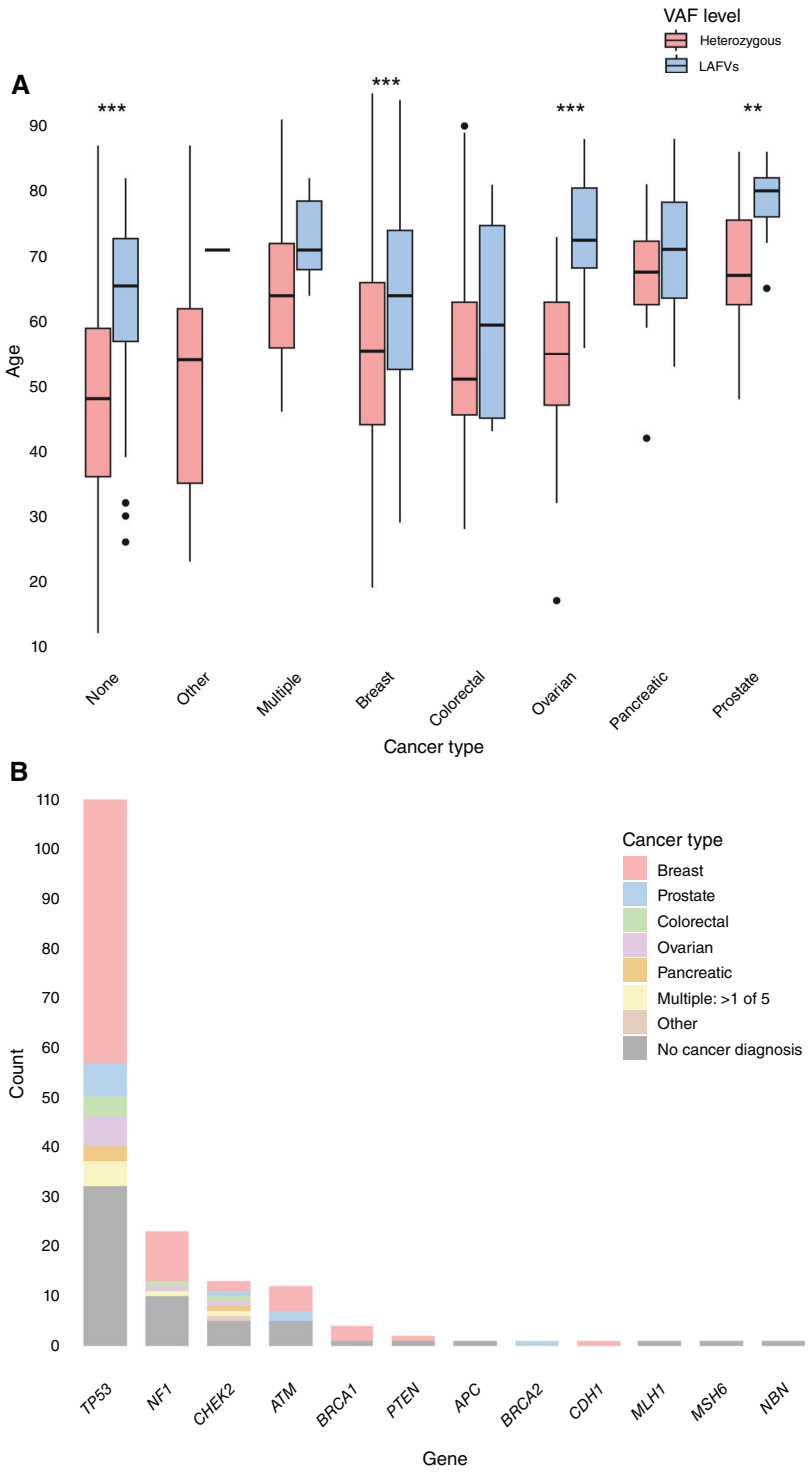

Figure 2.

Personal cancer diagnosis and age distribution differences between LAFVs and heterozygous variants. **A,** Box plot of age distribution by cancer type and VAF among PVs. **B,** Pathogenic/likely pathogenic LAFVs by gene and cancer type. *None* indicates no cancer diagnosis, *Multiple* indicates multiple cancer diagnoses (>1 of the five cancers previously defined), and *Other* indicates not being part of the five cancers (breast, colorectal, ovarian, pancreatic, and prostate). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

Discussion

Although LAFVs are uncommon findings in cancer predisposition testing, they can have substantial clinical implications. We present one of the largest real-world data studies, including 363,405 consecutive individuals who underwent MGPT for cancer predisposition genes. In total, 55,041 PVs and VUS were detected, with 965 variants with 10% to 30% VAF, and after confirming with Sanger sequencing, 463 PVs and VUS were classified as LAFVs, representing 0.1% (463/363,405) of the total cases tested. Our results are consistent with those of previous studies showing that LAFVs in MGPT are rarely detected (17, 22). The lower detection rate in our

study may be due to the availability of confirmatory Sanger sequencing, which was performed to eliminate low-level variants that are not reproducible.

In this study, ORs were calculated within a defined cohort of 1,197 individuals with overlapping variants, which included 1,027 with inferred heterozygous PVs and 170 with pathogenic LAFVs. These estimates do not reflect the general population of sequenced patients but rather a selected subset based on predefined criteria, ensuring a direct comparison between LAFVs and heterozygous variants.

Similar to prior work, we found that older age was associated with LAFVs in cancer-related genes (13, 23). Among individuals with either an inferred heterozygous PV or an LAVF, people aged 50 years and older were four times more likely to have an LAFV than a heterozygous PV (OR = 4.35; $P < 0.001$). Our study found that individuals with LAFVs and a personal history of breast, ovarian, or prostate cancer, as well as those with no history of cancer, were generally older. The higher prevalence of LAFVs in older individuals suggests a link between aging and acquisition of LAFVs, as previously reported (24, 25). This finding supports the hypothesis that LAFVs may represent age-related mutational events rather than solely inherited variants. Although the clinical impact of LAFVs is still under investigation, their predominance in older individuals underscores the importance of considering age when interpreting these variants.

Personal cancer history and cytotoxic treatments have also been linked to the detection of LAFVs (26–29). Specifically, the detection rate of CH can exceed 25% in individuals with a personal history of cancer (2, 5, 14). Notably, in our study, among the prespecified cohort of individuals with either an inferred heterozygous PV or an LAFV, women with a history of breast cancer were 1.5 times more likely to have LAFVs than those with heterozygous variants, after adjusting for age. Conversely, the proportion of LAFVs was lower in the group without cancer diagnosis (34.1% vs. 53.9%; $P = 0.036$). This suggests a potential link between the detection of LAFVs and solid tumor cancer diagnoses, possibly as a result of cytotoxic treatment or radiation, indicating CH, which has been described in prior studies (30–34). Additionally, previous studies have demonstrated an increased risk of developing a myelodysplastic syndrome in the individuals with CH (6, 35–37).

We observed that compared with non-AJ Caucasians, individuals of AJ heritage were more likely to have LAFVs relative to the comparative group of heterozygous variants. However, data on the prevalence of LAFVs across ethnicities are limited. A previous study conducted in New York City found that AJ heritage was not significantly associated with CH; however, this group had more somatic mutations in DNMT3A and TET2 (38). This finding may be influenced by differences in the cohort demographics (mostly white), testing panel selection (with AJ individuals having the highest mean number of genes ordered), or detection thresholds in AJ populations. Further research is warranted to validate these observations and investigate potential contributing factors.

The identification of variants with allele frequencies below 30% has increased along with the widespread use of NGS (1, 2). Following confirmatory studies including Sanger sequencing, LAFVs can be classified as heterozygous variants, sequencing artifacts, CH, or CM (19, 21, 39). Although it is challenging, this differentiation is crucial for individual management. For instance, the correct classification of LAFVs in genes such as *TP53* or *NF1* is essential because of their association with Li–Fraumeni Syndrome and neurofibromatosis type 1, respectively (4, 40–42). Individuals with these conditions are at an increased risk of multiple cancers, making it crucial to accurately determine the origin of LAFVs for clinical management. There are several methods for distinguishing LAFVs between CM and CH (4, 39, 43, 44). In our study, the most frequently detected genes in the LAFV cohort were *TP53* and *NF1*, consistent with previous reports (2, 17, 22). A notable contribution of this study is the inclusion of LAFVs detected in cancer-associated genes beyond *TP53*, specifically *APC, ATM, BRCA1, BRCA2, CHEK2, NF1*, and *PTEN*. Additionally, alternative specimen types and familial testing were used to confirm CM. Of the 62 cases available for complementary studies, 11 (17.7%) were confirmed as having CM. Although the sample sizes were limited, CM was confirmed in two of 11 cases for *PTEN*, two of 11 cases for *ATM*, and one case each for *BRCA1*, *BRCA2*, *APC*, and *CHEK2* (Supplementary Table S5). These results suggest that complementary testing and identification of CM have meaningful implications for patient and family risk-reduction strategies and surveillance.

Our study enumerated the prevalence of LAFVs in the entire cohort of non-selected individuals and analyzed their clinical differences from heterozygous variants within the predefined cohort. Despite the strengths of this large cohort study, several limitations must be acknowledged. Our gene panel did not encompass all CH-related genes, such as *DNMT3A*, *TET2*, *ASXL1*, *JAK2*, *SF3B1*, *SRSF2*, or *IDH1/IDH2*, making it challenging to exclude CH entirely. The use of different genetic testing panels among patients could be a potential confounder. Although stratifying by panel type would have resulted in smaller subgroup sizes, limiting meaningful comparisons, we recognize that panel selection may not have been uniform across age groups and could have influenced the findings. We excluded variants with allele frequencies below 10%, and our study lacked detailed clinical information on cytotoxic treatments, smoking, and other comorbidities. We used a >30% VAF cutoff to classify variants as heterozygous which was based on a predefined cutoff for this study, with the inherent limitations of such thresholds. Although Sanger sequencing has historically been considered a gold standard, it is less sensitive to alterations with lower allele frequencies, typically below a threshold of 10% to 15% (45). However, this limitation is less relevant to our study as we focused on variants above 10%. Despite the fact that we performed ancillary testing in 44 individuals and 23 different families, the origins of the indeterminate LAFVs remain unknown as neither the individuals were available for testing of other tissues, including additional somatic tissue (40, 44), nor were all family members available for testing.

LAFVs can also be detected in tumor tissue or liquid biopsies and are primarily used for clinical and therapeutic decision-making in cancer treatment (46, 47). Although our study focused on germline testing, we acknowledge that mosaicism and certain germline alterations also play a role in clinical decision-making, and they can be identified through tumor tissue or liquid biopsies (48). Moreover, the increasing number of germline variants used for therapeutic guidance highlights the evolving landscape of precision oncology (49). Given these considerations, future studies integrating data from tumor and liquid biopsies along with germline testing may provide a more comprehensive understanding of the clinical significance of LAFVs (50, 51).

CM in cancer associated genes has been previously described although it is often underdiagnosed or overlooked. It seems to be less frequent in certain cancer predisposition genes (52). A significant challenge in detecting mosaicism is obtaining tissue samples containing the variant. This limitation can be addressed by analyzing DNA from multiple tissue sources (53). Currently, LAFVs are documented in commercial germline genetics reports as being

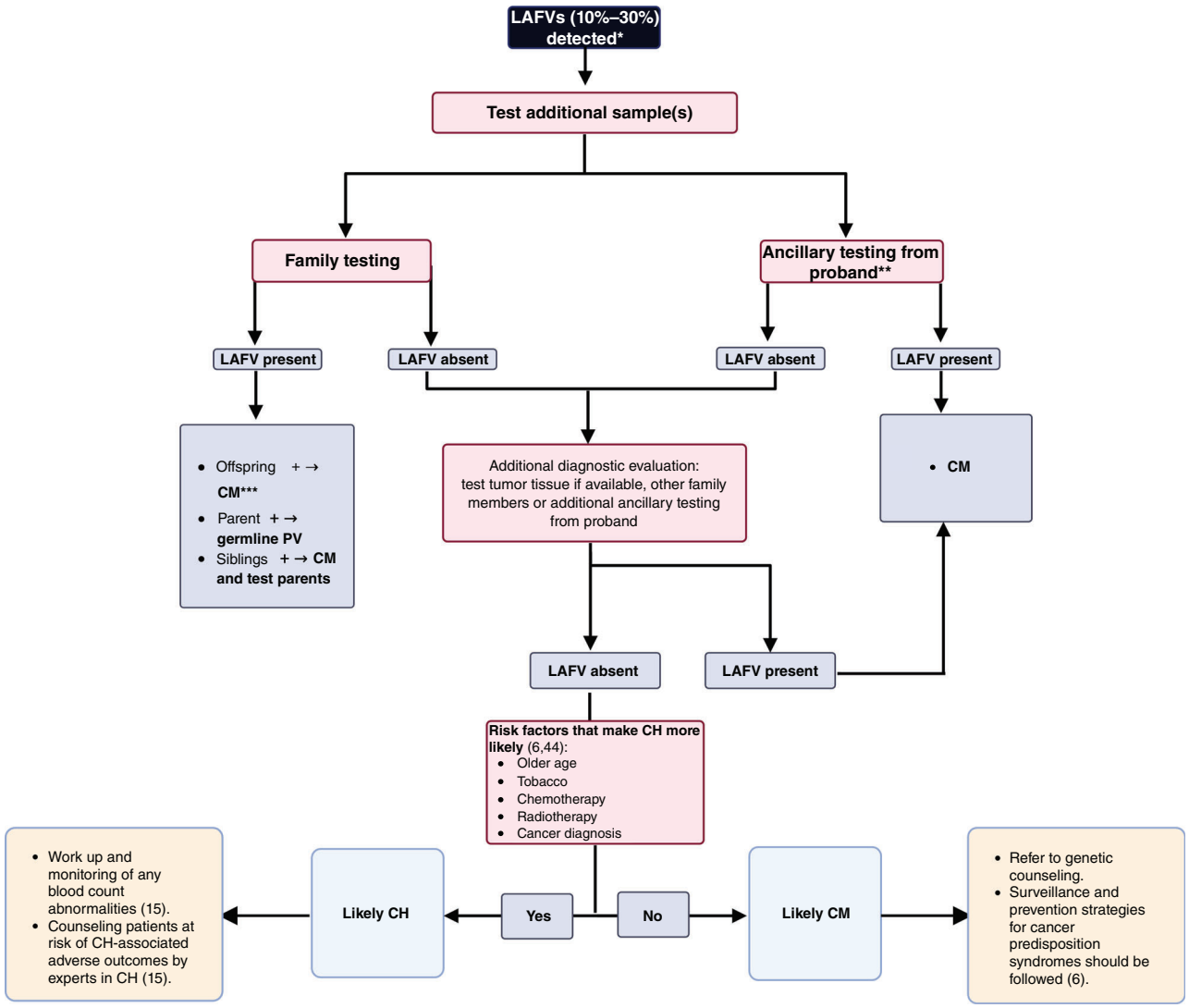

Figure 3.

Flowchart to aid the interpretation and management of LAFVs. *, This table has been adapted from Batalini and colleagues (8) and Schwartz and colleagues (39). **, To assess whether these variants were CM, additional tests were performed using either an alternative sample type (such as cultured fibroblasts or separately collected isolated DNA) from the proband or, when possible, through familial testing. ***, Post-zygotic alteration in DNA that occurs during embryonic development or later in the proband, involving the germ cells (sperm or egg).

detected with the possibility of somatic origin; therefore, it is essential to detail these findings comprehensively, including the affected gene, variant type, allele frequency detected in blood, and potential clinical implications. Including the VAF in reports can aid in identifying possible cases of CM, prompting further testing of individuals or their family members. After detecting LAFVs, we propose the following: determining the VAF; ancillary testing in different tissues in the same individual, tumor tissue or other benign tissues (polyps, skin, etc.); or testing family members to detect CM (**Fig. 3**). DNA from ectodermal tissues can be collected using buccal swabs, nail clippings, or hair root bulbs; mesodermal tissues can be examined through blood or saliva samples; and endodermal DNA can be sourced from urothelial cells found in urine (53). If the PV is detected through complementary testing, the individual should be managed as having CM. If the PV is not detected, CM should still be considered, particularly when no additional tissue from the proband is available or when an insufficient number of family members have been tested. The combination of methodologic and interpretive challenges reinforces the importance of implementing robust workflows to avoid missing CM. Detecting CM is crucial as its absence can result in inaccurate cancer risk assessments and clinical decisions. In our study, we underscore that CM should be considered as a potential diagnosis when an LAFV is detected and share concrete steps to adjudicate LAFVs (**Fig. 3**).

Overall, our results highlight the frequency and distribution of LAFVs in a clinical testing cohort. Our study underscores the fact that LAFVs can be detected in MGPT for cancer-related genes beyond those associated with hematologic malignancies. Despite the limited number of cases, CM can be found, and care should follow the

recommendations for specific familial cancer predisposition syndromes. This could affect preventive strategies and cancer treatments (54, 55). When CH is suspected, additional testing is often recommended for risk stratification (56). Specialized care for monitoring the risk of myelodysplastic syndrome and to provide preventative care for cardiovascular risks associated with these findings may be indicated (15, 57–60). Further research is needed to elucidate the origins of LAFVs and understand their clinical implications.

Authors' Disclosures

L.M. Polfus reports personal fees from Ambry Genetics outside the submitted work, as well as employment with Ambry Genetics. A. Prat reports grants and personal fees from Roche, Reveal Genomics, Daiichi Sankyo, AstraZeneca, and Novartis and personal fees from Ona Therapeutics and Peptomyc outside the submitted work. C. Horton reports personal fees from Ambry Genetics. No disclosures were reported by the other authors.

Authors' Contributions

A. Rodriguez-Hernandez: Conceptualization, resources, data curation, formal analysis, validation, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **L.M. Polfus:** Conceptualization, resources, data curation, formal analysis, investigation, visualization, methodology, writing–original draft, writing–review and editing. **B.L. Bychkovsky:** Conceptualization, supervision, investigation, methodology, writing–original draft. **B. Souders:** Conceptualization, resources, supervision, investigation, methodology, writing–original draft. **A. Prat:** Conceptualization, investigation, methodology. **B. Adamo:** Conceptualization, supervision, validation, writing–review and editing. **J.E. Garber:** Conceptualization, resources, supervision, validation, investigation, methodology, writing–original draft. **C. Horton:** Conceptualization, resources, data curation, formal analysis, supervision, investigation, visualization, methodology, writing–original draft, project administration, writing–review and editing. **H.Q. Rana:** Conceptualization, resources, supervision, investigation, methodology, writing–original draft, project administration, writing–review and editing.

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Note

Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

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Supplemental material First publication

eTable 1: ICD10 clinical data used to create an Odds Ratio measure

eTable 2: Genetic Panel Distribution of Individuals in Heterozygous and LAFV Categories

eTable 3: Median age by Cancer overall and by VAF status

eTable 4: Gene distribution by cancer type among LAFVs

eTable 5: Ancillary testing on a secondary tissue from the proband (n=44) or through familial testing (n=23 families)

eTable 1: ICD10 clinical data used to create an Odds Ratio measure

ICD10 Code	ICD10 Definition	Curation Category
C74	Malignant neoplasm of adrenal gland	Adrenal
C74.0	Malignant neoplasm of cortex of adrenal gland	Adrenal
C74.00	Malignant neoplasm of cortex of unspecified adrenal gland	Adrenal
C74.01	Malignant neoplasm of cortex of right adrenal gland	Adrenal
C74.02	Malignant neoplasm of cortex of left adrenal gland	Adrenal
C74.1	Malignant neoplasm of medulla of unspecified adrenal gland	Adrenal
C74.10	Malignant neoplasm of medulla of unspecified adrenal gland	Adrenal
C74.11	Malignant neoplasm of medulla of right adrenal gland	Adrenal
C74.12	Malignant neoplasm of medulla of left adrenal gland	Adrenal
C74.9	Malignant neoplasm of unspecified part of adrenal gland	Adrenal
C74.90	Malignant neoplasm of unspecified part of unspecified adrenal gland	Adrenal
C74.91	Malignant neoplasm of unspecified part of right adrenal gland	Adrenal
C74.92	Malignant neoplasm of unspecified part of left adrenal gland	Adrenal
D44.1	Neoplasm of uncertain behaviour of adrenal gland	Adrenal-Uncertain
D44.10	Neoplasm of uncertain behaviour of unspecified adrenal gland	Adrenal-Uncertain
D44.11	Neoplasm of uncertain behaviour of right adrenal gland	Adrenal-Uncertain
D44.12	Neoplasm of uncertain behaviour of left adrenal gland	Adrenal-Uncertain
C22	Malignant neoplasm of liver and intrahepatic bile ducts	Biliary Tract
C22.0	Liver cell carcinoma	Biliary Tract
C22.1	Intrahepatic bile duct carcinoma	Biliary Tract
C22.2	Hepatoblastoma	Biliary Tract
C22.3	Angiosarcoma of liver	Biliary Tract
C22.4	Other sarcomas of liver	Biliary Tract
C22.7	Other specified carcinomas of liver	Biliary Tract
C22.8	Malignant neoplasm of liver, primary, unspecified as to type	Biliary Tract
C22.9	Malignant neoplasm of liver, not specified as primary or secondary	Biliary Tract
C23	Malignant neoplasm of gallbladder	Biliary Tract
C24	Malignant neoplasm of other and unspecified parts of biliary tract	Biliary Tract
C24.0	Malignant neoplasm of extrahepatic bile duct	Biliary Tract
C24.1	Malignant neoplasm of ampulla of Vater	Biliary Tract
C24.8	Malignant neoplasm of overlapping sites of biliary tract	Biliary Tract
C24.9	Malignant neoplasm of biliary tract, unspecified	Biliary Tract
D37.6	Neoplasm of uncertain behaviour of liver, gallbladder and bile ducts	Biliary Tract-Uncertain
D01.5	Carcinoma in situ of liver, gallbladder and bile ducts	Biliary Tract-in situ
C71	Malignant neoplasm of brain	Brain
C71.0	Malignant neoplasm of cerebrum, except lobes and ventricles	Brain
C71.1	Malignant neoplasm of frontal lobe	Brain
C71.2	Malignant neoplasm of temporal lobe	Brain
C71.3	Malignant neoplasm of parietal lobe	Brain
C71.4	Malignant neoplasm of occipital lobe	Brain
C71.5	Malignant neoplasm of cerebral ventricle	Brain
C71.6	Malignant neoplasm of cerebellum	Brain
C71.7	Malignant neoplasm of brain stem	Brain
C71.8	Malignant neoplasm of overlapping sites of brain	Brain
C71.9	Malignant neoplasm of brain, unspecified	Brain
C72.9	Malignant neoplasm of central nervous system, unspecified	Brain
D43	Neoplasm of uncertain behaviour of brain and central nervous system	Brain-Uncertain
D43.0	Neoplasm of uncertain behaviour of brain, supratentorial	Brain-Uncertain
D43.1	Neoplasm of uncertain behaviour of brain, infratentorial	Brain-Uncertain
D43.2	Neoplasm of uncertain behaviour of brain, unspecified	Brain-Uncertain
D43.3	Neoplasm of uncertain behaviour of cranial nerves	Brain-Uncertain
D43.4	Neoplasm of uncertain behaviour of spinal cord	Brain-Uncertain
D43.8	Neoplasm of uncertain behaviour of other specified parts of central nervous system	Brain-Uncertain
D43.9	Neoplasm of uncertain behaviour of central nervous system, unspecified	Brain-Uncertain
D49.6	Neoplasm of unspecified behaviour of brain	Brain
Z85.841	Personal history of malignant neoplasm of brain	Brain
C50	Malignant neoplasm of breast	Breast
C50.0	Malignant neoplasm of nipple and areola	Breast
C50.01	Malignant neoplasm of nipple and areola, female	Breast
C50.011	Malignant neoplasm of nipple and areola, right female breast	Breast
C50.012	Malignant neoplasm of nipple and areola, left female breast	Breast
C50.019	Malignant neoplasm of nipple and areola, unspecified female breast	Breast

C50.02	Malignant neoplasm of nipple and areola, male	Breast
C50.021	Malignant neoplasm of nipple and areola, right male breast	Breast
C50.022	Malignant neoplasm of nipple and areola, left male breast	Breast
C50.029	Malignant neoplasm of nipple and areola, unspecified male breast	Breast
C50.1	Malignant neoplasm of central portion of breast	Breast
C50.11	Malignant neoplasm of central portion of breast, female	Breast
C50.111	Malignant neoplasm of central portion of right female breast	Breast
C50.112	Malignant neoplasm of central portion of left female breast	Breast
C50.119	Malignant neoplasm of central portion of unspecified female breast	Breast
C50.12	Malignant neoplasm of central portion of breast, male	Breast
C50.121	Malignant neoplasm of central portion of right male breast	Breast
C50.122	Malignant neoplasm of central portion of left male breast	Breast
C50.129	Malignant neoplasm of central portion of unspecified male breast	Breast
C50.2	Malignant neoplasm of upper-inner quadrant of breast	Breast
C50.21	Malignant neoplasm of upper-inner quadrant of breast, female	Breast
C50.211	Malignant neoplasm of upper-inner quadrant of right female breast	Breast
C50.212	Malignant neoplasm of upper-inner quadrant of left female breast	Breast
C50.219	Malignant neoplasm of upper-inner quadrant of unspecified female breast	Breast
C50.22	Malignant neoplasm of upper-inner quadrant of breast, male	Breast
C50.221	Malignant neoplasm of upper-inner quadrant of right male breast	Breast
C50.222	Malignant neoplasm of upper-inner quadrant of left male breast	Breast
C50.229	Malignant neoplasm of upper-inner quadrant of unspecified male breast	Breast
C50.3	Malignant neoplasm of lower-inner quadrant of breast	Breast
C50.31	Malignant neoplasm of lower-inner quadrant of breast, female	Breast
C50.311	Malignant neoplasm of lower-inner quadrant of right female breast	Breast
C50.312	Malignant neoplasm of lower-inner quadrant of left female breast	Breast
C50.319	Malignant neoplasm of lower-inner quadrant of unspecified female breast	Breast
C50.32	Malignant neoplasm of lower-inner quadrant of breast, male	Breast
C50.321	Malignant neoplasm of lower-inner quadrant of right male breast	Breast
C50.322	Malignant neoplasm of lower-inner quadrant of left male breast	Breast
C50.329	Malignant neoplasm of lower-inner quadrant of unspecified male breast	Breast
C50.4	Malignant neoplasm of upper-outer quadrant of breast	Breast
C50.41	Malignant neoplasm of upper-outer quadrant of breast, female	Breast
C50.411	Malignant neoplasm of upper-outer quadrant of right female breast	Breast
C50.412	Malignant neoplasm of upper-outer quadrant of left female breast	Breast
C50.419	Malignant neoplasm of upper-outer quadrant of unspecified female breast	Breast
C50.42	Malignant neoplasm of upper-outer quadrant of breast, male	Breast
C50.421	Malignant neoplasm of upper-outer quadrant of right male breast	Breast
C50.422	Malignant neoplasm of upper-outer quadrant of left male breast	Breast
C50.429	Malignant neoplasm of upper-outer quadrant of unspecified male breast	Breast
C50.5	Malignant neoplasm of lower-outer quadrant of breast	Breast
C50.51	Malignant neoplasm of lower-outer quadrant of breast, female	Breast
C50.511	Malignant neoplasm of lower-outer quadrant of right female breast	Breast
C50.512	Malignant neoplasm of lower-outer quadrant of left female breast	Breast
C50.519	Malignant neoplasm of lower-outer quadrant of unspecified female breast	Breast
C50.52	Malignant neoplasm of lower-outer quadrant of breast, male	Breast
C50.521	Malignant neoplasm of lower-outer quadrant of right male breast	Breast
C50.522	Malignant neoplasm of lower-outer quadrant of left male breast	Breast
C50.529	Malignant neoplasm of lower-outer quadrant of unspecified male breast	Breast
C50.6	Malignant neoplasm of axillary tail of breast	Breast
C50.61	Malignant neoplasm of axillary tail of breast, female	Breast
C50.611	Malignant neoplasm of axillary tail of right female breast	Breast
C50.612	Malignant neoplasm of axillary tail of left female breast	Breast
C50.619	Malignant neoplasm of axillary tail of unspecified female breast	Breast
C50.62	Malignant neoplasm of axillary tail of breast, male	Breast
C50.621	Malignant neoplasm of axillary tail of right male breast	Breast
C50.622	Malignant neoplasm of axillary tail of left male breast	Breast
C50.629	Malignant neoplasm of axillary tail of unspecified male breast	Breast
C50.8	Malignant neoplasm of overlapping sites of breast	Breast
C50.81	Malignant neoplasm of overlapping sites of breast, female	Breast
C50.811	Malignant neoplasm of overlapping sites of right female breast	Breast
C50.812	Malignant neoplasm of overlapping sites of left female breast	Breast
C50.819	Malignant neoplasm of overlapping sites of unspecified female breast	Breast
C50.82	Malignant neoplasm of overlapping sites of breast, male	Breast

C50.821	Malignant neoplasm of overlapping sites of right male breast	Breast
C50.822	Malignant neoplasm of overlapping sites of left male breast	Breast
C50.829	Malignant neoplasm of overlapping sites of unspecified male breast	Breast
C50.9	Malignant neoplasm of breast of unspecified site	Breast
C50.91	Malignant neoplasm of breast of unspecified site, female	Breast
C50.911	Malignant neoplasm of unspecified site of right female breast	Breast
C50.912	Malignant neoplasm of unspecified site of left female breast	Breast
C50.919	Malignant neoplasm of unspecified site of unspecified female breast	Breast
C50.92	Malignant neoplasm of breast of unspecified site, male	Breast
C50.921	Malignant neoplasm of unspecified site of right male breast	Breast
C50.922	Malignant neoplasm of unspecified site of left male breast	Breast
C50.929	Malignant neoplasm of unspecified site of unspecified male breast	Breast
D05	Carcinoma in situ of breast	Breast
D05.0	Lobular carcinoma in situ of breast	Breast-in situ
D05.00	Lobular carcinoma in situ of unspecified breast	Breast-in situ
D05.01	Lobular carcinoma in situ of right breast	Breast-in situ
D05.02	Lobular carcinoma in situ of left breast	Breast-in situ
D05.1	Intraductal carcinoma in situ of breast	Breast
D05.10	Intraductal carcinoma in situ of unspecified breast	Breast
D05.11	Intraductal carcinoma in situ of right breast	Breast
D05.12	Intraductal carcinoma in situ of left breast	Breast
D05.8	Other specified type of carcinoma in situ of breast	Breast
D05.80	Other specified type of carcinoma in situ of unspecified breast	Breast
D05.81	Other specified type of carcinoma in situ of right breast	Breast
D05.82	Other specified type of carcinoma in situ of left breast	Breast
D05.9	Unspecified type of carcinoma in situ of breast	Breast
D05.90	Unspecified type of carcinoma in situ of unspecified breast	Breast
D05.91	Unspecified type of carcinoma in situ of right breast	Breast
D05.92	Unspecified type of carcinoma in situ of left breast	Breast
D48.6	Neoplasm of uncertain behaviour of breast	Breast-Uncertain
D48.60	Neoplasm of uncertain behaviour of unspecified breast	Breast-Uncertain
D48.61	Neoplasm of uncertain behaviour of right breast	Breast-Uncertain
D48.62	Neoplasm of uncertain behaviour of left breast	Breast-Uncertain
D49.2	Neoplasm of uncertain behaviour of left breast	Breast-Uncertain
D49.3	Neoplasm of unspecified behaviour of breast	Breast
Z85.3	Personal history of malignant neoplasm of breast	Breast
Z86.000	Personal history of in-situ neoplasm of breast	Breast
C18.0	Malignant neoplasm of cecum	Colon
C18.1	Malignant neoplasm of appendix	Colon
C18.2	Malignant neoplasm of ascending colon	Colon
C18.3	Malignant neoplasm of hepatic flexure	Colon
C18.4	Malignant neoplasm of transverse colon	Colon
C18.5	Malignant neoplasm of splenic flexure	Colon
C18.6	Malignant neoplasm of descending colon	Colon
C18.7	Malignant neoplasm of sigmoid colon	Colon
C18.8	Malignant neoplasm of overlapping sites of colon	Colon
C18.9	Malignant neoplasm of colon, unspecified	Colon
C19	Malignant neoplasm of rectosigmoid junction	Colon
C20	Malignant neoplasm of rectum	Colon
C21.0	Malignant neoplasm of anus, unspecified	Colon
C21.1	Malignant neoplasm of anal canal	Colon
C21.2	Malignant neoplasm of cloacogenic zone	Colon
C21.8	Malignant neoplasm of overlapping sites of rectum, anus and anal canal	Colon
D37.3	Neoplasm of uncertain behaviour of appendix	Colon-Uncertain
D37.4	Neoplasm of uncertain behaviour of colon	Colon-Uncertain
D37.5	Neoplasm of uncertain behaviour of rectum	Colon-Uncertain
Z85.03	Personal history of malignant neoplasm of large intestine	Colon
Z85.04	Personal history of malignant neoplasm of rectum, rectosigmoid junction, and anus	Colon
C7A.02	Malignant carcinoid tumors of the appendix, large intestine, and rectum	Colon-Carcinoid
C7A.021	Malignant carcinoid tumor of the cecum	Colon-Carcinoid
C7A.022	Malignant carcinoid tumor of the ascending colon	Colon-Carcinoid
C7A.023	Malignant carcinoid tumor of the transverse colon	Colon-Carcinoid
C7A.024	Malignant carcinoid tumor of the descending colon	Colon-Carcinoid
C7A.025	Malignant carcinoid tumor of the sigmoid colon	Colon-Carcinoid

C7A.026	Malignant carcinoid tumor of the rectum	Colon-Carcinoid
C7A.029	Malignant carcinoid tumor of the large intestine, unspecified portion	Colon-Carcinoid
Z85.030	Personal history of malignant carcinoid tumor of large intestine	Colon-Carcinoid
Z85.038	Personal history of other malignant neoplasm of large intestine	Colon-Carcinoid
Z85.040	Personal history of malignant carcinoid tumor of rectum	Colon-Carcinoid
Z85.048	Personal history of other malignant neoplasm of rectum, rectosigmoid junction, and anus	Colon-Carcinoid
D01.0	Carcinoma in situ of colon	Colon-in situ
D01.1	Carcinoma in situ of rectosigmoid junction	Colon-in situ
D01.2	Carcinoma in situ of rectum	Colon-in situ
D01.3	Carcinoma in situ of anus and anal canal	Colon-in situ
D01.4	Carcinoma in situ of other and unspecified parts of intestine	Colon-in situ
D01.40	Carcinoma in situ of unspecified part of intestine	Colon-in situ
D01.49	Carcinoma in situ of other parts of intestine	Colon-in situ
C16	Malignant neoplasm of stomach	Gastric
C16.0	Malignant neoplasm of cardia	Gastric
C16.1	Malignant neoplasm of fundus of stomach	Gastric
C16.2	Malignant neoplasm of body of stomach	Gastric
C16.3	Malignant neoplasm of pyloric antrum	Gastric
C16.4	Malignant neoplasm of pylorus	Gastric
C16.5	Malignant neoplasm of lesser curvature of stomach, unspecified	Gastric
C16.6	Malignant neoplasm of greater curvature of stomach, unspecified	Gastric
C16.8	Malignant neoplasm of overlapping sites of stomach	Gastric
C16.9	Malignant neoplasm of stomach, unspecified	Gastric
D37.1	Neoplasm of uncertain behaviour of stomach	Gastric-Uncertain
D85.028	Personal history of other malignant neoplasm of stomach	Gastric
Z85.02	Personal history of malignant neoplasm of stomach	Gastric
Z85.028	Personal history of other malignant neoplasm of stomach	Gastric
C7A.092	Malignant carcinoid tumor of the stomach	Gastric-Carcinoid
C7A.094	Malignant carcinoid tumor of the foregut, unspecified	Gastric-Carcinoid
C7A.095	Malignant carcinoid tumor of the midgut, unspecified	Gastric-Carcinoid
C7A.096	Malignant carcinoid tumor of the hindgut, unspecified	Gastric-Carcinoid
Z85.020	Personal history of malignant carcinoid tumor of stomach	Gastric-Carcinoid
D00.2	Carcinoma in situ of stomach	Gastric-in situ
Z86.003	Personal history of in-situ neoplasm of oral cavity, oesophagus and stomach	Gastric-in situ
C64	Malignant neoplasm of kidney, except renal pelvis	Kidney
C64.1	Malignant neoplasm of right kidney, except renal pelvis	Kidney
C64.2	Malignant neoplasm of left kidney, except renal pelvis	Kidney
C64.9	Malignant neoplasm of unspecified kidney, except renal pelvis	Kidney
C65	Malignant neoplasm of renal pelvis	Kidney
C65.1	Malignant neoplasm of right renal pelvis	Kidney
C65.2	Malignant neoplasm of left renal pelvis	Kidney
C65.9	Malignant neoplasm of unspecified renal pelvis	Kidney
C67.0	Malignant neoplasm of trigone of bladder	Kidney
C68.0	Malignant neoplasm of urethra	Kidney
D41.0	Neoplasm of uncertain behaviour of kidney	Kidney-Uncertain
D41.00	Neoplasm of uncertain behaviour of unspecified kidney	Kidney-Uncertain
D41.01	Neoplasm of uncertain behaviour of right kidney	Kidney-Uncertain
D41.02	Neoplasm of uncertain behaviour of left kidney	Kidney-Uncertain
D49.51	Neoplasm of unspecified behaviour of kidney	Kidney
D49.511	Neoplasm of unspecified behaviour of right kidney	Kidney
D49.512	Neoplasm of unspecified behaviour of left kidney	Kidney
D49.519	Neoplasm of unspecified behaviour of unspecified kidney	Kidney
Z85.52	Personal history of malignant neoplasm of kidney	Kidney
Z85.528	Personal history of other malignant neoplasm of kidney	Kidney
Z85.53	Personal history of malignant neoplasm of renal pelvis	Kidney
C7A.093	Malignant carcinoid tumor of the kidney	Kidney-Carcinoid
Z85.520	Personal history of malignant carcinoid tumor of kidney	Kidney-Carcinoid
C43	Malignant melanoma of skin	Melanoma
C43.0	Malignant melanoma of lip	Melanoma
C43.1	Malignant melanoma of eyelid, including canthus	Melanoma
C43.10	Malignant melanoma of unspecified eyelid, including canthus	Melanoma
C43.11	Malignant melanoma of right eyelid, including canthus	Melanoma
C43.111	Malignant melanoma of right upper eyelid, including canthus	Melanoma
C43.112	Malignant melanoma of right lower eyelid, including canthus	Melanoma

C43.12	Malignant melanoma of left eyelid, including canthus	Melanoma
C43.121	Malignant melanoma of left upper eyelid, including canthus	Melanoma
C43.122	Malignant melanoma of left lower eyelid, including canthus	Melanoma
C43.2	Malignant melanoma of ear and external auricular canal	Melanoma
C43.20	Malignant melanoma of unspecified ear and external auricular canal	Melanoma
C43.21	Malignant melanoma of right ear and external auricular canal	Melanoma
C43.22	Malignant melanoma of left ear and external auricular canal	Melanoma
C43.3	Malignant melanoma of other and unspecified parts of face	Melanoma
C43.30	Malignant melanoma of unspecified part of face	Melanoma
C43.31	Malignant melanoma of nose	Melanoma
C43.39	Malignant melanoma of other parts of face	Melanoma
C43.4	Malignant melanoma of scalp and neck	Melanoma
C43.5	Malignant melanoma of trunk	Melanoma
C43.51	Malignant melanoma of anal skin	Melanoma
C43.52	Malignant melanoma of skin of breast	Melanoma
C43.59	Malignant melanoma of other part of trunk	Melanoma
C43.6	Malignant melanoma of upper limb, including shoulder	Melanoma
C43.60	Malignant melanoma of unspecified upper limb, including shoulder	Melanoma
C43.61	Malignant melanoma of right upper limb, including shoulder	Melanoma
C43.62	Malignant melanoma of left upper limb, including shoulder	Melanoma
C43.7	Malignant melanoma of lower limb, including hip	Melanoma
C43.70	Malignant melanoma of unspecified lower limb, including hip	Melanoma
C43.71	Malignant melanoma of right lower limb, including hip	Melanoma
C43.72	Malignant melanoma of left lower limb, including hip	Melanoma
C43.8	Malignant melanoma of overlapping sites of skin	Melanoma
C43.9	Malignant melanoma of skin, unspecified	Melanoma
Z85.820	Personal history of malignant melanoma of skin	Melanoma
D03	Melanoma in situ	Melanoma-in situ
D03.0	Melanoma in situ of lip	Melanoma-in situ
D03.1	Melanoma in situ of eyelid, including canthus	Melanoma-in situ
D03.10	Melanoma in situ of unspecified eyelid, including canthus	Melanoma-in situ
D03.11	Melanoma in situ of right eyelid, including canthus	Melanoma-in situ
D03.111	Melanoma in situ of right upper eyelid, including canthus	Melanoma-in situ
D03.112	Melanoma in situ of right lower eyelid, including canthus	Melanoma-in situ
D03.12	Melanoma in situ of left eyelid, including canthus	Melanoma-in situ
D03.121	Melanoma in situ of left upper eyelid, including canthus	Melanoma-in situ
D03.122	Melanoma in situ of left lower eyelid, including canthus	Melanoma-in situ
D03.2	Melanoma in situ of ear and external auricular canal	Melanoma-in situ
D03.20	Melanoma in situ of unspecified ear and external auricular canal	Melanoma-in situ
D03.21	Melanoma in situ of right ear and external auricular canal	Melanoma-in situ
D03.22	Melanoma in situ of left ear and external auricular canal	Melanoma-in situ
D03.3	Melanoma in situ of other and unspecified parts of face	Melanoma-in situ
D03.30	Melanoma in situ of unspecified part of face	Melanoma-in situ
D03.39	Melanoma in situ of other parts of face	Melanoma-in situ
D03.4	Melanoma in situ of scalp and neck	Melanoma-in situ
D03.5	Melanoma in situ of trunk	Melanoma-in situ
D03.51	Melanoma in situ of anal skin	Melanoma-in situ
D03.52	Melanoma in situ of breast (skin) (soft tissue)	Melanoma-in situ
D03.59	Melanoma in situ of other part of trunk	Melanoma-in situ
D03.6	Melanoma in situ of upper limb, including shoulder	Melanoma-in situ
D03.60	Melanoma in situ of unspecified upper limb, including shoulder	Melanoma-in situ
D03.61	Melanoma in situ of right upper limb, including shoulder	Melanoma-in situ
D03.62	Melanoma in situ of left upper limb, including shoulder	Melanoma-in situ
D03.7	Melanoma in situ of lower limb, including hip	Melanoma-in situ
D03.70	Melanoma in situ of unspecified lower limb, including hip	Melanoma-in situ
D03.71	Melanoma in situ of right lower limb, including hip	Melanoma-in situ
D03.72	Melanoma in situ of left lower limb, including hip	Melanoma-in situ
D03.8	Melanoma in situ of other sites	Melanoma-in situ
D03.9	Melanoma in situ, unspecified	Melanoma-in situ
Z86.006	Personal history of melanoma in-situ	Melanoma-in situ
C56.1	Malignant neoplasm of right ovary	Ovarian
C56.2	Malignant neoplasm of left ovary	Ovarian
C56.3	Malignant neoplasm of bilateral ovaries	Ovarian
C56.9	Malignant neoplasm of unspecified ovary	Ovarian

D39.1	Neoplasm of uncertain behaviour of ovary	Ovarian-Uncertain
D39.10	Neoplasm of uncertain behaviour of unspecified ovary	Ovarian-Uncertain
D39.11	Neoplasm of uncertain behaviour of right ovary	Ovarian-Uncertain
D39.12	Neoplasm of uncertain behaviour of left ovary	Ovarian-Uncertain
Z85.43	Personal history of malignant neoplasm of ovary	Ovarian
C48.0	Malignant neoplasm of retroperitoneum	Ovarian
C48.1	Malignant neoplasm of specified parts of peritoneum	Ovarian
C48.2	Malignant neoplasm of peritoneum, unspecified	Ovarian
C48.8	Malignant neoplasm of overlapping sites of retroperitoneum and peritoneum	Ovarian
C49.A0	Malignant neoplasm of overlapping sites of retroperitoneum and peritoneum	Ovarian
D48.4	Neoplasm of uncertain behaviour of peritoneum	Ovarian-Uncertain
C25	Malignant neoplasm of pancreas	Pancreatic
C25.0	Malignant neoplasm of head of pancreas	Pancreatic
C25.1	Malignant neoplasm of body of pancreas	Pancreatic
C25.2	Malignant neoplasm of tail of pancreas	Pancreatic
C25.3	Malignant neoplasm of pancreatic duct	Pancreatic
C25.4	Malignant neoplasm of endocrine pancreas	Pancreatic
C25.7	Malignant neoplasm of other parts of pancreas	Pancreatic
C25.8	Malignant neoplasm of overlapping sites of pancreas	Pancreatic
C25.9	Malignant neoplasm of pancreas, unspecified	Pancreatic
Z85.07	Personal history of malignant neoplasm of pancreas	Pancreatic
C61	Malignant neoplasm of prostate	Prostate
D40.0	Neoplasm of uncertain behaviour of prostate	Prostate-Uncertain
Z85.46	Personal history of malignant neoplasm of prostate	Prostate
D07.5	Carcinoma in situ of prostate	Prostate-in situ
C49	Malignant neoplasm of other connective and soft tissue	Sarcoma
C49.0	Malignant neoplasm of connective and soft tissue of head, face and neck	Sarcoma
C49.1	Malignant neoplasm of connective and soft tissue of upper limb, including shoulder	Sarcoma
C49.10	Malignant neoplasm of connective and soft tissue of unspecified upper limb, including should	Sarcoma
C49.11	Malignant neoplasm of connective and soft tissue of right upper limb, including shoulder	Sarcoma
C49.12	Malignant neoplasm of connective and soft tissue of left upper limb, including shoulder	Sarcoma
C49.2	Malignant neoplasm of connective and soft tissue of lower limb, including hip	Sarcoma
C49.20	Malignant neoplasm of connective and soft tissue of unspecified lower limb, including hip	Sarcoma
C49.21	Malignant neoplasm of connective and soft tissue of right lower limb, including hip	Sarcoma
C49.22	Malignant neoplasm of connective and soft tissue of left lower limb, including hip	Sarcoma
C49.3	Malignant neoplasm of connective and soft tissue of thorax	Sarcoma
C49.4	Malignant neoplasm of connective and soft tissue of abdomen	Sarcoma
C49.5	Malignant neoplasm of connective and soft tissue of pelvis	Sarcoma
C49.6	Malignant neoplasm of connective and soft tissue of trunk, unspecified	Sarcoma
C49.8	Malignant neoplasm of overlapping sites of connective and soft tissue	Sarcoma
C49.9	Malignant neoplasm of connective and soft tissue, unspecified	Sarcoma
C17.0	Malignant neoplasm of duodenum	Small Intestine
C17.1	Malignant neoplasm of jejunum	Small Intestine
C17.2	Malignant neoplasm of ileum	Small Intestine
C17.3	Meckel's diverticulum, malignant	Small Intestine
C17.8	Malignant neoplasm of overlapping sites of small intestine	Small Intestine
C17.9	Malignant neoplasm of small intestine, unspecified	Small Intestine
D37.2	Neoplasm of uncertain behaviour of small intestine	Small Intestine-Uncertain
Z85.06	Personal history of malignant neoplasm of small intestine	Small Intestine
Z85.060	Personal history of malignant carcinoid tumor of small intestine	Small Intestine
Z85.068	Personal history of other malignant neoplasm of small intestine	Small Intestine
C7A.01	Malignant carcinoid tumors of the small intestine	Small Intestine-Carcinoid
C7A.010	Malignant carcinoid tumor of the duodenum	Small Intestine-Carcinoid
C7A.011	Malignant carcinoid tumor of the jejunum	Small Intestine-Carcinoid
C7A.012	Malignant carcinoid tumor of the ileum	Small Intestine-Carcinoid
C7A.019	Malignant carcinoid tumor of the small intestine, unspecified portion	Small Intestine-Carcinoid
C73	Malignant neoplasm of thyroid gland	Thyroid
D44.0	Neoplasm of uncertain behaviour of thyroid gland	Thyroid-Uncertain
Z85.850	Personal history of malignant neoplasm of thyroid	Thyroid
D09.3	Carcinoma in situ of thyroid and other endocrine glands	Thyroid-in situ
C66	Malignant neoplasm of ureter	Ureter
C66.1	Malignant neoplasm of right ureter	Ureter
C66.2	Malignant neoplasm of left ureter	Ureter
C66.9	Malignant neoplasm of unspecified ureter	Ureter

C67.6	Malignant neoplasm of ureteric orifice	Ureter
D41.2	Neoplasm of uncertain behaviour of ureter	Ureter-Uncertain
D41.20	Neoplasm of uncertain behaviour of unspecified ureter	Ureter-Uncertain
D41.21	Neoplasm of uncertain behaviour of right ureter	Ureter-Uncertain
D41.22	Neoplasm of uncertain behaviour of left ureter	Ureter-Uncertain
Z85.54	Personal history of malignant neoplasm of ureter	Ureter
C54.0	Malignant neoplasm of isthmus uteri	Uterine
C54.1	Malignant neoplasm of endometrium	Uterine
C54.2	Malignant neoplasm of myometrium	Uterine
C54.3	Malignant neoplasm of fundus uteri	Uterine
C54.8	Malignant neoplasm of overlapping sites of corpus uteri	Uterine
C54.9	Malignant neoplasm of corpus uteri, unspecified	Uterine
C55	Malignant neoplasm of uterus, part unspecified	Uterine
C57.0	Malignant neoplasm of fallopian tube	Uterine
C57.00	Malignant neoplasm of unspecified fallopian tube	Uterine
C57.01	Malignant neoplasm of right fallopian tube	Uterine
C57.02	Malignant neoplasm of left fallopian tube	Uterine
C57.4	Malignant neoplasm of uterine adnexa, unspecified	Uterine
D39.0	Neoplasm of uncertain behaviour of uterus	Uterine-Uncertain
Z85.42	Personal history of malignant neoplasm of other parts of uterus	Uterine
D07.0	Carcinoma in situ of endometrium	Uterine-in situ

eTable 2: Genetic Panel Distribution of Individuals in Heterozygous and LAFV Categories

	Genes tested (n)	Het variants (n %)	LAFVs (n%)	p-value
APC,ATM,BARD1,BRCA1,BRCA2,BRIP1,BMPR1A,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	34	218 (21.2%)	51 (30.0%)	0,013
APC,ATM,AXIN2,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD51C,RAD51D,RECQL,SMAD4,SMARCA4,STK11,TP53	36	188 (18.3%)	27 (15.9%)	0,518
AIP,ALK,APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDC73,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,CTNNA1,DICER1,EGFR,EGLN1,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,KIF1B,KIT,LZTR1,MAX,MEN1,MET,MITF,MLH1,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PDGFRA,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PTCH1,PTEN,RAD51C,RAD51D,RB1,RECQL,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	77	109 (10.6%)	16 (9.4%)	0,786
AIP,ALK,APC,ATM,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,DICER1,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,MAX,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,NF2,PALB2,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	67	105 (10.2%)	21 (12.4%)	0,418
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,SMARCA4,STK11,TP53	25	40 (3.9%)	5 (2.9%)	0,667
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,MRE11A,MUTYH,NBN,NF1,PALB2,PTEN,RAD50,RAD51C,RAD51D,TP53	17	30 (2.9%)	8 (4.7%)	0,235
AIP,ALK,APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CASR,CDC73,CDH1,CDK4,CDKN1B,CDKN2A,CFTR,CHEK2,CPA1,CTNNA1,CTRC,DICER1,EGFR,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,KIT,MAX,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PDGFRA,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PRSS1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,SPINK1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	81	29 (2.8%)	5 (2.9%)	0,808
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CASR, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CFTR, CHEK2, CPA1, CTNNA1, CTRC, DICER1, EGFR, EGLN1, EPCAM, FAM175A, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIF1B, KIT, LZTR1, MAX, MEN1, MET, MITF, MLH1, MLH3, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PALLD, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PRSS1, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL, RET, RINT1, RPS20, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, SPINK1, STK11, SUFU, TERT, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	91	25 (2.4%)	2 (1.2%)	0,411
ATM,BRCA1,BRCA2,CDH1,CHEK2,PALB2,PTEN,TP53	8	23 (2.2%)	1 (0.6%)	0,236
AIP,ALK,APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDC73,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,CTNNA1,DICER1,EGFR,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,KIT,MAX,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PDGFRA,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	75	21 (2.0%)	4 (2.4%)	0,772
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,MLH1,MSH2,MSH6,NBN,NF1,PALB2,PMS2,PTEN,RAD51C,RAD51D,RECQL,SMARCA4,STK11,TP53	23	20 (1.9%)	3 (1.8%)	1
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, CTNNA1, DICER1, EGFR, EGLN1, EPCAM, FAM175A, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIF1B, KIT, LZTR1, MAX, MEN1, MET, MITF, MLH1, MLH3, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PALLD, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL, RET, RINT1, RPS20, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TERT, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	85	18 (1.8%)	1 (0.6%)	0,502
ATM,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,EPCAM,MLH1,MSH2,MSH6,NBN,NF1,PALB2,PMS2,PTEN,RAD51C,RAD51D,TP53	18	15 (1.5%)	0 (0.0%)	0,148
ATM,BRCA1,BRCA2,CHEK2,EPCAM,HOXB13,MLH1,MSH2,MSH6,NBN,PALB2,PMS2,RAD51D,TP53	14	8 (0.8%)	3 (1.8%)	0,197
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	36	7 (0.7%)	0 (0.0%)	0,602
APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, GREM1, HOXB13, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, POT1, PTEN, RAD51C, RAD51D, RB1, RECQL, SMAD4, SMARCA4, STK11, TP53	40	5 (0.5%)	0 (0.0%)	1
APC,ATM,AXIN2,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,CTNNA1,DICER1,EPCAM,GREM1,HOXB13,KIT,MEN1,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PDGFRA,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SDHA,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,TP53,TSC1,TSC2,VHL	48	5 (0.5%)	0 (0.0%)	1
AIP,ALK,APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CASR,CDC73,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,CPA1,CTNNA1,CTRC,DICER1,EGFR,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,KIT,MAX,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PDGFRA,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PRSS1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,SPINK1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	80	4 (0.4%)	1 (0.6%)	0,536
AIP,ALK,APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDC73,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,CTNNA1,DICER1,EGFR,EGLN1,EPCAM,FAM175A,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,KIF1B,KIT,LZTR1,MAX,MEN1,MET,MITF,MLH1,MLH3,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PALLD,PDGFRA,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RECQL,RET,RINT1,RPS20,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TERT,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	85	4 (0.4%)	0 (0.0%)	1
APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNA1, DICER1, EGFR, EPCAM, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, MET, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PALLD, PDGFRA, PMS2, POLD1, POLE, POT1, PTCH1, PTEN, RAD51C, RAD51D, RECQL, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, SUFU, TERT, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	62	4 (0.4%)	0 (0.0%)	1
ATM,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,EPCAM,MLH1,MSH2,MSH6,NBN,NF1,PALB2,PMS2,PTEN,RAD51C,RAD51D,STK11,TP53	19	4 (0.4%)	0 (0.0%)	1
APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNA1, DICER1, EGFR, EPCAM, FH, FLCN, GREM1, HOXB13, KIT, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PDGFRA, PMS2, POLD1, POLE, POT1, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL, RET, RPS20, SDHA, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, SUFU, TP53, TSC1, TSC2, VHL	62	3 (0.3%)	0 (0.0%)	1
APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNA1, DICER1, EPCAM, FH, FLCN, GREM1, HOXB13, KIT, MAX, MEN1, MET, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NTHL1, PALB2, PDGFRA, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TMEM127, TP53, VHL	51	3 (0.3%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,CTNNA1,DICER1,EPCAM,FH,FLCN,GREM1,HOXB13,KIT,MAX,MEN1,MET,MLH1,MSH2,MSH3,MSH6,MUTYH,NBN,NTHL1,PALB2,PDGFRA,PMS2,POLD1,POLE,PTEN,RAD51C,RAD51D,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,TMEM127,TP53,VHL	51	3 (0.3%)	0 (0.0%)	1
APC,BMPR1A,CDH1,CHEK2,EPCAM,GREM1,MLH1,MSH2,MSH6,MUTYH,PMS2,POLD1,POLE,PTEN,SMAD4,STK11,TP53	17	3 (0.3%)	0 (0.0%)	1
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, CTNNA1, DICER1, EGFR, EGLN1, EPCAM, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIF1B, KIT, LZTR1, MAX, MEN1, MET, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RECQL, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	77	2 (0.2%)	1 (0.6%)	0,369
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, CTNNA1, DICER1, EGFR, EPCAM, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIT, MAX, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	75	2 (0.2%)	0 (0.0%)	1
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, GREM1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, STK11, TP53	36	2 (0.2%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,CTNNA1,DICER1,EGFR,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PDGFRA,PMS2,POLD1,POLE,POT1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	61	2 (0.2%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,CTNNA1,DICER1,EPCAM,FH,FLCN,GREM1,HOXB13,KIT,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PDGFRA,PMS2,POLD1,POLE,POT1,PTEN,RAD50,RAD51C,RAD51D,RB1,SDHA,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,TP53,TSC1,TSC2,VHL	55	2 (0.2%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDKN2A,CHEK2,EPCAM,FH,FLCN,GREM1,HOXB13,MEN1,MET,MITF,MLH1,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PMS2,POLD1,POLE,POT1,PTEN,RAD51C,RAD51D,RET,SDHB,SDHC,SDHD,SMAD4,STK11,TP53,TSC1,TSC2,VHL	46	2 (0.2%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	34	2 (0.2%)	0 (0.0%)	1
APC,AXIN2,BMPR1A,CDH1,CHEK2,EPCAM,GREM1,MLH1,MSH2,MSH3,MSH6,MUTYH,NTHL1,PMS2,POLD1,POLE,PTEN,SMAD4,STK11,TP53	20	2 (0.2%)	0 (0.0%)	1
ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53	20	2 (0.2%)	0 (0.0%)	1
NF1	1	2 (0.2%)	0 (0.0%)	1
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CASR, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CFTR, CHEK2, CPA1, CTNNA1, CTRC, DICER1, EGFR, EGLN1, EPCAM, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIF1B, KIT, LZTR1, MAX, MEN1, MET, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PRSS1, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, SPINK1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	83	1 (0.1%)	0 (0.0%)	1

AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CASR, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CFTR, CHEK2, CPA1, CTNNA1, CTRC, DICER1, EGFR, EPCAM, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIT, MAX, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PRSS1, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, SPINK1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	81	1 (0.1%)	0 (0.0%)	1
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CASR, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, CPA1, CTNNA1, CTRC, DICER1, EGFR, EGLN1, EPCAM, FAM175A, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIF1B, KIT, LZTR1, MAX, MEN1, MET, MITF, MLH1, MLH3, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PALLD, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL, RET, RINT1, RPS20, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TERT, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	88	1 (0.1%)	0 (0.0%)	1
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, CPA1, CTNNA1, DICER1, EGFR, EGLN1, EPCAM, FAM175A, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIF1B, KIT, LZTR1, MAX, MEN1, MET, MITF, MLH1, MLH3, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PALLD, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL, RET, RPS20, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TERT, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	85	1 (0.1%)	0 (0.0%)	1
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, CTNNA1, DICER1, EGFR, EGLN1, EPCAM, FAM175A, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIF1B, KIT, LZTR1, MAX, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL, RET, RINT1, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TERT, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	81	1 (0.1%)	0 (0.0%)	1
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, CTNNA1, DICER1, EPCAM, FAM175A, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, MAX, MEN1, MET, MITF, MLH1, MLH3, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PALLD, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD50, RAD51C, RAD51D, RB1, RECQL, RET, RINT1, RPS20, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TERT, TMEM127, TP53, TSC1, TSC2, VHL, XRCC2	78	1 (0.1%)	0 (0.0%)	1
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, DICER1, EGFR, EPCAM, FH, FLCN, GREM1, HOXB13, KIT, MAX, MEN1, MET, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PDGFRA, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL	67	1 (0.1%)	0 (0.0%)	1
AIP, ALK, APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, DICER1, EPCAM, GREM1, HOXB13, LZTR1, MEN1, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TP53, TSC1, TSC2, VHL	55	1 (0.1%)	0 (0.0%)	1
AIP, ALK, APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, DICER1, EPCAM, GREM1, HOXB13, LZTR1, MEN1, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PHOX2B, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TP53, TSC1, TSC2, VHL	52	1 (0.1%)	0 (0.0%)	1
AIP, APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CASR, CDC73, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, DICER1, EGFR, EPCAM, FH, FLCN, GALNT12, GREM1, HOXB13, KIT, LZTR1, MAX, MEN1, MET, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PDGFRA, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, SMARCE1, STK11, SUFU, TMEM127, TP53, TSC1, TSC2, VHL	70	1 (0.1%)	0 (0.0%)	1
AIP,ALK,APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CASR,CDC73,CDH1,CDK4,CDKN1B,CDKN2A,CFTR,CHEK2,CPA1,CTNNA1,CTRC,DICER1,EGFR,EGLN1,EPCAM,FAM175A,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,KIF1B,KIT,LZTR1,MAX,MEN1,MET,MITF,MLH1,MLH3,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PALLD,PDGFRA,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PRSS1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RECQL,RET,RINT1,RPS20,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,SPINK1,STK11,SUFU,TERT,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	91	1 (0.1%)	1 (0.6%)	0,264
AIP,ALK,APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CASR,CDC73,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,CPA1,CTNNA1,CTRC,DICER1,EGFR,EGLN1,EPCAM,FAM175A,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,KIF1B,KIT,LZTR1,MAX,MEN1,MET,MITF,MLH1,MLH3,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PALLD,PDGFRA,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PRSS1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RECQL,RET,RINT1,RPS20,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,SPINK1,STK11,SUFU,TERT,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	90	1 (0.1%)	0 (0.0%)	1
AIP,ALK,APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDC73,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,CTNNA1,DICER1,EGFR,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,KIT,MAX,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PDGFRA,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PRSS1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	75	1 (0.1%)	0 (0.0%)	1
AIP,ALK,APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,DICER1,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,MAX,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	70	1 (0.1%)	0 (0.0%)	1
AIP,ALK,APC,ATM,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CASR,CDH1,CDK4,CDKN1B,CDKN2A,CFTR,CHEK2,CPA1,CTRC,DICER1,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,MAX,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,PALB2,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PRSS1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,SPINK1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	73	1 (0.1%)	0 (0.0%)	1
AIP,ALK,APC,ATM,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,DICER1,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,KIT,MAX,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,PALB2,PDGFRA,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	69	1 (0.1%)	0 (0.0%)	1
AIP,ALK,APC,ATM,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,DICER1,EPCAM,FANCC,FH,FLCN,GALNT12,GREM1,HOXB13,MAX,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,PALB2,PHOX2B,PMS2,POLD1,POLE,POT1,PRKAR1A,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TMEM127,TP53,TSC1,TSC2,VHL,XRCC2	69	1 (0.1%)	0 (0.0%)	1
AIP,ALK,APC,ATM,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CASR,CDH1,CDK4,CDKN1B,CDKN2A,CFTR,CHEK2,CPA1,CTRC,DICER1,EGFR,EPCAM,FANCC,MEN1,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,PALB2,PHOX2B,PMS2,POT1,PRKAR1A,PRSS1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,SMAD4,SMARCA4,SMARCB1,SMARCE1,SPINK1,STK11,SUFU,TP53,TSC1,TSC2,VHL	56	1 (0.1%)	0 (0.0%)	1
AIP,ALK,APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CASR,CDH1,CDKN1B,CDKN2A,CFTR,CHEK2,CPA1,CTRC,DICER1,EGFR,EPCAM,FANCC,MEN1,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,NF2,PALB2,PHOX2B,PMS2,POT1,PRKAR1A,PRSS1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,SMAD4,SMARCA4,SMARCB1,SMARCE1,SPINK1,STK11,SUFU,TP53,TSC1,TSC2,VHL	53	1 (0.1%)	0 (0.0%)	1
AIP,ALK,APC,ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,MEN1,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,NF2,PALB2,PHOX2B,PMS2,POT1,PRKAR1A,PTCH1,PTEN,RAD50,RAD51C,RAD51D,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TP53,TSC1,TSC2,VHL	40	1 (0.1%)	0 (0.0%)	1
ALK,APC,ATM,AXIN2,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,FH,FLCN,GREM1,HOXB13,MEN1,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,POT1,PTEN,RAD50,RAD51C,RAD51D,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,TMEM127,TP53	51	1 (0.1%)	0 (0.0%)	1
ALK,APC,ATM,AXIN2,BAP1,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EGFR,EPCAM,FH,FLCN,GALNT12,GREM1,HOXB13,MEN1,MET,MITF,MLH1,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTCH1,PTEN,RAD51C,RAD51D,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMARCA4,STK11,TP53,TSC1,TSC2,VHL	51	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BAP1, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, FAM175A, FANCC, FH, FLCN, GALNT12, GREM1, MET, MITF, MLH1, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, RECQL, RINT1, RPS20, SDHA, SDHB, SDHC, SDHD, SMAD4, STK11, TP53, TSC1, TSC2, VHL, XRCC2	52	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN1B, CDKN2A, CHEK2, DICER1, EPCAM, FH, FLCN, GREM1, HOXB13, KIT, MAX, MEN1, MET, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NTHL1, PALB2, PDGFRA, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, SMAD4, SMARCA4, TMEM127, TP53, VHL	51	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNA1, DICER1, EGFR, EPCAM, FANCC, FH, FLCN, GALNT12, GREM1, HOXB13, KIT, MEN1, MET, MITF, MLH1, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NF2, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, RECQL, RINT1, RPS20, SDHA, SDHB, SDHC, SDHD, SMAD4, STK11, TP53, TSC1, TSC2, VHL, XRCC2	61	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, GREM1, HOXB13, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, POT1, PTEN, RAD51C, RAD51D, RB1, RECQL, SMAD4, SMARCA4, STK11, TERT, TP53	41	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, EPCAM, FH, FLCN, GREM1, HOXB13, MET, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, SMAD4, STK11, TP53, TSC1, TSC2, VHL	39	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, FAM175A, FANCC, GREM1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, RINT1, SMAD4, SMARCA4, STK11, TP53, XRCC2	41	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, FH, FLCN, GREM1, HOXB13, KIT, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PDGFRA, PMS2, POLD1, POLE, POT1, PRKAR1A, PTCH1, PTEN, RAD51C, RAD51D, RB1, RECQL, SDHA, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, SUFU, TP53, TSC1, TSC2	51	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BARD1, BLM, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, DICER1, EPCAM, GALNT12, GREM1, MLH1, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, RECQL, RPS20, SMAD4, SMARCA4, STK11, TP53	38	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNA1, DICER1, EPCAM, GREM1, HOXB13, KIT, MEN1, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PDGFRA, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, SDHA, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TP53, TSC1, TSC2, VHL	47	1 (0.1%)	0 (0.0%)	1

APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, FAM175A, FANCC, GREM1, HOXB13, MLH1, MLH3, MRE11A, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, RECQL, RINT1, SDHA, SDHB, SDHC, SDHD, SMAD4, SMARCA4, SMARCB1, STK11, TP53, XRCC2	48	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, GALNT12, GREM1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, STK11, TP53	37	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, GREM1, HOXB13, MLH1, MLH3, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, RPS20, SMAD4, SMARCA4, STK11, TP53	38	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, GREM1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, RPS20, SMAD4, SMARCA4, STK11, TP53	37	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, GREM1, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, STK11, TP53, VHL	36	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BMPR1A, BRCA1, BRCA2, CDH1, CHEK2, EPCAM, GREM1, HOXB13, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51D, SMAD4, STK11, TP53	27	1 (0.1%)	0 (0.0%)	1
APC, ATM, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, GREM1, HOXB13, MITF, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, POLD1, POLE, PTEN, RAD50, RAD51C, RAD51D, RB1, SMAD4, SMARCA4, STK11, TP53	37	1 (0.1%)	0 (0.0%)	1
APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CASR, CDH1, CDKN2A, CFTR, CHEK2, CPA1, CTRC, DICER1, EPCAM, FANCC, HOXB13, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PALLD, PMS2, PRSS1, PTEN, RAD50, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, SPINK1, STK11, TP53, TSC1, TSC2, VHL	43	1 (0.1%)	0 (0.0%)	1
APC, ATM, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CASR, CDH1, CDKN2A, CFTR, CHEK2, CPA1, CTRC, DICER1, EPCAM, FANCC, MEN1, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PALLD, PMS2, PRSS1, PTEN, RAD50, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, SPINK1, STK11, TP53, TSC1, TSC2, VHL	42	1 (0.1%)	0 (0.0%)	1
APC, ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CDKN2A, CHEK2, DICER1, EPCAM, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, RECQL, SMARCA4, STK11, TP53	25	1 (0.1%)	0 (0.0%)	1
APC, AXIN2, BMPR1A, BRCA1, BRCA2, CDH1, CHEK2, EPCAM, GREM1, MLH1, MSH2, MSH3, MSH6, MUTYH, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, SMAD4, STK11, TP53	23	1 (0.1%)	0 (0.0%)	1
APC, AXIN2, BMPR1A, CDH1, CHEK2, EPCAM, FLCN, GREM1, MEN1, MLH1, MSH2, MSH3, MSH6, MUTYH, NF1, NTHL1, PMS2, POLD1, POLE, PTEN, RET, SMAD4, STK11, TP53	24	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EGFR,EPCAM,FH,FLCN,GALNT12,GREM1,HOXB13,KIT,MEN1,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PDGFRA,PMS2,POLD1,POLE,POT1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,RET,SDHA,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,TP53,TSC1,TSC2,VHL	58	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,FH,FLCN,GALNT12,GREM1,HOXB13,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,RET,SMAD4,SMARCA4,STK11,TP53,VHL	45	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,CTNNA1,DICER1,EGFR,EPCAM,FH,FLCN,GALNT12,GREM1,HOXB13,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,POT1,PTCH1,PTEN,RAD50,RAD51C,RAD51D,SDHA,SDHB,SDHC,SDHD,SMAD4,SMARCA4,SMARCB1,SMARCE1,STK11,SUFU,TP53,TSC1,TSC2,VHL	57	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,CTNNA1,DICER1,EPCAM,FH,FLCN,GREM1,HOXB13,MET,MITF,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SDHA,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,TP53,TSC1,TSC2,VHL	50	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EGFR,EPCAM,FH,FLCN,GREM1,HOXB13,MET,MITF,MLH1,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD51C,RAD51D,RECQL,SDHA,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,TP53,TSC1,TSC2,VHL	49	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,EPCAM,FANCC,FH,FLCN,GREM1,HOXB13,KIT,MAX,MEN1,MET,MLH1,MSH2,MSH6,MUTYH,NBN,PALB2,PDGFRA,PMS2,POLD1,POLE,POT1,PTEN,RAD51C,RAD51D,RET,SDHA,SDHAF2,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,TMEM127,TP53,VHL	49	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BAP1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GALNT12,GREM1,HOXB13,MLH1,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	36	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,FANCC,GALNT12,GREM1,HOXB13,MEN1,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,RET,SMAD4,SMARCA4,STK11,TP53,XRCC2	43	1 (0.1%)	1 (0.6%)	0,264
APC,ATM,AXIN2,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,FANCC,GALNT12,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53,XRCC2	41	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN1B,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MEN1,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NF2,NTHL1,PALB2,PMS2,POLD1,POLE,PRKAR1A,PTCH1,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,SUFU,TP53,TSC1,TSC2,VHL	46	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GALNT12,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	38	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDKN2A,CHEK2,EPCAM,GREM1,HOXB13,MLH1,MSH2,MSH3,MSH6,MUTYH,NBN,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD51C,RAD51D,SMAD4,STK11,TP53	31	1 (0.1%)	0 (0.0%)	1
APC,ATM,AXIN2,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	35	1 (0.1%)	0 (0.0%)	1
APC,ATM,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MITF,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,POT1,PTEN,RAD50,RAD51C,RAD51D,RB1,SMAD4,SMARCA4,STK11,TP53	38	1 (0.1%)	0 (0.0%)	1
APC,ATM,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,EPCAM,GREM1,MITF,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,RB1,SMAD4,STK11,TP53	34	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,FANCC,GALNT12,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53,XRCC2	38	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,FANCC,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53,XRCC2	37	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BLM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	35	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CASR,CDH1,CDK4,CDKN2A,CFTR,CHEK2,CPA1,CTRC,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PRSS1,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,SPINK1,STK11,TP53	40	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,CTNNA1,DICER1,EPCAM,GREM1,HOXB13,MEN1,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	36	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EGFR,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	37	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MEN1,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	35	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,RET,SMAD4,SMARCA4,STK11,TP53	37	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MSH2,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	35	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,RB1,SMAD4,SMARCA4,STK11,TP53	35	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,EPCAM,GREM1,MEN1,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,STK11,TP53	30	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,EPCAM,GREM1,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,STK11,TP53	29	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CDKN2A,CHEK2,DICER1,EPCAM,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,SMARCA4,STK11,TP53	27	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CDKN2A,CHEK2,EPCAM,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,STK11,TP53	25	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CDKN2A,CHEK2,EPCAM,MLH1,MSH2,MSH6,NBN,NF1,PALB2,PMS2,PTEN,RAD51C,RAD51D,STK11,TP53	22	1 (0.1%)	0 (0.0%)	1
APC,ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,FANCC,MLH1,MSH2,MSH6,NBN,NF1,PALB2,PMS2,PRKAR1A,PTEN,RAD50,RAD51C,RAD51D,RET,SMARCA4,STK11,TP53	27	1 (0.1%)	0 (0.0%)	1
APC,ATM,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	31	1 (0.1%)	0 (0.0%)	1
APC,ATM,BMPR1A,BRCA1,BRCA2,CDH1,CHEK2,EPCAM,GREM1,MLH1,MSH2,MSH6,MUTYH,PALB2,PMS2,POLD1,POLE,PTEN,SMAD4,STK11,TP53	21	1 (0.1%)	0 (0.0%)	1
APC,ATM,BRCA1,BRCA2,BRIP1,CDH1,CDKN2A,CHEK2,EPCAM,MLH1,MSH2,MSH6,NBN,NF1,PALB2,PMS2,PTEN,RAD51C,RAD51D,STK11,TP53	21	1 (0.1%)	0 (0.0%)	1
APC,BRCA1,EPCAM,MEN1,MLH1,MSH2,MSH6,NF1,PMS2,SDHA,SDHAF2,SDHB,SDHC,SDHD,TP53	15	1 (0.1%)	0 (0.0%)	1

ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, HOXB13, MLH1, MSH2, MSH6, NBN, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53	21	1 (0.1%)	0 (0.0%)	1
ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, NBN, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53	19	1 (0.1%)	1 (0.6%)	0,264
ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH6, NF1, PALB2, PMS2, PTEN, RAD51C, RAD51D, STK11, TP53	19	1 (0.1%)	0 (0.0%)	1
ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, EPCAM, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, PTEN, RAD51C, RAD51D, TP53	21	1 (0.1%)	0 (0.0%)	1
ATM, BRCA1, BRCA2, BRIP1, CHEK2, EPCAM, HOXB13, MLH1, MSH2, MSH6, NBN, PALB2, PMS2, RAD51C, RAD51D, TP53	16	1 (0.1%)	0 (0.0%)	1
ATM, BRCA1, BRCA2, BRIP1, CHEK2, NBN, PALB2, RAD51C, RAD51D	9	1 (0.1%)	0 (0.0%)	1
ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, STK11, TP53	9	1 (0.1%)	0 (0.0%)	1
ATM, BRCA1, BRCA2, CHEK2, EPCAM, MLH1, MSH2, MSH6, PALB2, PMS2	10	1 (0.1%)	0 (0.0%)	1
ATM, BRCA1, BRCA2, CHEK2, MLH1, MSH2, MSH6, PALB2, PMS2	9	1 (0.1%)	0 (0.0%)	1
ATM,BAP1,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,MITF,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTCH1,PTEN,RAD50,RAD51C,RAD51D,RB1,SMARCA4,STK11,TP53	31	1 (0.1%)	0 (0.0%)	1
ATM,BAP1,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,SMARCA4,STK11,TP53	26	1 (0.1%)	0 (0.0%)	1
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,SMARCA4,STK11,TP53	26	1 (0.1%)	1 (0.6%)	0,264
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,HOXB13,MLH1,MSH2,MSH6,MUTYH,NBN,PALB2,PMS2,PTEN,RAD51C,RAD51D,STK11,TP53	22	1 (0.1%)	0 (0.0%)	1
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMARCA4,STK11,TP53	27	1 (0.1%)	0 (0.0%)	1
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTCH1,PTEN,RAD50,RAD51C,RAD51D,SMARCA4,STK11,TP53	26	1 (0.1%)	0 (0.0%)	1
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,EPCAM,FANCC,MLH1,MRE11A,MSH2,MSH6,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,STK11,TP53,XRCC2	24	1 (0.1%)	0 (0.0%)	1
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,EPCAM,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,TP53	23	1 (0.1%)	0 (0.0%)	1
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,FANCC,MRE11A,MUTYH,NBN,NF1,PALB2,PTEN,RAD50,RAD51C,RAD51D,STK11,TP53,XRCC2	20	1 (0.1%)	0 (0.0%)	1
ATM,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,NBN,NF1,PALB2,PTEN,RAD51C,RAD51D,TP53	14	1 (0.1%)	0 (0.0%)	1
ATM,BARD1,BRCA1,BRCA2,CDH1,CHEK2,EPCAM,FANCC,MLH1,MRE11A,MSH2,MSH6,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,STK11,TP53,XRCC2	23	1 (0.1%)	0 (0.0%)	1
ATM,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,EPCAM,MLH1,MSH2,MSH6,NBN,PALB2,PMS2,PTEN,RAD51C,RAD51D,STK11,TP53	18	1 (0.1%)	0 (0.0%)	1
ATM,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,EPCAM,MLH1,MSH2,MSH6,PALB2,PMS2,PTEN,RAD51C,RAD51D,TP53	16	1 (0.1%)	0 (0.0%)	1
ATM,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,NBN,PALB2,PTEN,TP53	10	1 (0.1%)	0 (0.0%)	1
ATM,BRCA1,BRCA2,CHEK2,HOXB13,MLH1,MSH2,MSH6,PALB2,PMS2	10	1 (0.1%)	0 (0.0%)	1
BAP1,BRCA2,CDK4,CDKN2A,MITF,POT1,PTEN,RB1,TP53	9	1 (0.1%)	0 (0.0%)	1
BAP1,CHEK2,EPCAM,FH,FLCN,MET,MITF,MLH1,MSH2,MSH6,PMS2,PTEN,SDHA,SDHB,SDHC,SDHD,TP53,TSC1,TSC2,VHL	20	1 (0.1%)	0 (0.0%)	1
BRCA1, BRCA2, BRIP1, CHEK2, EPCAM, MLH1, MSH2, MSH6, PALB2, PMS2, PTEN, RAD51C, RAD51D, SMARCA4	14	1 (0.1%)	0 (0.0%)	1
BRCA1,BRCA2	2	1 (0.1%)	0 (0.0%)	1
BRCA1,BRCA2,BRIP1,EPCAM,MLH1,MSH2,MSH6,PALB2,PMS2,PTEN,RAD51C,RAD51D,TP53	13	1 (0.1%)	0 (0.0%)	1
BRCA1,BRCA2,CHEK2,TP53	4	1 (0.1%)	0 (0.0%)	1
BRCA1,BRCA2,MSH6	3	1 (0.1%)	0 (0.0%)	1
CHEK2	1	1 (0.1%)	0 (0.0%)	1
CHEK2, EPCAM, MEN1, MLH1, MSH2, MSH6, PMS2, PRKAR1A, TP53	9	1 (0.1%)	0 (0.0%)	1
EGLN1, FH, KIF1B, MAX, MEN1, NF1, RET, SDHA, SDHAF2, SDHB, SDHC, SDHD, TMEM127, TP53, VHL	15	1 (0.1%)	0 (0.0%)	1
PTEN	1	1 (0.1%)	0 (0.0%)	1
APC, ATM, AXIN2, BAP1, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, DICER1, EPCAM, FH, FLCN, GREM1, HOXB13, MET, MITF, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, SDHA, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TP53, TSC1, TSC2, VHL	48	0 (0.0%)	2 (1.2%)	0,02
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CDK4, CDKN2A, CHEK2, CTNNA1, DICER1, EPCAM, GREM1, HOXB13, KIT, MEN1, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PDGFRA, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, SDHA, SDHB, SDHC, SDHD, SMAD4, SMARCA4, STK11, TP53, TSC1, TSC2, VHL	47	0 (0.0%)	1 (0.6%)	0,142
APC, ATM, AXIN2, BARD1, BMPR1A, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, DICER1, EPCAM, GREM1, MLH1, MSH2, MSH3, MSH6, MUTYH, NBN, NF1, NTHL1, PALB2, PMS2, POLD1, POLE, PTEN, RAD51C, RAD51D, RECQL, SMAD4, SMARCA4, STK11, TP53	33	0 (0.0%)	1 (0.6%)	0,142
APC, AXIN2, BMPR1A, BRCA1, BRCA2, CDH1, CHEK2, EPCAM, GREM1, MLH1, MSH2, MSH3, MSH6, MUTYH, NTHL1, PMS2, POLD1, POLE, PTEN, SMAD4, STK11, TP53	22	0 (0.0%)	1 (0.6%)	0,142
APC,ATM,AXIN2,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	37	0 (0.0%)	1 (0.6%)	0,142
APC,ATM,AXIN2,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,EPCAM,GREM1,HOXB13,MLH1,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTEN,RAD51C,RAD51D,SMAD4,STK11,TP53	33	0 (0.0%)	1 (0.6%)	0,142
APC,ATM,BAP1,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,FH,FLCN,GREM1,HOXB13,MET,MITF,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,RB1,SDHA,SDHB,SDHC,SDHD,SMAD4,SMARCA4,STK11,TP53,TSC1,TSC2,VHL	47	0 (0.0%)	1 (0.6%)	0,142
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH3,MSH6,MUTYH,NBN,NF1,NTHL1,PALB2,PMS2,POLD1,POLE,PTCH1,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,SUFU,TP53	38	0 (0.0%)	1 (0.6%)	0,142
APC,ATM,BARD1,BMPR1A,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,GREM1,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	31	0 (0.0%)	1 (0.6%)	0,142
APC,ATM,BARD1,BMPR1A,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,GREM1,HOXB13,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,POLD1,POLE,PTEN,RAD50,RAD51C,RAD51D,SMAD4,SMARCA4,STK11,TP53	32	0 (0.0%)	1 (0.6%)	0,142
APC,ATM,BRCA1,BRCA2,BRIP1,CDH1,CDKN2A,CHEK2,EPCAM,MLH1,MSH2,MSH6,PALB2,PMS2,PTEN,RAD51C,RAD51D,STK11,TP53	19	0 (0.0%)	1 (0.6%)	0,142
APC,ATM,BRCA1,BRCA2,CDKN2A,EPCAM,MLH1,MSH2,MSH6,PALB2,PMS2,STK11,TP53	13	0 (0.0%)	1 (0.6%)	0,142
ATM, BARD1, BRCA1, BRCA2, BRIP1, CHEK2, FAM175A, NBN, PALB2, PTEN, RAD51C, RAD51D, RECQL, TP53	14	0 (0.0%)	1 (0.6%)	0,142
ATM,BAP1,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CDK4,CDKN2A,CHEK2,DICER1,EPCAM,MITF,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,SMARCA4,STK11,TP53	29	0 (0.0%)	1 (0.6%)	0,142
ATM,BAP1,BARD1,BRCA1,BRCA2,BRIP1,CDH1,CHEK2,DICER1,EPCAM,FH,FLCN,MITF,MLH1,MRE11A,MSH2,MSH6,MUTYH,NBN,NF1,PALB2,PMS2,PTEN,RAD50,RAD51C,RAD51D,SMARCA4,STK11,TP53,TSC1,TSC2	31	0 (0.0%)	1 (0.6%)	0,142
BAP1, BRCA1, BRCA2, CDK4, CDKN2A, EPCAM, MITF, MLH1, MSH2, MSH6, PALB2, PMS2, POT1, PTEN, RB1, TP53	16	0 (0.0%)	1 (0.6%)	0,142

Fisher's exact test assessing differences in panel genes tested between individuals with low allele frequency variants (LAFVs) and those with heterozygous variants (Het).

eTable 3: Median age by Cancer overall and by VAF status

		Overall	Het variants	LAFVs	p-value
Cancer	n	median (Q1, Q3)	median (Q1, Q3)	median (Q1, Q3)	
No cancer diagnosis	612	50.0 (37.0-60.0)	48.0 (36.0-59.0)	65.5 (57.0-72.8)	<0.001***
Other cancers	24	56.0 (36.0-62.5)	54.0 (35.0-62.0)	71.0 (71.0-71.0)	NA^^
Multiple cancer diagnosis (2-5)*	30	66.5 (57.25-77.0)	64.0 (56.0-72.0)	71.0 (68.0-78.5)	0,105
Breast	393	57.0 (45.0-67.0)	55.5 (44.0-66.0)	64.0 (52.5-74.0)	<.001***
Colorectal	41	51.0 (45.0-64.0)	51.0 (45.5-63.0)	59.5 (45.0-74.8)	0,376
Ovarian	43	58.0 (49.0-70.0)	55.0 (47.0-63.0)	72.5 (68.25-80.5)	<0.001***
Pancreatic	16	68.0 (62.5-73.5)	67.5 (62.5-72.3)	71.0 (63.5-78.3)	0,599
Prostate	38	70.5 (64.25-79.5)	67.0 (62.5-75.5)	80.0 (76.0-82.0)	0.006**

VAF; variant allele frequency

Non parametric since does not meet normality assumption for t test. ^^ Not enough observations to calculate p value. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

*Multiple Cancer Diagnosis: more than one of the five types previously specified (breast, ovarian, prostate, colorectal, and/or pancreatic cancer).

eTable 4: Gene distribution by cancer type among LAFVs

Type of cancer	TP53	NF1	CHEK2	ATM	BRCA1	PTEN	APC	BRCA2	CDH1	MLH1	MSH6	NBN
Breast	53 (48.2%	10 (43.5%	2 (15.4%	5 (41.7%	3 (75.0%	1 (50.0%	0 (0.0%)	0 (0.0%)	1 (100.0%	0 (0.0%)	0 (0.0%)	0 (0.0%)
Colorectal	4 (3.6%)	1 (4.4%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Pancreatic	3 (2.7%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Prostate	7 (6.4%)	0 (0.0%)	1 (7.7%)	2 (16.7%	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (100.0%	0 (0.0%)	0 (0.0%)	0 (0.0%)
Ovarian	6 (5.5%)	1 (4.4%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other cancer diagnosis	0 (0.0%)	0 (0.0%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Multiple Cancer Diagnosis (2-5)*	5 (4.6%)	1 (4.4%)	1 (7.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
No cancer diagnosis	32 (29.1%	10 (43.5%	5 (38.5%	5 (41.7%	1 (25.0%	1 (50.0%	1 (100.0%	0 (0.0%)	0 (0.0%)	1 (100.0%	1 (100.0%	1 (100.0%
Total LAFVs	110	23	13	12	4	2	1	1	1	1	1	1

LAFVs: low allele frequency variant

*Multiple Cancer Diagnosis: more than one of the five types previously specified (breast, ovarian, prostate, colorectal, and/or pancreatic cancer).

eTable 5: Ancillary testing on a secondary tissue from the proband (n=44) or through familial testing (n=23 families)

Case I.D	Cancer history	Result of germline testing	VAF	Results of cultured fibroblasts	Results family testing	Clinical Interpretation
1	Breast	*TP53*	13,01	Negative for *TP53*	-	Indeterminate LAFV
2	Breast	*TP53*	11,53	Negative for *TP53*	Negative for *TP53* in 1 relative	Indeterminate LAFV
3	Breast	*PTEN*	16,83	***PTEN***	-	**Constitutional mosaicism**
4	No cancer	*NBN*	13,54	Negative for *NBN*	-	Indeterminate LAFV
5	No cancer	*CHEK2*	14,98	***CHEK2***	-	**Constitutional mosaicism**
6	Breast	*BRCA1*	15,61	***BRCA1***	Negative for *BRCA1* in 2 relatives	**Constitutional mosaicism**
7	No cancer	*TP53*	17,89	Negative for *TP53*	-	Indeterminate LAFV
8	No cancer	*TP53*	25,59	Negative for *TP53*	-	Indeterminate LAFV
9	No cancer	*NF1*	10,05	Negative for *NF1*	-	Indeterminate LAFV
10	Breast	*TP53*	18,61	Negative for *TP53*	-	Indeterminate LAFV
11	Other	*APC*	15,28	***APC***	-	**Constitutional mosaicism**
12	No cancer	*TP53*	23,37	Negative for *TP53*	Negative for *TP53* in 1 relative	Indeterminate LAFV
13	Breast	*TP53*	16,08	Negative for *TP53*	-	Indeterminate LAFV
14	Prostate	*TP53*	12,56	Negative for *TP53*	Negative for *TP53* in 2 relatives	Indeterminate LAFV
15	No cancer	*TP53*	18,61	Negative for *TP53*	-	Indeterminate LAFV
16	Breast, ovarian	*TP53*	13,28	Negative for *TP53*	Negative for *TP53* in 1 relative	Indeterminate LAFV
17	Breast	*TP53*	13,86	Negative for *TP53*	-	Indeterminate LAFV
18	Breast, ovarian	*ATM*	16,01	Negative for *ATM*	-	Indeterminate LAFV
19	No cancer	*BRCA1*	13,21	Negative for *BRCA1*	-	Indeterminate LAFV
20	Breast	*TP53*	19,95	Negative for *TP53*	-	Indeterminate LAFV
21	Breast	*BRCA1*	16,53	Negative for *BRCA1*	-	Indeterminate LAFV
22	Other	*TP53*	16,82	Negative for *TP53*	-	Indeterminate LAFV
23	Breast	*TP53*	19,21	Negative for *TP53*	-	Indeterminate LAFV
24	Other	*TP53*	18,94	Negative for *TP53*	-	Indeterminate LAFV
25	Breast	*TP53*	19,48	Negative for *TP53*	-	Indeterminate LAFV
26	Breast	*TP53*	12,09	Negative for *TP53*	-	Indeterminate LAFV
27	No cancer	*ATM*	24,92	***ATM***	-	**Constitutional mosaicism**
28	Breast	*TP53*	16,09	***TP53***	-	**Constitutional mosaicism**
29	Breast	*TP53*	11,16	Negative for *TP53*	-	Indeterminate LAFV
30	No cancer	*TP53*	22,12	***TP53***	-	**Constitutional mosaicism**
31	No cancer	*TP53*	11,11	Negative for *TP53*	-	Indeterminate LAFV
32	Prostate	*BRCA2*	15,6	***BRCA2***	-	**Constitutional mosaicism**
33	Breast	*TP53*	18,38	***TP53***	-	**Constitutional mosaicism**
34	No cancer	*ATM*	24,73	Negative for *ATM*	-	Indeterminate LAFV
35	No cancer	*PTEN*	10,32	***PTEN***	-	**Constitutional mosaicism**
36	Breast	*TP53*	14,75	Negative for *TP53*	-	Indeterminate LAFV
37	Breast	*TP53*	10,94	Negative for *TP53*	-	Indeterminate LAFV
38	Breast	*CHEK2*	11,53	Negative for *CHEK2*	-	Indeterminate LAFV
39	colon	*NF1*	12,78	Negative for *NF1*	-	Indeterminate LAFV
40	Breast	*NF1*	12,76	Negative for *NF1*	-	Indeterminate LAFV
41	No cancer	*TP53*	26,73	Negative for *TP53*	-	Indeterminate LAFV
42	Breast	*TP53*	16,63	Negative for *TP53*	-	Indeterminate LAFV
43	Breast, ovarian	*TP53*	27,89	Negative for *TP53*	-	Indeterminate LAFV
44	Breast	*TP53*	13,4	Negative for *TP53*	-	Indeterminate LAFV
45	Breast	*TP53*	18,27	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
46	No cancer	*CHEK2*	18,76	-	Negative for *CHEK2* in 2 relatives	Indeterminate LAFV
47	Prostate	*TP53*	14,01	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
48	Breast	***ATM***	13,84	-	Positive for *ATM* in 4 relatives	**Constitutional mosaicism**
49	Breast	*TP53*	14,76	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
50	Breast	*TP53*	14,8	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
51	No cancer	*TP53*	21,94	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
52	No cancer	*CHEK2*	14,83	-	Negative for *CHEK2* in 3 relatives	Indeterminate LAFV
53	Breast	*TP53*	18,96	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
54	Other	*TP53*	13,17	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
55	Breast	*NF1*	10,1	-	Negative for *NF1* in 2 relatives	Indeterminate LAFV
56	Breast	*TP53*	10,64	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
57	No cancer	*TP53*	23,8	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
58	Breast	*CHEK2*	13,9	-	Negative for *CHEK2* in 1 relative	Indeterminate LAFV
59	Other	*CHEK2*	13,9	-	Negative for *CHEK2* in 2 relatives	Indeterminate LAFV
60	No cancer	*TP53*	24,12	-	Negative for *TP53* in 1 relative	Indeterminate LAFV
61	Other	*NF1*	16,9	-	Negative for *NF1* in 1 relative	Indeterminate LAFV
62	Breast	*TP53*	16,24	-	Negative for *TP53* in 8 relatives	Indeterminate LAFV

I.D: identification number; LAFV neg: low allele frequency variant negative

Confirmed constitutional mosaicism results are highlighted in bold.

No cancer:1 of 5 main cancers (breast, colorectal, pancreatic, prostate and ovarian) or other (other cancer not part of the main 5).

Second publication: ***"Prevalence and clinical impact of germline pathogenic variants in breast cancer: a descriptive large single-center study"***

Abstract:

Background: Germline (likely) pathogenic variants (PVs) are identified in 5%-10% of patients with breast cancer (BC) and play a critical role in guiding clinical management, including the use of targeted therapies such as poly (ADP-ribose) polymerase (PARP) inhibitors (PARPi). High-risk genes such as *BRCA1*, *BRCA2,* and *PALB2*, and moderate-risk genes such as *CHEK2* and *ATM*, influence BC risk and treatment decisions. This study evaluates the prevalence and clinical impact of PVs in a large consecutive cohort.

Materials and methods: A retrospective analysis was conducted on 912 individuals with BC who underwent germline testing at the Hospital Clinic of Barcelona from 2016 to 2023. Genetic testing for 14 BC and Lynch syndrome genes was carried out using the TruSight Hereditary Cancer Panel. Statistical analyses were carried out to assess associations between germline results and clinical characteristics, including eligibility for PARPi therapy.

Results: Of the 912 individuals, 129 (14.1%) had a PV, with *BRCA2* (31.8%) and *BRCA1* (24%) being the most frequently altered genes. Additionally, 16.2% carried variants of uncertain significance, most commonly in *ATM* and *BRCA2* genes. Patients with PV were younger compared with PV-negative individuals (median age: 43.5 versus 48.2 years, P ¼ 0.006), more likely to have bilateral BC (13.3% versus 5.8%, P ¼ 0.002), and more frequently diagnosed with triple-negative BC (TNBC; 28.7% versus 20.8%, P ¼ 0.046). Of those with PVs, 39.1% completed a bilateral mastectomy, 36.7% had a risk-reducing salpingo-oophorectomy, and 22.7% had both surgeries. PV detection was associated with higher stages at diagnosis (stage IV: 13.0% versus 5.9%, P < 0.001). In the metastatic cohort, 12.9% received PARPi therapy, with 80.7% harboring *BRCA1/2* PVs. In early BC, 13.1% met the criteria for adjuvant PARPi.

Conclusions: The identification of germline PVs significantly influences surgical decisions and systemic therapies. Genetic testing for patients with BC optimizes care, particularly in selecting candidates for PARPi in both early and advanced BC, improving management and prevention strategies.

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ORIGINAL ARTICLE

Prevalence and clinical impact of germline pathogenic variants in breast cancer: a descriptive large single-center study

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Available online xxx

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Conclusions: The identification of germline PVs significantly influences surgical decisions and systemic therapies. Genetic testing for patients with BC optimizes care, particularly in selecting candidates for PARPi in both early and advanced BC, improving management and prevention strategies.

Key words: breast cancer, germline testing, pathogenic germline variants, PARP inhibitors

INTRODUCTION

Breast cancer (BC) remains the most common malignancy among females and a leading cause of cancer-related mortality.[1] Germline (likely) pathogenic variants (PVs), hereafter collectively referred to as PVs, are detected in 5%-10% of BC cases, and it is essential to identify them given

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their critical role in guiding treatment decisions.[2-5] High-risk genes such as *BRCA1, BRCA2, PALB2*, and *TP53*, and moderate-risk genes such as *CHEK2* and *ATM*, significantly influence the risk of developing BC.[2,6-8] The detection of PVs has implications for surgical choices, adjuvant therapies, and risk-reducing procedures, particularly in patients with a family history or early-onset BC.[9] Triple-negative breast cancer (TNBC) and estrogen receptor-positive (ER-positive) BC display varying associations with different PVs.[10-16] Understanding the prevalence and characteristics of PVs in patients with BC is crucial for optimizing management and improving outcomes.[2,6,17-25] This study presents a single-center experience of germline testing in 912 consecutive patients with BC, providing insights into PV detection and its clinical relevance.

MATERIALS AND METHODS

Study population

This retrospective, single-center study included 1060 consecutive individuals with a personal history of invasive BC who met regional criteria for germline genetic testing at the Hospital Clinic of Barcelona, Spain (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2025.104543). Patients were referred to the hereditary cancer unit between January 2016 and January 2023. Written informed consent was obtained from all participants to carry out the germline testing, and this study was approved by the Ethics Committee of the Hospital Clinic of Barcelona (HCB/2021/0062).

Patients with an *in situ* BC, a BC diagnosis before 2000 [due to differences in treatment options and immunohistochemistry (IHC) and molecular information], or missing clinical information were excluded, resulting in a final cohort of 912 patients (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2025.104543). The cohort was divided into two groups: (i) negative group (PV negative), comprising BC patients with no (likely) PV, (likely) benign, or with variant of uncertain significance (VUS) without clinical impact, and (ii) the PV carrier group, comprising those with PV or likely pathogenic variant (LPV) in BC-related genes and Lynch syndrome-related genes including *BRCA1, BRCA2, PALB2, ATM, CHEK2, BARD1, RAD51C, RAD51D, TP53,* and *PTEN,* and as opportunistic testing including *MLH1, MSH2*, and *MSH6. BRCA1, BRCA2, PALB2, PTEN*, and *TP53* were categorized as high-risk genes, while *ATM, CHEK2, BARD1, RAD51C, RAD51D*, and *MSH2* were considered moderate risk.[6]

Clinical data and family history were obtained from medical records. Germline testing criteria were based on current guidelines,[6,19] and the primary reason for testing was used for analysis (Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2025.104543). Family cancer history was documented for first-, second-, and third-degree relatives, including various cancer types. Personal cancer history included specific cancers but excluded *in situ* BC and BC relapses. To explore the use of poly (ADP-ribose) polymerase inhibitors (PARPi), all patients with metastatic disease (stage IV) either at inclusion or who progressed to stage IV during the study period were included in the analysis.

Hormone receptor status was assessed via immunohistochemistry (IHC), human epidermal growth factor receptor 2 (HER2) status via IHC and FISH, and intrinsic subtypes were based on Prosigna® or PAM50 data.[26]

Germline genetic testing

DNA were extracted from peripheral blood samples using the Magnapure 96 system (Roche, Indianapolis, IN). Next-generation sequencing (NGS) libraries were prepared using the TruSight Rapid Capture Kit and Hereditary Cancer Panel (Illumina, San Diego, CA), with sequencing carried out on the MiSeq platform. Analysis focused on BC susceptibility genes (*BRCA1, BRCA2, PALB2,* etc.) and Lynch syndrome-related genes (*MLH1, MSH2,* and *MSH6).*[14] *BARD1* was tested in patients referred from 2021, accounting for 35.2% (321/912) of the cohort.

Detected variants were classified per the American College of Medical Genetics (ACMG) guidelines into five categories: likely PVs, PVs, VUS, likely benign, or benign.[27] Sanger sequencing was used to confirm all indel PVs.

More details on the genetic analysis can be found in another study conducted by our group.[28] If needed, a draft of this manuscript can be provided for further reference.

Statistical analysis

For analysis, LPVs and PVs were combined and referred to as PV. Clinical characteristics were compared between the PV carrier and PV-negative groups using the chi-square or Fisher's exact test, as appropriate. Age at diagnosis was assessed using the Mann—Whitney *U* or Kruskal—Wallis test due to non-normal distribution. Logistic regression models were used to analyze the association between clinical variables and the presence of PVs, adjusting for age, sex, personal cancer history, TNBC subtype, bilateral BC, and stage at diagnosis. A P value of <0.05 was considered statistically significant, with a false discovery rate (Benjamini—Hochberg) correction applied for multiple comparisons. Data collection and cleaning were conducted between 2021 and 2023, and statistical analysis was carried out between 2023 and 2024. All analyses were carried out using R version 4.3.2.

RESULTS

Study population characteristics

Between January 2016 and January 2023, 912 individuals with a personal history of invasive BC underwent genetic counseling and germline testing at the Hospital Clinic of Barcelona (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2025.104543). The median age of the cohort was 47.6 years (range 24.4-88.6 years), and 98.5% were female ($n = 898$). The cohort was predominantly white or European ethnicity (95.0%), followed by a smaller percentage of Hispanic or South or Central

American (2.4%) (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2025.104543).

A total of 831 individuals (91.1%) met the clinical criteria for suspicion of hereditary cancer syndrome, which included family aggregation of cancers ($n = 357$), BC diagnosis before age 40 years ($n = 199$), TNBC before age 60 years ($n = 162$), related tumors ($n = 49$), multiple BCs in the same individual ($n = 36$), male BC ($n = 12$), or Ashkenazi Jewish ancestry ($n = 1$). Among the 81 individuals (6.9%) who did not meet these criteria, 63 (77.8%) underwent genetic testing for potential therapeutic benefits, with 39.7% ($n = 23$) of patients with stage IV *de novo* BC referred for genetic testing to explore the use of PARPi (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2025.104543).

The clinical characteristics of the overall cohort showed that the majority had hormone receptor-positive (HR-positive)/HER2-negative BC (62.4%, $n = 563$), while 22.0% ($n = 198$) had TNBC, and 15.6% ($n = 141$) had HER2-positive BC. Out of the 912 tested, 129 individuals (14.1%) carried a PV, and 783 individuals (85.9%) had no PV identified in BC-related genes (Figure 1A). *BRCA2* was the most frequently affected gene, with 41 PVs (31.8%), followed by *BRCA1* with 31 PVs (24.0%), *CHEK2* with 17 PVs (13.2%), and *ATM* with 15 PVs (11.6%). *PALB2* PVs were detected in 13 individuals (10.0%), *TP53* in 5 (3.9%), and other less frequent PVs included *BARD1, MSH2, PTEN*, and *RAD51C* (each detected in 1.6% of or fewer individuals) (Figure 1B) (Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2025.104543).

Two patients were found to have two distinct PVs: one carried both *BRCA2* and *MSH6* variants, while another carried both *PALB2* and *CHEK2* variants. VUS were identified in 16.2% ($n = 148$) of individuals (Figure 1A), with the majority occurring in *ATM* (25.7%), *BRCA2* (15.5%), and *PALB2* (10.8%) (Supplementary Table S4, available at https://doi.org/10.1016/j.esmoop.2025.104543).

Clinical predictors of PVs

Table 1 presents a comparison of demographic and clinical characteristics between PV carriers and PV-negative patients. Individuals with PVs were significantly younger at the time of BC diagnosis, with a median age of 43.5 years compared with 48.2 years for those without PVs ($P = 0.006$). Premenopausal status was more common in individuals with PVs (68.3% versus 56.9%, $P = 0.023$). Additionally, individuals with PVs were more likely to have a personal history of other cancers (19.4% versus 11.8%, $P = 0.022$). Differences in clinical criteria for germline testing between the PV carriers and PV-negative groups were not statistically significant ($P = 0.271$), but a higher proportion of individuals with PVs had a personal history of BC diagnosed at $\leq$40 years of age (27.9%) or TNBC diagnosed at $\leq$60 years of age (20.2%) compared with the negative group (20.8% and 17.4%, respectively).

Histologically, no significant differences were observed between groups. Invasive ductal carcinoma was the predominant histology in both groups (84.8% in the PV carrier group and 85.9% in the PV-negative group). Invasive lobular carcinoma and other subtypes were similarly distributed between the two groups. No significant differences were observed in histological grade, tumor size, or node involvement between groups (Table 1).

In multivariable logistic regression analysis, age at BC diagnosis as a continuous variable was inversely associated with PV-positive results [odds ratio (OR) 0.96, 95%

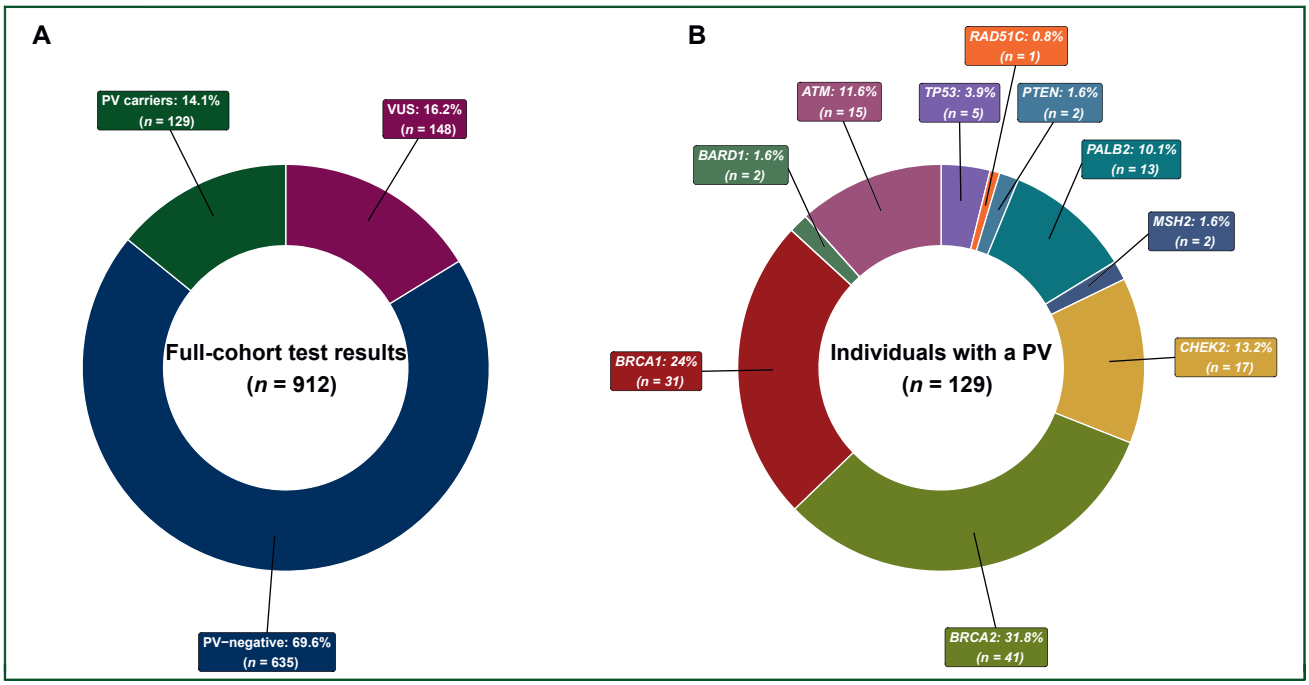

Figure 1. Germline genetic testing results. (A) Results of germline testing in patients with breast cancer ($n = 912$). (B) Distribution of (likely) pathogenic variants among PV carriers ($n = 129$).
PV, (likely) pathogenic variant; VUS, variant of uncertain significance.

Table 1. Differences in clinical characteristics between the PV-negative and the PV-carriers group

Characteristics	PV-negative (n = 783)	PV-carriers (n = 129)	P-value
Sex, *n* (%)			
Female	774 (98.9)	124 (96.1)	**0.036**
Male	9 (1.2)	5 (3.9)	
Age at diagnosis, years, median (range)	48.16 (24.44-88.55)	43.46 (28.27-74.13)	**0.006**
Age at diagnosis (groups), *n* (%)			
≤50 years	445 (56.8)	84 (65.1)	0.082
>50 years	338 (43.2)	45 (34.9)	
Age at diagnosis (groups), *n* (%)			
≤40 years	178 (22.7)	46 (35.7)	**0.002**
>40 years	605 (77.3)	83 (64.3)	
Menopausal status, *n* (%)			
Premenopausal	431 (56.9%)	84 (68.%)	**0.023**
Postmenopausal	326 (43.1)	39 (31.7)	
Ethnicity, *n* (%)			
White or European	738 (94.3)	120 (93.0)	0.208
Hispanic or South or Central American	20 (2.6)	2 (1.6)	
Asian or Southeast Asian	12 (1.5)	3 (2.3)	
Middle Eastern or North African	3 (0.4)	2 (1.6)	
Black, African, or Caribbean	1 (0.1)	1 (0.8)	
Ashkenazi Jews	1 (0.1)	0	
Criteria for genetic testing, *n* (%)			
Family aggregation	315 (40.2)	42 (32.6)	0.271
Breast cancer and aged ≤40 years	163 (20.8)	36 (27.9)	
TNBC and aged ≤60 years	136 (17.4)	26 (20.2)	
Therapeutic benefit	56 (7.2)	7 (5.4)	
Related tumors in the same patient	42 (5.3)	7 (5.5)	
≥ 2 breast cancers	31 (4.0)	5 (3.9)	
Not meeting clinical criteria	16 (2.0)	2 (1.6)	
Family not informative	15 (1.9)	0	
Male breast cancer	8 (1.0)	4 (3.1)	
Ashkenazi Jews	1 (0.1)	0	
Family cancer history, *n* (%)[a]			
No	173 (22.3)	23 (18.4)	0.392
Yes	604 (77.7)	102 (81.6)	
Personal cancer history[b], *n* (%)			
No	689 (88.2)	104 (80.6%)	**0.024**
Yes	92 (11.8)	25 (19.4)	
Histology, *n* (%)			
Invasive ductal carcinoma	642 (85.9)	106 (84.8)	0.942
Invasive lobular carcinoma	52 (7.0)	10 (8.0)	
Others	53 (7.1)	9 (7.2)	
Grade of differentiation, *n* (%)			
Low grade (1 and 2)	425 (69.8)	65 (63.7)	0.267
High grade (3)	184 (30.2)	37 (36.3)	
Stage at diagnosis, *n* (%)			
I	286 (39.4)	30 (26.1)	**0.005**
II	315 (43.4)	58 (50.4)	
III	82 (11.3)	12 (10.4)	
IV	43 (5.9)	15 (13.0)	
cT, *n* (%)			
≤2 cm	323 (45.0)	37 (34.3)	0.097
2-5 cm	278 (38.7)	52 (48.1)	
>5 cm	117 (16.3)	19 (17.6)	
cN, *n* (%)			
Negative	472 (65.6)	67 (60.9)	0.374
Positive	248 (34.4)	43 (39.1)	
IHC subtype, *n* (%)			
HR+/HER2−	489 (63.3)	74 (57.4)	0.065
HR+/HER2+	83 (10.7)	16 (12.4)	
HR−/HER2+	40 (5.2)	2 (1.6)	
TNBC	161 (20.8)	37 (28.7)	
Bilateral breast cancer, *n* (%)			
No	729 (94.2)	111 (86.7)	**0.002**
Yes	45 (5.8)	17 (13.3)	
TNBC, *n* (%)			
No	612 (79.2)	92 (71.3)	**0.046**
Yes	161 (20.8)	37 (28.7)	

Continued

Table 1. Continued

Characteristics	PV-negative (n = 783)	PV-carriers (n = 129)	P-value
Intrinsic subtypes, *n* (%)			
Luminal A	148 (43.9)	28 (38.4)	0.247
Luminal B	85 (25.2)	18 (25.7)	
HER2-enriched	53 (15.7)	9 (12.3)	
Basal-like	51 (15.1)	18 (24.7)	
Neoadjuvant chemotherapy, *n* (%)			
No	397 (54.8)	61 (53.0)	0.767
Yes	327 (45.2)	54 (47.0)	
Type of surgery, *n* (%)			
Lumpectomy	419 (58.4)	56 (49.6)	**0.014**
Mastectomy	281 (39.2)	49 (43.4)	
Bilateral mastectomy	17 (2.4)	8 (7.1)	
Adjuvant chemotherapy, *n* (%)			
No	442 (61.9)	57 (51.4)	**0.044**
Yes	272 (38.1)	54 (48.6)	
Adjuvant hormone therapy, *n* (%)			
No	201 (27.5)	40 (35.7)	0.065
Yes	531 (72.5)	72 (64.3)	
Carboplatin NA/A *n* (%)			
No	618 (88.4)	94 (81.0)	**0.041**
Yes	81 (11.6)	22 (19.0)	
Radiotherapy, *n* (%)			
No	149 (21.0)	35 (31.0)	**0.022**
Yes	560 (79.0)	78 (69.0)	
Risk-reducing mastectomy (RRM), *n* (%)			
No	687 (95.2)	78 (60.9)	**<0.001**
Yes	35 (4.8)	50 (39.1)	
Risk-reducing salpingo-oophorectomy (RRSO), *n* (%)			
No	707 (97.7)	81 (63.3)	**<0.001**
Yes	17 (2.3)	47 (36.7)	
RRM + RRSO, *n* (%)			
No	719 (99.6)	99 (77.3)	**<0.001**
Yes	3 (0.4)	29 (22.7)	

Statistically significant results are highlighted in bold ($P < 0.05$).
cN, node involvement at diagnosis; cT, tumor size at diagnosis; ER, estrogen receptor; ER, estrogen receptor; HR+, ER ≥1%; HR−, ER <1%; HER2, human epidermal growth factor receptor 2; HER2+, positive (IHC 3+ or FISH positive); HR, hormone receptor; IHC, immunohistochemical subtype; LPV, likely pathogenic variant; NA/A, neoadjuvant and/or adjuvant chemotherapy; PV, pathogenic variant; TNBC, triple-negative breast cancer; VUS, variant of uncertain significance.
[a]Family history of cancer: first-, second-, and third-degree relatives.
[b]Personal cancer history included the diagnosis of other cancers except a second breast cancer.

confidence interval (CI) 0.94-0.98, $P < 0.001$]. Male sex was significantly associated with PVs (OR 5.32, 95% CI 1.01-22.26, $P = 0.052$) as were a personal history of cancer other than BC (OR 2.30, 95% CI 1.27-4.04, $P = 0.011$), stage IV disease at diagnosis (OR 3.84, 95% CI 1.82-7.92, $P = 0.001$), and bilateral breast cancer (OR 2.69, 95% CI 1.35-5.14, $P = 0.010$). TNBC was significantly associated with PVs in the univariable analysis ($P = 0.047$); however, this association was not statistically significant in the multivariable analysis (OR 1.55, 95% CI 0.96-2.46, $P = 0.093$) (Table 2).

High-risk and moderate-risk PVs compared with the negative group

Compared with PV-negative patients, PV carrier with PVs in high-risk genes (i.e. *BRCA1, BRCA2, PALB2, TP53*, and *PTEN*) ($n = 92$) were younger at diagnosis (42.8 versus 48.2 years; $P = 0.002$) and more likely to have bilateral BC (15.4% versus 5.8%; $P = 0.001$), stage IV disease (12.3% versus 5.9%; $P = 0.008$), and TNBC (38.0% versus 20.8%; $P < 0.001$) (Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2025.104543). In multivariable logistic regression analysis, clinical variables associated with high-risk PVs were younger age (OR 0.95, 95% CI 0.93-0.97, $P < 0.001$), personal history of cancer other than BC (OR 2.33, 95% CI 1.14-4.56, $P = 0.029$), stage IV at diagnosis (OR 4.35, 95% CI 1.72-10.42, $P = 0.002$), bilateral breast cancer (OR 3.83, 95% CI 1.80-7.82, $P = 0.001$), and TNBC subtype (OR 2.41, 95% CI 1.43-4.02, $P = 0.002$) (Supplementary Table S6, available at https://doi.org/10.1016/j.esmoop.2025.104543). In contrast, individuals with moderate-risk PVs (*ATM, CHEK2, BARD1, RAD51C,* and *MSH2*) did not exhibit any clinical difference compared with PV-negative individuals (Supplementary Tables S7 and S8, available at https://doi.org/10.1016/j.esmoop.2025.104543).

IHC subtype distribution by PV gene

The IHC subtype distribution among PV carriers revealed that 57.4% had HR-positive/HER2-negative BC, 28.7% had TNBC, and 14.0% had HER2-positive BC. Notably, *BRCA1* PVs were predominantly associated with TNBC (77.4%), whereas *BRCA2* PVs were primarily associated with HR-positive/HER2-negative BC (70.7%), with only 14.6% having TNBC and 14.6% HER2-positive BC. Among *PALB2* carriers, 69.2% had HR-positive/HER2-negative and 30.8% had TNBC, while

Table 2. Association of clinical variables with PV carriers

Variables	Univariable analysis (OR, 95% CI)	*P* value	Multivariable analysis (OR, 95% CI)[a]	*P* value	Adj *P* value[b]
Age[c]	0.97 (0.96-0.99)	**0.008**	**0.96 (0.94-0.98)**	**<0.001**	**<0.001**
Sex: male versus female	3.46 (1.05-10.20)	**0.028**	5.32 (1.01-22.26)	**0.029**	0.052
Personal cancer history yes versus no	1.80 (1.09-2.89)	**0.018**	**2.30 (1.27-4.04)**	**0.005**	**0.011**
Stage II versus stage I at diagnosis	1.75 (1.11-2.84)	**0.019**	1.57 (0-96-2.59)	0.073	0.093
Stage III versus stage I at diagnosis	1.40 (0.66-2.78)	0.360	1.38 (0.64-2.83)	0.388	0.388
Stage IV versus stage I at diagnosis	3.33 (1.62-6.61)	**<0.001**	**3.84 (1.82-7.92)**	**<0.001**	**0.001**
Bilateral breast cancer yes versus no	2.48 (1.34-4.41)	**0.003**	**2.69 (1.35-5.14)**	**0.003**	**0.010**
TNBC yes versus no	1.52 (1.00-2.30)	**0.047**	1.55 (0.96-2.46)	0.065	0.093

Statistically significant results are highlighted in bold ($P < 0.05$).
Adj, adjusted; CI, confidence of interval; OR, odds ratio; PV, pathogenic variant; TNBC, triple-negative breast cancer.
[a]Multivariable analyses were adjusted with age, sex, family history, personal cancer history, stage at diagnosis, bilateral breast cancer, and TNBC subtype.
[b]Adjusted (Adj) *P* values were calculated using the false discovery rate (Benjamini—Hochberg) correction method.
[c]Age as a continuous variable.

among *CHEK2* carriers, 76.5% had HR-positive/HER2-negative (Figure 2). Multinomial analysis demonstrated significant associations between *BRCA1* and TNBC (OR 10.41, 95% CI 4.40-24.62, $P < 0.001$), and *TP53* and HER2-positive BC (OR 11.92, 95% CI 1.23-115.58, $P = 0.032$) (Supplementary Table S9, available at https://doi.org/10.1016/j.esmoop.2025.104543).

While the distribution of Prosigna/PAM50-based intrinsic subtypes did not differ significantly between the PV carriers and PV-negative groups ($P = 0.247$), a higher numerical proportion of basal-like subtypes was observed in tumors from PV carriers (24.7% versus 15.1%; $P = 0.055$). This difference was statistically significant when comparing high-risk PV carriers to PV-negative individuals (32.7% versus 15.1%; $P = 0.031$) (Supplementary Table S5, available at https://doi.org/10.1016/j.esmoop.2025.104543), but not in individuals with moderate-risk PVs (4.8% versus 15.1%; $P = 0.284$) (Supplementary Table S7, available at https://doi.org/10.1016/j.esmoop.2025.104543). The luminal A, luminal B, and HER2-enriched subtypes were similarly distributed between groups (Table 1).

Impact of germline results on treatment

The presence of PVs significantly influenced both surgical and systemic treatment decisions. PV carriers underwent more surgical interventions, with 43.4% undergoing therapeutic mastectomy compared with 39.2% in the PV-negative group ($P = 0.014$). Similarly, bilateral mastectomies were carried out more frequently in individuals with PVs (7.1% versus 2.4%; $P = 0.014$). Regarding adjuvant treatment, individuals with PVs were more likely to receive adjuvant chemotherapy (48.6% versus 38.1%; $P = 0.044$) and platinum-based chemotherapy (19.0% versus 11.6%; $P = 0.041$). Although the rates of neoadjuvant chemotherapy were similar between groups (47.0% versus 45.2%; $P = 0.767$), individuals with PVs showed a non-significant trend toward being less likely to receive adjuvant hormone therapy (64.3% versus 72.5%; $P = 0.065$).

In those who received neoadjuvant chemotherapy, there was no difference in pathological complete response (pCR) between the PV carriers and PV-negative groups (38.5% versus 38.1%; $P = 1.00$). Among individuals with PVs, 39.1% underwent risk-reducing mastectomy (RRM), and 36.7% had a risk-reducing salpingo-oophorectomy (RRSO). Notably, 22.7% underwent both surgeries (RRM + RRSO) (Table 1).

A closer examination of individuals with high-risk PVs (e.g. *BRCA1, BRCA2, PALB2, TP53*) showed a statistically non-significant higher prevalence of mastectomies and bilateral mastectomies (56.1%) ($n = 46$) compared with those with PVs in moderate-risk genes [35.5% ($n = 11$); $P = 0.134$]. High-risk PV carriers also received more platinum-based chemotherapy (24.1% versus 6.1%; $P = 0.034$) and less adjuvant hormone therapy (54.3% versus 90.3%; $P < 0.001$). Regarding risk-reducing surgeries, 46.2% ($n = 42$) of individuals with high-risk PVs underwent RRM, compared with 21.6% ($n = 8$) of those with moderate-risk PVs ($P = 0.018$). Similarly, RRSO was more prevalent in high-risk PV carriers, with 47.3% ($n = 43$) undergoing the procedure, compared with only 10.8% ($n = 4$) in the moderate-risk group ($P < 0.001$). All individuals who underwent both RRM and RRSO carried a high-risk PV ($n = 29$; 100%).

In the cohort of individuals with stage IV BC at diagnosis or relapse during follow-up ($n = 203$), 12.9% ($n = 26$) received treatment with PARPi. Among these patients, 18 (69.2 PV%) had a germline PV in *BRCA1/2*, 1 had in *CHEK2*, and 1 in *RAD51C*. Three individuals had somatic pathogenic variants in *BRCA1/2*, while three were negative for germline PV and unknown *BRCA1/2* somatic variants. The patients without *BRCA1/2* variants were treated within clinical trials. Regarding treatment with PARPi, 76.9% ($n = 20$) received olaparib, including three who were treated in combination with immunotherapy, while 23.1% ($n = 6$) received talazoparib. Among patients with germline *BRCA1/2* PVs, 44.4% (8/18) achieved a complete radiological response (CR) or partial response (PR), and the median duration of response was 5.4 months (range 1.2-20.2 months). Among patients with somatic PVs in *BRCA1/2* ($n = 3$), two experienced a PR and one had progressive disease. The patient with a germline PV in *CHEK2* who received PARPi as sixth-line therapy achieved a PR, while the patient with a germline PV in *RAD51C* treated

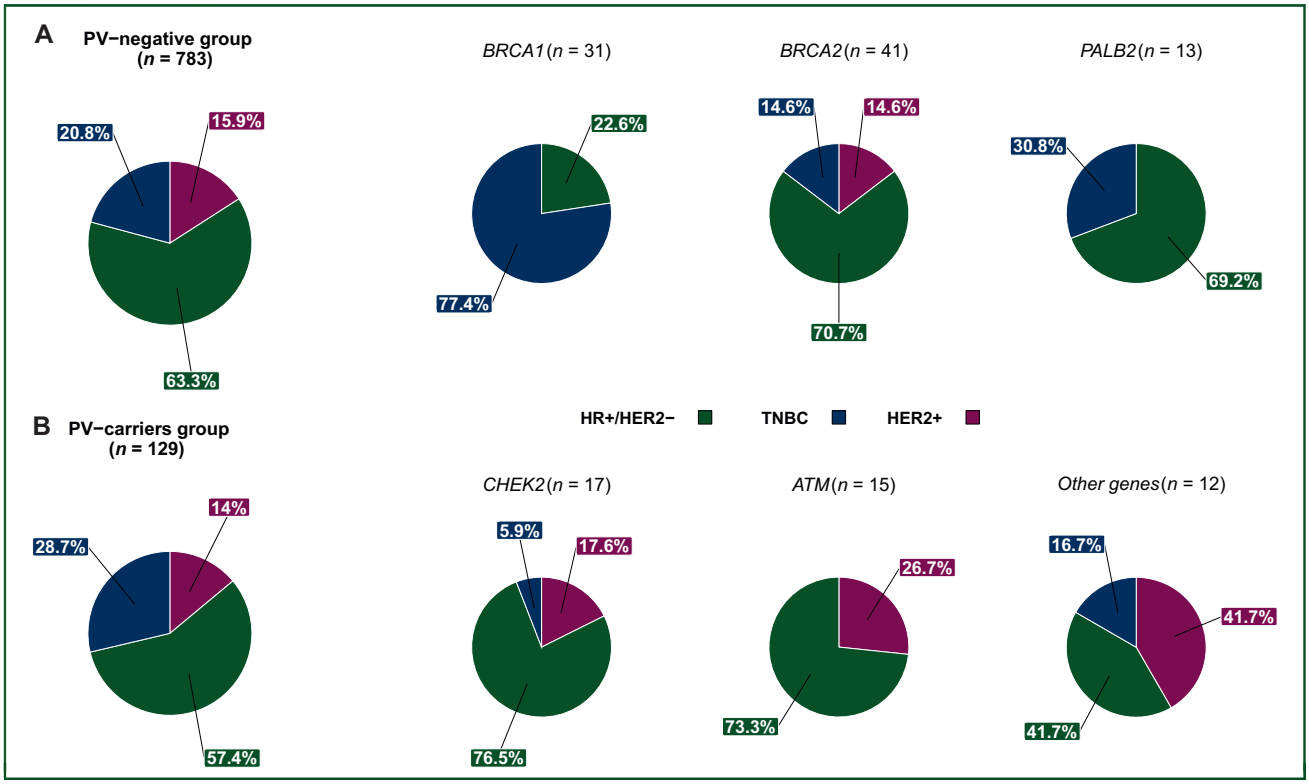

Figure 2. The distribution by immunohistochemistry subtypes. (A) Patients with negative genetic testing ($n = 783$). (B) PV carriers ($n = 129$), per gene [*BRCA1* ($n = 31$), *BRCA2* ($n = 41$), *PALB2* ($n = 13$), *CHEK2* ($n = 17$), *ATM* ($n = 15$)], and other genes [(*BARD1* ($n = 1$), *MSH2* ($n = 2$), *PTEN* ($n = 2$), *RAD51C* ($n = 1$), *TP53* ($n = 5$)]. HER2-2, human epidermal growth factor receptor-2; HR, hormone receptor; PV, (likely) pathogenic variant; TNBC, triple-negative breast cancer.

in the second line had stable disease (SD) for 5.4 months (Figure 3). In early-stage BC, 99 individuals met the OlympiA trial criteria for adjuvant olaparib.[29] Of these, 13.1% ($n = 13$) carried a germline PV in *BRCA1/2*. None of the early-stage individuals in our cohort received PARPi in the adjuvant setting (Supplementary Table S10, available at https://doi.org/10.1016/j.esmoop.2025.104543).

DISCUSSION

Our study reports findings from a cohort of 912 patients with BC who underwent germline testing for 14 BC and Lynch syndrome-related genes. We detected PVs in 14.1% of patients, a higher rate than typically reported in unselected cohorts,[30] which likely reflects our cohort's selection based on clinical criteria such as family history, early-onset disease, and TNBC. High-risk PVs in genes such as *BRCA1, BRCA2, PALB2,* and *TP53* significantly influence management decisions, including surgical options like RRMs and the use of adjuvant therapies.[2,26,31]

The distribution of PVs in our cohort revealed *BRCA2* as the most prevalent mutation, in contrast to earlier studies where *BRCA1* was more commonly found.[32,33] This shift may be enhanced by the broader application of genetic testing in HR-positive BC, where *BRCA2* variants are more frequent,[34] and improvement in NGS techniques. Additionally, 6.9% of our cohort had stage IV disease at diagnosis, where *BRCA2* variants have been more commonly described than *BRCA1* PVs.[35,36] The inclusion of moderate-risk genes (*ATM, CHEK2, RAD51C*, and others) further highlights the complexity of genetic predisposition in BC.[18]

We also found that 16.9% of our cohort carried VUS, predominantly in *ATM*. Among the genes tested, *ATM* is one of the largest, with 66 exons, but its exons are not frequently mutated. Consequently, clinical and functional data for many *ATM* variants are limited.[37] While VUS have been reported in 10%-20% of BC cases, their clinical implications remain unclear, and patient management should not rely solely on these findings.[38] Continuous monitoring and potential reclassification of VUS are essential, as some variants may later be reclassified as pathogenic or benign.[39]

Consistent with prior knowledge, key clinical variables, such as younger age at BC onset, male sex, bilateral BC, and a personal history of cancer other than a second BC, were significantly associated with PV detection, particularly for high-risk genes. In contrast, moderate-risk genes were less clearly linked to specific clinical characteristics.[12,30,40,41] Despite the fact that TNBC was highly predictive of *BRCA1* variants, more than half of the PVs were found in patients with a luminal phenotype, underscoring the importance of testing beyond TNBC.[10,11,32,42] In our cohort, we present novel findings on intrinsic subtypes. High-risk PV carriers were enriched in the basal-like subtype, with no significant differences in other intrinsic subtypes (LumA, LumB, HER2-enriched), as previously reported.[43]

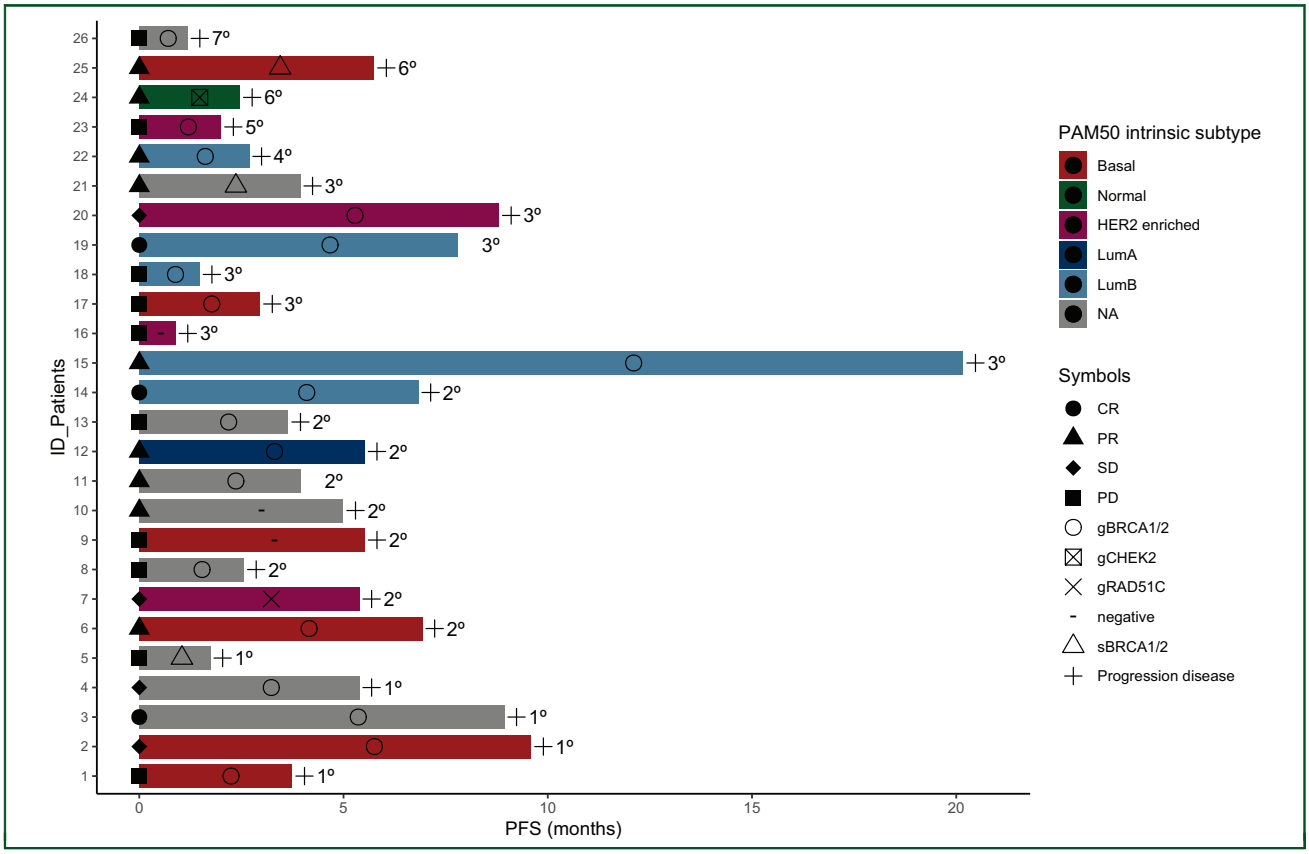

Figure 3. Swimmer plot of metastatic breast cancer patients treated with PARP inhibitors. A total of 26 patients received treatment with PARP inhibitors in a metastatic setting, across different lines of therapy and with various *BRCA1/2* statuses.
CR, complete response; g*BRCA1/2*, germline pathogenic variants (PV) in *BRCA1/2*; g*CHEK2*, germline PV in *CHEK2*; g*RAD51C*, germline PV in *RAD51C*; PD, progression disease; PFS, progression-free survival; PR, partial response; s*BRCA1/2*, somatic PV in *BRCA1/2*; SD, stable disease.

In line with prior results, surgical decisions were influenced by PV status, with patients carrying PVs being more likely to undergo bilateral mastectomies at diagnosis or eventual risk-reducing surgeries.[9,44,45] Notably, 21.6% of moderate-risk PV carriers also underwent RRMs, despite limited evidence supporting this intervention. This points to the need for refined guidelines to prevent overtreatment in moderate-risk PV carriers.[45,46]

The advent of PARPi has transformed treatment for patients with BC with *BRCA1/2* PVs. PARPi have demonstrated significant improvements in survival outcomes, both in early and metastatic settings, leading to expanded recommendations for genetic testing.[20-22,25,26,47] Germline testing guided PARPi therapy for 12.9% of metastatic patients and identified 13.1% of patients with early-stage BC who would have met the criteria for adjuvant PARPi, highlighting the growing importance of germline testing in personalized treatment strategies.

Despite the strengths of our study, such as the relatively large sample size, there are limitations to consider. The retrospective design and selection bias based on clinical criteria may have affected some of the clinical variables and outcomes. Additionally, the relatively small number of PVs detected for individual genes limits our ability to make definitive conclusions about the clinical differences between PV carriers and PV-negative individuals. Although PV rates decrease as testing criteria are broadened for therapeutic purposes, our clinical predictors of PVs remain consistent with previously reported findings.[12,40,41,48]

Our findings have important clinical implications, particularly for optimizing genetic testing strategies in low-resource settings. Given the financial and infrastructural constraints that limit access to comprehensive genetic testing, the use of clinically available variables—such as age at diagnosis, sex, cancer stage, bilateral BC, histologic subtype, and personal and family history—can serve as a practical and cost-effective approach to identifying individuals at higher risk of carrying a PV. By leveraging these factors, health care providers can prioritize genetic testing for those most likely to benefit, ensuring that limited resources are allocated efficiently. Additionally, our study's reliance on a targeted gene panel comprising well-established BC susceptibility and Lynch syndrome genes further supports the feasibility of this approach in settings where extensive sequencing may not be accessible. Expanding access to genetic testing through strategies such as mainstreaming, point-of-care testing, and telemedicine-based genetic counseling could help overcome existing barriers.[49–51] Future research should focus on refining predictive models based on clinical data and evaluating the

real-world impact of integrating these strategies into routine oncology care, with the goal of improving early detection and personalized management of hereditary BC worldwide.

In conclusion, while germline testing in BC has been generalized to facilitate therapeutic approach, clinical and pathologic features remain important and useful predictors of PV in high-risk-associated genes in clinical practice. Our study underscores the clinical importance of germline testing in BC, particularly in patients with high-risk factors such as early-onset disease, bilateral BC, or a personal history of cancer. Germline information significantly impacts surgical and systemic treatment decisions, particularly in advanced-stage disease and adjuvant settings where PARPi can be a key therapeutic option. It is crucial to ensure equitable access to genetic testing while prioritizing patients most likely to benefit from early detection. Future research should focus on understanding the implications of PVs in moderate-risk genes and further validate these findings across diverse populations through prospective, multicenter studies.

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DISCLOSURE

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DATA SHARING

The data from this study are not publicly accessible, as participants did not consent to public sharing. However, researchers may request access to the data for academic purposes, contingent on a data transfer agreement and approval from the ethics committee.

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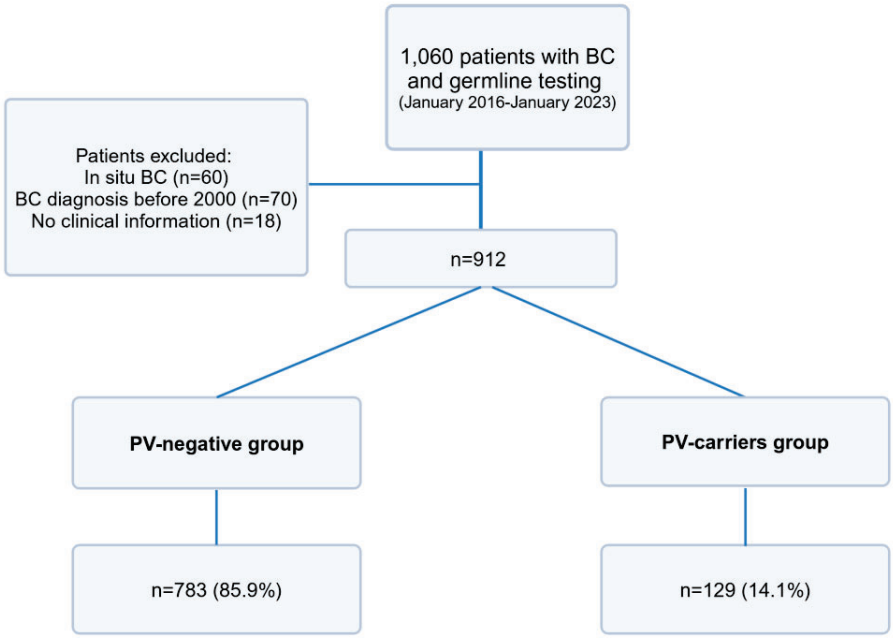
Supplemental Figure 1



Supplemental Material

eTable1. Selection criteria for genetic testing at Hospital Clinic of Barcelona.

	Clinical Criteria
Breast cancer	- Breast cancer ≤ 40 years old - Triple-negative breast cancer ≤ 60 years old - Breast cancer in male - ≥ 2 breast cancer (Two breast cancer cases at least one of them diagnosed ≤ 50 years old) - Therapeutic benefit (metastatic or early breast cancer suitable for PARPi treatment) - Related tumors in the same patient (breast and/or ovarian cancer) - Family aggregation (Family cancer history suggested of cancer-hereditary syndromes) - Non-informative family - Ancestry with founder mutations

*These criteria were adapted for this study and do not fully correlate with the specific genetic testing criteria for familial breast assessment.

PARPi: PARP inhibitors

eTable 2: Clinical characteristics in patients with germline testing

Characteristics	**All patients (n=912)**
Sex	
Female	898 (98.5%)
Male	14 (1.5%)
Age at diagnosis (median, range)	47.61 (24.44-88.55)
Age at diagnosis (groups)	
≤ 50 years	529 (58.0%)
>50 years	383 (42.0%)
Age at diagnosis (groups)	
≤ 40 years	224 (24.6%)
>40 years	688 (74.4%)
Menopausal status	
Premenopausal	515 (57.6%)
Postmenopausal	365 (40.8%)
Male	14 (1.6%)
Unknown	18
Germline testing	
PV	129 (14.1%)
VUS	154 (16.9%)
Negative	629 (69.0%)
Race and ethnicity:	

White or European	858 (95.0%)
Hispanic or South or Central America	22 (2.4%)
Asian or Southeast Asian	15 (1.7%)
Middle Eastern or North Africa	5 (0.6%)
Black, African, or Caribbean	2 (0.2%)
Ashkenazi Jews	1 (0.1%)
Unknown	9
Criteria for genetic testing:	
Family aggregation	357 (39.1%)
Breast cancer ≤ 40 years	199 (21.8%)
TNBC < = 60 years	162 (17.7%)
Therapeutic benefit	63 (6.9%)
Related tumors in the same patient	49 (5.4%)
≥o 2 breast cancers	36 (3.9%)
Not meet clinical criteria	18 (2.0%)
Non- informative family	15 (1.6%)
Male Breast cancer	12 (1.3%)
Ashkenazi Jews	1 (0.1%)
Family Cancer History (1º,2º and 3º)	
No	196 (21.7%)
Yes	706 (78.3%)
Unknown	10
Personal Cancer History	
No	793 (87.1%)
Yes	117 (12.9%)
Unknown	2
Histology	
Invasive Ductal carcinoma	748 (85.8%)
Invasive Lobular carcinoma	62 (7.1%)
Others	62 (7.1%)
Unknown	40
Stage at diagnosis	
I	316 (37.6%)
II	373 (44.4%)
III	94 (11.2%)
IV	58 (6.9%)
Unknown	71
cT	
≤ 2 cm	360 (43.6%)
2-5 cm	330 (40.0%)
> 5 cm	136 (16.5%)
Unknown	86
cN	
Negative	539 (65.0%)
Positive	291 (35.0%)

Unknown	82
Grade of differentiation	
Low-grade (1 and 2)	490 (68.9%)
High grade (3)	221 (31.1%)
IHC subtype	
HR+/HER2-	563 (62.4%)
HR+/HER2+	99 (11.0%)
HR-/HER2+	42 (4.7%)
TNBC	198 (22.0%)
Unknown	10
Bilateral Breast Cancer	
No	840 (93.1%)
Yes	62 (6.9%)
Unknown	10
TNBC	
No	704 (78.1%)
Yes	198 (21.9%)
Unknown	10
Intrinsic subtypes	
LumA	176 (39.7%)
LumB	104 (23.4%)
HER2 enriched	62 (14.00%)
Basal-like	69 (15.6%)
Normal-like	33 (7.4%)
Unknown	469
Neoadjuvant chemotherapy (NA)	
No	458 (54.6%)
Yes	381 (45.4%)
Unknown	73
Type of surgery	
Lumpectomy	475 (57.2%)
Mastectomy	330 (39.8%)
Bilateral Mastectomy	25 (3.0%)
Unknown	82
Adjuvant chemotherapy (A)	
No	499 (60.5%)
Yes	326 (39.5%)
Unknown	87
Adjuvant hormonotherapy	
No	241 (28.6%)
Yes	603 (71.4%)
Unknown	68
Carboplatin NA/A	
No	712 (87.36%)
Yes	103 (12.63%)

Unknown	97
Radiotherapy	
No	184 (22.4%)
Yes	638 (77.6%)
Unknown	90
Risk Reduction Mastectomy (RRM)	
No	765 (90.0%)
Yes	85 (10.0%)
Unknown	62
Risk Reduction Salpingo-oophorectomy (RRSO)	
No	788 (92.5%)
Yes	64 (7.5%)
Unknown	60
RRM + RRSO	
No	818 (96.2%)
Yes	32 (3.8%)
Unknown	62

PV: (likely) pathogenic, VUS: variant of uncertain significant, cT: tumor size at diagnosis, cN: node involvement at diagnosis, ER: estrogen receptor, TNBC: triple negative breast cancer, LumA: Luminal A, LumB: Luminal B

*Family cancer history: 1º, 2º and 3º degree relatives

**Personal Cancer History included the diagnosis of other cancer except a second breast cancer
***IHC: immunohistochemical subtype, HR: hormone receptor, HR +: estrogen receptor >= 1%, HR -: estrogen receptor <1%, HER2: Human Epidermal growth factor Receptor 2;

HER2+: positive (IHC 3+ or FISH positive), TNBC: Triple Negative Breast Cancer (HR 1-10%)

eTable 3: Likely pathogenic and pathogenic variants detected in the cohort

Gene affected	Type of mutation	DNA change	Protein change	dbSNP rs ID	Number
ATM	nonsense	c.8272delG	p.(Val2758PhefsTer48)	-	1
ATM	nonsense	c.3801delG	p.(Val1268Ter)	rs587779834	1
ATM	deletion	c.3712_3716delTTATT	p.(Leu1238LysfsTer6)	rs1423067039	1
ATM	missense	c.6203T>C	p.(Leu2068Ser)	rs1555114558	2

ATM	frameshift	c.8264_8268delATAAG	p.(Tyr2755CysfsTer12)	rs730881294	1
ATM	splice donor	c.2921+2dup	-	rs1565424654	1
ATM	nonsense	c.3802delG	p.(Val1268Ter)	rs587779834	1
ATM	nonsense	c.4507C>T	p.(Gln1503Ter)	-	1
ATM	frameshift	c.3628_3629delAT	p.(Met1210GlyfsTer18)	-	1
ATM	frameshift	c.8292_8293delTG	p.(Ser2764ArgfsTer)	rs879254036	1
ATM	nonsense	c.7220C>A	p.(Ser2407Ter)	rs1555122149	1
ATM	nonsense	c.748C>T	p.(Arg250Ter)	rs772821016	1
ATM	frameshift	c.3381_3384delTCAG	p.(Gln1128LysfsTer)	rs587781971	1
ATM	nonsense	c.3802delG	p.(Val1268Ter)	rs587779834	1
BARD1	nonsense	c.1921C>T	p.(Arg641Ter)	rs587781948	1
BARD1	nonsense	c.1156G>T	p.(Glu386Ter)	rs1553622218	1
BRCA1	missense	c.5096G>A	p.(Arg1699Gln)	rs41293459	1
BRCA1	frameshift	c.124delA	p.(Ile42Tyrfs)	rs80357943	2
BRCA1	nonsense	c.5154G>A	p.(Trp1718Ter)	rs80357239	1
BRCA1	frameshift	c.2285_2286delGA	p.(Arg762IlefsTer5)	-	1
BRCA1	frameshift	c.68_69delAG	p.(Glu23ValfsTer17)	rs80357713	3
BRCA1	nonsense	c.3839_3843delinsAGGC	p.(Ser1280Ter)	rs273900717	1
BRCA1	-	-	-	-	1
BRCA1	frameshift	c.2020_2021delA	p(Lys701fs)	rs431825389	1
BRCA1	nonsense	c.3205C>T	p.(Gln1069Ter)	rs2053627043	1
BRCA1	missense	c.211A>G	p.(Arg71Gly)	rs80357382	3
BRCA1	frameshift	deletion exon 24	-	-	1
BRCA1	-	exons 17 and 18 according to LRG_292t1	-	-	1
BRCA1	nonsense	c.220C>T	p.(Gln74Ter)	rs80357234	1
BRCA1	-	-	-	-	1
BRCA1	missense	c.4484G>T	p.(Arg1495Met)	rs80357389	1

BRCA 1	frameshift	c.2217dupA	p.(Val740SerfsTer3)	rs80357802	1
BRCA 1	nonsense	c.2214dup	p.(Lys739Ter)	rs80357574	1
BRCA 1	missense	c.5309G>T	p.(Gly1770Val)	rs863224765	1
BRCA 1	-	-	-	-	1
BRCA 1	-	-	-	-	1
BRCA 1	frameshift	c.3756_3759delGTCT	p.(Ser1253ArgfsTer10)	-	1
BRCA 1	frameshift	c.1961delA	p.(Lys654SerfsTer47)	rs80357522	1
BRCA 1	frameshift	c. 5266dupC	p.(Gln1756ProfsTer7)	rs80357906	1
BRCA 1	frameshift	c.815_824dup	p.(Thr276fs)	rs387906563	1
BRCA 1	frameshift	c.3770_3771delAG	p.(Glu1257Glyfs)	rs80357993	1
BRCA 1	missense	c.5558A>G	p.(Tyr1853Cys)	rs80357258	1
BRCA 2	frameshift	c.658_659delGT	p.(Val220Ilefs)	rs80359604	1
BRCA 2	frameshift	c.8179delG	p.(Ala2727Leufs*6)	-	1
BRCA 2	synonymous	c.9117G>A	p.(Pro3039=)	rs28897756	1
BRCA 2	duplication	c.3264dupT	p.(Gln1089SerfsTer10)	rs80359380	2
BRCA 2	nonsense	c.9018C>A	p.(Tyr3006Ter)	rs80359154	1
BRCA 2	nonsense	c.8485C>T	p.(Gln2829Ter)	rs80359099	1
BRCA 2	-	-	-	-	1
BRCA 2	frameshift	c.9026_9030delATCAT	p.(Tyr3009SerfsTer7)	rs80359741	4
BRCA 2	frameshift	c.1257delT	p.(Cys419TrpfsTer11)	rs80359272	1
BRCA 2	frameshift	c.3170_3174delAGAAA	p.(Lys1057ThrfsTer8)	rs80359373	1
BRCA 2	frameshift	c.4829_4830delTG	p.(Val1610GlyfsTer4)	rs80359468	1
BRCA 2	frameshift	c.6001_6002delTC	c.(6005A>T)	-	1
BRCA 2	nonsense	c.3455T>G	p.(Leu1152Ter)	rs80358593	1
BRCA 2	frameshift	c.7932delT	p.(Asn2644Lysfs*4)	-	1
BRCA 2	frameshift	c.2808_2811delACAA	p.(Ala938Profs*21)	rs80359351	2

BRCA 2	frameshift	c.2701delC	p.(Ala902LeufsTer2)	rs397507637	1
BRCA 2	deletion	c.9326_9327insATTA	p.(Asn3110Leufs*2)	-	1
BRCA 2	missense	c.8023A>G	p.(Ile2675Val)	rs397507954	1
BRCA 2	nonsense	c.4912A>T	p.(Lys1638Ter)	rs886040553	1
BRCA 2	frameshift	c.429delT	p.(Val144LeufsTer8)	rs587781945	1
BRCA 2	missense	c.9004 G>A	p.(Glu3002Lys)	-	1
BRCA 2	nonsense	c.9382C>T	p.(Arg3128Ter)	rs80359212	1
BRCA 2	frameshift	c.4936_4939delGAAA	p.(Glu1646GlnfsTer23)	rs80359473	1
BRCA 2	frameshift	c.4456_4459del	p.(Val1486AsnfsTer5)	rs80359449	1
BRCA 2	-	-	-	-	1
BRCA 2	frameshift	c.4131_4134del	p.(Thr1378Argfs*9)	-	1
BRCA 2	nonsense	c.9286G>T	p.(Glu3096Ter)	rs80359199	1
BRCA 2	frameshift	c.8174_8185delinsTT	p.(Trp2725PhefsTer5)	rs730881615	1
BRCA 2	frameshift	c.5722_5723delCT	p.(Leu1908ArgfsTer2)	rs80359530	1
BRCA 2	frameshift	c.1310_1313delAAGA	p.(Lys437IlefsTer2)	rs80359277	1
BRCA 2	deletion	c.4963delT	p.(Tyr1655ThrfsTer15)	rs886040557	1
BRCA 2	missense	c8167 G>C	p.(Asp2723His)	rs41293511	1
BRCA 2	deletion	c.538delA	p.(Ile180PhefsTer5)	-	1
BRCA 2	frameshift	c.6405_6409delCTTAA	p.(Asn2135LysfsTer3)	rs80359584	1
BRCA 2	missense	c.3922G>T	p.(Glu1308Ter)	rs80358638	1
BRCA 2	frameshift	c.3744_3747del	p.(Ser1248fs)	rs80359403	1
CHEK2	missense	c.470T>C	p.(Ile157Thr)	rs17879961	1
CHEK2	nonsense	c.507delT	p.(Phe169LeufsTer2)	rs587780183	1
CHEK2	missense	c.499G>A	p.(Gly167Arg)	rs72552322	1
CHEK2	frameshift	c.591delA	p.(Val198PhefsTer7)	rs587782245	1
CHEK2	missense	c.349A>G	p.(Arg117Gly)	rs28909982	4
CHEK2	frameshift	c.870delC	p.(Phe292LeufsTer12)	rs1555916987	1

CHEK2	nonsense	c.363T>A	p.(Cys121Ter)	rs1209379774	1
CHEK2	truncate	c.1100delC	p.(Thr367MetfsTer15)	rs555607708	4
CHEK2	frameshift	c.1368dup	p.(Glu457ArgfsTer33)	rs730881700	1
CHEK2	nonsense	c.283C>T	p.(Arg95Ter)	rs587781269	1
CHEK2	splice donor	c.846+4_846+7delAGTA	-	rs764884641	1
MSH2	nonsense	c.1861C>T	p.(Arg621Ter)	rs63750508	1
MSH2	frameshift	c.1705_1706del	p.(Glu569ILefsTer2)	rs63750393	1
MSH6	missense	c.3725G>A	p.(Arg1242His)	rs63750119	1
PALB2	deletion	c.(3113+1_3114-1)_(3201+1_3202-1)del	-	-	1
PALB2	frameshift	c.2205dupA	p.(Ala736SerfsTer93)	-	2
PALB2	nonsense	c.1240C>T	p.(Arg414Ter)	rs180177100	2
PALB2	frameshift	c3067C>T	p. (Glu1023Ter)	-	1
PALB2	nonsense	c.3256C>T	p.(Arg1086Ter)	rs587776527	1
PALB2	frameshift	deletion exon 7 to exon 11	-	-	1
PALB2	nonsense	c.1653T>A	p.(Tyr551Ter)	rs118203997	2
PALB2	missense	c.2816T>G	p.(Leu939Trp)	rs45478192	1
PALB2	frameshift	c.976dupT	p.(Ser326PhefsTer6)	rs1555461517	1
PALB2	splice donor	c.3113+1G>A	-	rs1597079557	1
PALB2	deletion	deletion exon 1 to 10	-	-	1
PTEN	splice donor	c.253+1G>T	-	rs587776667	1
PTEN	missense	c.822G>T	p.(Trp274Cys)	-	1
RAD51C	nonsense	c.1044_1045insGA	p.(Thr349GlufsTer16)	-	1
TP53	deletion	deletion exon 1 to 11	-	-	1
TP53	deletion	c.754_762delCTCACCATC	p.(L252_I254delLTI)	rs2073243450	1
TP53	missense	c.524G>A	p.(Arg175His)	rs28934578	1
TP53	missense	c.733G>A in exon 7	p.(Gly245Ser)	rs28934575	1
TP53	missense	c.472C>T	p.(Arg158Cys)	rs587780068	1

Number: number of individuals with this variant

eTable 4: Variants of uncertain significant detected in the cohort

Affected gene	Type of mutation	DNA change	Protein change	dbSNP ID	Number
ATM	missense	c.9149C>T	p.(Pro3050Leu)	rs778267979	1
ATM	missense	c.8311A>G	p.(Thr2771Ala)	rs876660587	1
ATM	-	-	-	-	1
ATM	missense	c.5938G>A	p.(Gly1980Arg)	rs786203765	1
ATM	missense	c.4856G>A	p.(Arg1619Lys)	rs730881393	1
ATM	missense	c.7187C>G	p.(Thr2396Ser)	rs370559102	2
ATM	missense	c.1106C>T	p.(Ser369Phe)	rs762557654	1
ATM	missense	c.7475T>G	p.(Leu2492Arg)	rs56399857	1
ATM	missense	c.3925G>A	p.(Ala1309Thr)	rs149711770	1
ATM	missense	c.922T>C	p.(Trp308Arg))	rs2079878501	1
ATM	missense	c.2932T>C	p.(Ser978Pro)	rs139552233	1
ATM	missense	c.610G>A	p.(Gly204Arg)	rs147915571	1
ATM	missense	c.8810T>C	p.(Val2937Ala)	rs587782149	2
ATM	missense	c.8327T>C	p.(Ile2776Thr)	rs746475628	1
ATM	-	-	-	-	1
ATM	missense	c.7793G>A	p.(Arg2598Gln)	rs140263969	1
ATM	missense	c.3728A>G	p(Asn1243Ser)	rs730881363	1
ATM	-	-	-	-	1
ATM	missense	c.2650C>T	p.(Pro884Ser)	rs55982963	1
ATM	missense	c.8560C>T	p.(Arg2854Cys)	rs201958469	1
ATM	missense	c.5490G>C	p.(Met1830Ile)	-	1
ATM	missense	c.4546C>T	p.(His1516Tyr)	-	1
ATM	synonymous	c.6975G>A	p.(Ala2325=)	rs556778314	1

ATM	missense	c.2689T>A	p.(Phe897Ile)	rs147122522	2
ATM	missense	c.8851G>A	p.(Val2951Ile))	rs1555151205	1
ATM	missense	c.295A>G	p.(Ser99Gly)	rs137882485	1
ATM	missense	c.5189G>A	p.(Arg1730Gln)	rs373789346	1
ATM	missense	c.1009C>T	p.(Arg337Cys)	rs138398778	1
ATM	missense	c.5278A>G	p.(Met1760Val)	rs151327241	1
ATM	missense	c.2305G>A	p.(Glu769Lys)	rs1591546453	1
ATM	missense	c.6067G>A	p.(Gly2023Arg)	rs11212587	3
ATM	missense	c.1595G>A	p.(Cys532Tyr)	rs35963548	2
BARD1	missense	c.238G>A	p.(Gly80Arg)	-	1
BARD1	missense	c.724T>C	p.(Phe242Leu)	rs745837404	1
BARD1	inframe deletion	c.26_40del	-	rs587781979	1
BRCA1	missense	c.5066T>C	p.(Met1689Thr)	rs80357061	1
BRCA1	missense	c.956A>G	p.(Asn319Ser)	rs397507258	1
BRCA1	missense	c. 1712 T >C	p.(Ile571Thr)	rs80357159	1
BRCA1	missense	c.3808T>G	p.(Ser1566Ala)	rs730881493	1
BRCA1	-	-	-	-	1
BRCA1	missense	c.4454C>T	p.(Thr1485Ile)	rs80356870	1
BRCA1	missense	c.2666C>T	p.(Ser889Phe)	rs769712441	1
BRCA1	missense	c.4730C>A	p(Ser1577Tyr)	rs273901741	1
BRCA1	missense	c.4393A>G	p.(Ile1465Val)	rs1567779778	1
BRCA1	missense	c.2662C>T	p.(His888Tyr)	rs80357480	2
BRCA2	missense	c.1196C>T	p.(Thr399Ile)	-	1
BRCA2	missense	c.4295G>T	p.(Ser1432Ile)	rs564757581	1
BRCA2	intro variant	c.7007+31A>T	-	rs555236509	1
BRCA2	missense	c.7967T>G	p.(Leu2656Arg)	-	1

BRCA 2	synonymous	c.3801T>G	p.(Asp1267Glu)	-	2
BRCA 2	missense	c.10033G>A	p.(Ala3345Thr)	rs546597661	1
BRCA 2	missense	c.7931A>G	p.(Asn2644Ser)	rs80359020	1
BRCA 2	missense	c.7271G>C	p.(Arg2424Thr)	rs756057155	1
BRCA 2	missense	c.9155G>A	p.(Arg3052Gln)	rs80359171	1
BRCA 2	missense	c.9296A> G	p.(Asn3099Ser)	rs730881571	1
BRCA 2	missense	c.8113A>G	p.(Ser2705Gly)	rs756105620	1
BRCA 2	deletion	c.1909+22delT	-	rs276174816	1
BRCA 2	missense	c.7598C>G	p.(Ser2533Cys)	rs80358987	1
BRCA 2	missense	c.6957A>C	p.(Arg2319Ser)	rs398122573	1
BRCA 2	missense	c.8482A>G	p.(Ile2828Val)	rs80359098	1
BRCA 2	missense	c.7928C>G	p.(Ala2643Gly)	rs80359018	1
BRCA 2	missense	c.7783G>T	p.(Ala2595Ser)	rs80359007	1
BRCA 2	missense	c.2924T>G	p.(Ile975Ser)	rs398122756	1
BRCA 2	missense	c.1277A>C	p.(Lys426Thr)	rs1421854019	1
BRCA 2	missense	c.5155A>T	p.(Asn1719Tyr)	rs398122794	1
BRCA 2	inframe/insertion	c.8588_8590dupAAG	p.(Glu2863dup)	rs730881616	1
BRCA 2	missense	c.9104A>C	p.(Tyr3035Ser)	rs80359165	1
BRIP1	missense	c.2477A>G	p.(Asn826Ser)	rs760127237	1
BRIP1	missense	c.1735C>T	p.(Arg579Cys)	rs28997571	1
BRIP1	missense	c.2624A>G	p.(Glu875Gly)	-	1
BRIP1	missense	c.2258A>G	p.(Asp753Gly)	rs745578572	1
BRIP1	missense	c.2441G>A	p.(Arg814His)	rs45468199	1
BRIP1	missense	c.679C>G	p.(Gln227Glu)	rs45459799	1
BRIP1	missense	c.139C>G	p.(Pro47Ala)	rs28903098	1
CHEK 2	single nucleotide variant	c.320-5T>A	-	rs121908700	1
CHEK 2	missense	c.520C>T	p.(Leu174Phe)	rs876659400	1

CHEK 2	missense	c.434G>A	(p.(Arg145Gln)	rs587781667	1
CHEK 2	missense	c.715G>A	p.(Glu239Lys)	rs121908702	2
CHEK 2	missense	c.1427C>T	p.(Thr476Met)	rs142763740	2
CHEK 2	missense	c.1228T>G	p.(Cys410Gly)	rs1555913689	1
MLH1	missense	c.96>G	p.(Ile32Met)	-	1
MLH1	missense	c.91_92delinsTG	p.(Ala31Cys)	rs63749994	1
MLH1	missense	c.1565G>A	p.(Arg522Gln)	rs63751630	1
MLH1	missense	c.1538T>C	p.(Ile513Thr)	rs876658689	1
MLH1	missense	c.1013A>G	p.(Asn338Ser)	rs63751467	1
MLH1	missense	c.1166G>A	p.(Arg389Gln)	rs63750361	1
MLH1	missense	c.1876T>C	p.(Phe626Leu)	rs377241633	1
MLH1	missense	c.439G>C	p.(Gly147Arg)	rs267607747	1
MSH2	missense	c.1159C>G	p.(Leu387Val)	rs751249745	1
MSH2	missense	c.980C>T	p.(Ala327Val)	rs1553353141	1
MSH2	missense	c.1130A> G	p.(Gln377Arg)	rs776174711	1
MSH2	missense	c.1408G>C	p.(Val470Leu)	-	1
MSH2	missense	c.1748A>G	p.(Asn583Ser)	rs201118107	1
MSH2	missense	c.20A>T	p.(Glu7Val)	rs530071578	1
MSH2	missense	c.939T>A	p.(Phe313Leu)	-	1
MSH2	missense	c.1787A>G	p.(Asn596Ser)	rs41295288	1
MSH6	missense	c.3788G>A	p.(Arg1263His)	-	1
MSH6	missense	c.3727A>T	p.(Thr1243Ser)	rs147453999	1
MSH6	missense	c.2281A> G	p.(Arg761Gly)	rs199876321	2
MSH6	missense	c.3299C>T	p.(Thr1100Met)	rs63750442	1
MSH6	synonymous	c.1677C>T	-	-	1
MSH6	missense	c.4004A>C	p.(Glu1335Ala)	rs564434147	1
MSH6	missense	c.2175C>G	p.(Ile725Met)	rs63750304	1
MSH6	missense	c.1894A>G	p.(Lys632Glu)	rs755847154	1

MSH6	missense	c.532C>T	p.(Arg178Cys)	rs730881813	1
MSH6	inframe/insertion	c.2640_2641insAAA	p.(Asp880_Gly881insLys)	rs1252374906	1
MSH6	missense	c.1497G>A	p.(Met499Ile)	-	1
MSH6	synonymous	c.1902G>A	p.(Leu634Leu)	rs1572724876	1
PALB2	missense	c.2816T>G	p.(Leu939Trp)	rs45478192	1
PALB2	missense	c.1241G>A	p.(Arg414Gln)	rs74946108	1
PALB2	missense	c.3251C>T	p.(Ser1084Leu)	rs62625271	1
PALB2	missense	c.2780A>C	p.(Asp927Ala)	-	2
PALB2	missense	c.833_834delinsAT	p.(Leu278His)	rs587778582	1
PALB2	missense	c.1837C>G	p.(Gln613Glu)	rs1966865214	1
PALB2	missense	c.2401G>A	p.(Asp801Asn)	rs587782765	1
PALB2	missense	c.2612A>G	p.(Asp871Gly)	rs515726090	1
PALB2	missense	c.13C>T	p.(Pro5Ser)	rs377085677	1
PALB2	missense	c.1013C>T	p.(Pro338Leu)	rs1316239336	1
PALB2	missense	c.3428T>A	p.(Leu1143His)	rs62625284	1
PALB2	missense	c.344G>T	p.(Gly115VaL)	rs145598272	1
PALB2	missense	c.194C>T	p.(Pro65Leu)	rs62625272	1
PALB2	missense	c.656A>G	p.(Asp219Gly)	rs45594034	2
RAD51C	missense	c.746G>A	p.(Arg249His)	rs730881925	1
RAD51C	missense	c.696A>T	p.(Glu232Asp)	rs786201177	1
RAD51C	missense	c.492T>G	p.(Phe164Leu)	rs573992101	1
RAD51C	missense	c.3G>A	p.(Met1Ile)	rs769053886	1
RAD51C	missense	c.904G>T	p.(Gly302Trp)	-	1
RAD51D	missense	c.137C>G	p.(Ser46Cys)	rs587780102	1
RAD51D	missense	c.823C>T	p.(Arg275Trp)	rs752780416	1
RAD51D	missense	c.824G>A	p.(Arg275Gln)	rs368914740	1
TP53	missense	c.1066G>C	p.(Gly356Arg)	rs766786605	1

TP53	-	-	-	-	1
TP53	missense	c.504C>G	p.(His168Gln)	-	1
TP53	missense	c.335G>A	p.(Gly112Asp)	-	1
TP53	missense	c.79C>A	p.(Pro27Thr)	rs922736614	1

Number: number of individuals with this variant

eTable 5: Differences in clinical characteristics between negative patients and High-risk PV (*BRCA1, BRCA2, PALB2, TP53* and *PTEN*) based on germline testing

	No. (%)		
Clinical Variable	**PV-Negative (n=783)**	**High-risk PV (n=92)**	**p value**
Sex			
Female (intercept)	774 (98.9%)	89 (96.7%)	.123
Male	9 (1.1%)	3 (3.3%)	
Age at diagnosis (median, range)	48.16 (24.44-88.55)	42.83 (23.27-73.65)	**.002**
Age at diagnosis (groups)			
≤ 50 years	445 (56.8%)	62 (67.4%)	.072
>50 years	338 (43.2%)	30 (32.6%)	
Menopausal status			
Premenopausal	431 (56.9%)	61 (68.5%)	**.047**
Postmenopausal	326 (43.1%)	28 (31.5%)	
Gene			
BRCA1		31 (33.7%)	
BRCA2		41 (44.6%)	
PALB2		13 (14.1%)	
TP53		5 (5.4%)	
PTEN		2 (2.2%)	
Race and ethnicity:			
White or European	738 (94.3%)	83 (90.2%)	.072
Hispanic or South or Central America	20 (2.6%)	2 (2.2%)	
Asian or Southeast Asian	12 (1.5%)	23 (3.3%)	
Middle Eastern or North Africa	3 (0.4%)	2 (2.2%)	
Black, African, or Caribbean	1 (0.1%)	1 (1.1%)	
Ashkenazi Jews	1 (0.1%)	0	
Criteria for genetic testing:			
Family aggregation	315 (40.2%)	28 (30.4%)	.066
Breast cancer ≤ 40 years	163 (20.8%)	26 (28.3%)	
TNBC < = 60 years	136 (17.4%)	26 (28.3%)	
Therapeutic Benefit	56 (7.2%)	3 (3.3%)	

Related tumors in the same patient	42 (5.3%)	4 (4.3%)	
≥o 2 breast cancer	31 (4.0%)	3 (3.3%)	
Not meet clinical criteria	16 (2.0%)	0	
Family no informative	15 (1.9%)	0	
Male Breast cancer	8 (1.0%)	2 (2.1%)	
Ashkenazi Jews	1 (0.1%)	0	
Family History*			
No	173 (22.3%)	17 (19.3%)	.619
Yes	604 (77.7%)	71 (80.7%)	
Personal Cancer History**			
No	689 (88.2%)	74 (80.4%)	**.049**
Yes	92 (11.8%)	18 (19.6%)	
Histology			
Invasive Ductal carcinoma	642 (85.9%)	75 (84.3%)	.810
Invasive Lobular carcinoma	52 (7.0%)	6 (6.7%)	
Others	53 (7.1%)	8 (9.0%)	
Grade of differentiation			
Low-grade (1 and 2)	425 (69.8%)	41 (55.4%)	**.018**
High grade (3)	184 (30.2%)	33 (44.6%)	
Stage at diagnosis			
I	286 (39.4%)	18 (22.2%)	.008
II	315 (43.4%)	43 (53.1%)	
III	82 (11.3%)	10 (12.3%)	
IV	43 (5.9%)	10 (12.3%)	
cT			
≤ 2 cm	323 (45.0%)	20 (27.0)	**.012**
2-5 cm	278 (38.7%)	38 (51.4%)	
> 5 cm	117 (16.3%)	16 (21.6%)	
cN			
Negative	472 (65.6%)	49 (64.5%)	.901
Positive	248 (34.4%)	27 (35.5%)	
IHC subtype***			
HR+/HER2-	489 (63.3%)	48 (52.2%)	**.002**
HR+/HER2+	83 (10.7%)	8 (8.7%)	
HR-/HER2+	40 (5.2%)	1 (1.1%)	
TNBC	161 (20.8%)	35 (38.0%)	
Bilateral Breast Cancer			
No	729 (94.2%)	77 (84.6%)	**.001**
Yes	45 (5.8%)	14 (15.4%)	
TNBC			
No	612 (79.2%)	57 (62.0%)	**<.001**
Yes	161 (20.8%)	35 (38.0%)	
Intrinsic subtypes (PAM50)			
LumA	148 (43.9%)	18 (34.6%)	**.031**
LumB	85 (25.2%)	12 (23.1%)	

HER2 enriched	53 (15.7%)	5 (9.6%)	
Basal	51 (15.1%)	17 (32.7%)	
Neoadjuvant chemotherapy			
No	397 (54.8%)	43 (51.2%)	.553
Yes	327 (45.2%)	41 (48.8%)	
pCR			
No	192 (61.9%)	24 (61.5%)	1.00
Yes	118 (38.1%)	15 (38.5%)	
Type of surgery			
Lumpectomy	419 (58.4%)	36 (43.9%)	**.008**
Mastectomy	281 (39.2%)	40 (48.8%)	
Bilateral Mastectomy	17 (2.4%)	6 (7.3%)	
Adjuvant chemotherapy			
No	442 (61.9%)	39 (48.8%)	**.031**
Yes	272 (38.1%)	41 (51.2%)	
Adjuvant hormonotherapy			
No	201 (27.5%)	37 (45.7)	**.001**
Yes	531 (72.5%)	44 (54.3)	
Carboplatin NA/A			
No	618 (88.4%)	63 (75.9%)	**.001**
Yes	81 (11.6%)	20 (24.1%)	
Radiotherapy			
No	149 (21.0%)	26 (32.5%)	**.019**
Yes	560 (79.0%)	54 (67.5%)	
Risk Reduction Mastectomy (RRM)			**<.001**
No	687 (95.2%)	49 (53.8%)	
Yes	35 (4.8%)	42 (46.2%)	
Risk Reduction Salpingo-oophorectomy (RRSO)			**<.001**
No	707 (97.7%)	48 (52.7%)	
Yes	17 (2.3%)	43 (47.3%)	
RRM + RRSO			**<.001**
No	719 (99.6%)	62 (68.1%)	
Yes	3 (0.4%)	29 (31.9%)	

Statistically significant results are highlighted in bold ($p < 0.05$).
PV: (likely) pathogenic variant, cT: tumor size at diagnosis, cN: node involvement at diagnosis, ER: estrogen receptor, TNBC: triple negative breast cancer, pCR, LumA, LumB
*Family cancer history: 1º, 2º and 3º degree relatives
**Personal Cancer History included the diagnosis of other cancer except a second breast cancer
***Family aggregation: Multiple relatives on the same side of the family with the same or related types of cancer (e.g., breast, ovarian, prostate, pancreatic cancer, colorectal cancer, melanoma, endometrial, and others).
****IHC: immunohistochemical subtype, HR: hormone receptor, HR +: estrogen receptor >= 1%, HR -: estrogen receptor <1%, HER2: Human Epidermal growth factor Receptor 2;

HER2 +: positive (IHC 3+ or FISH positive), TNBC: Triple Negative Breast Cancer (HR 1-10%)
High-risk PV: *BRCA1, BRCA2, PALB2, PTEN*, and *TP53*

eTable 6. Association of clinical variables with high-risk PVs-carriers.

Variables	Univariable analysis (OR, 95% CI)	p value	Multivariable analysis (OR, 95% CI) ^	p value	Adj p value^^
Age*	0.97 (0.95-0.99)	**.004**	0.95 (0.93-0.97)	<.001	**<.001**
Sex: male vs female	2.90 (0.63-9.92)	.115	4.53 (0.22-31.53)	.190	.201
Personal cancer history yes vs no	1.82 (1.02-3.12)	**.035**	2.33 (1.14-4.56)	.016	**.029**
Stage II vs stage I at diagnosis	2.16 (1.24-3.93)	**.008**	1.81 (1.00-3.40)	.055	.074
Stage III vs stage I at diagnosis	1.94 (0.83-4.29)	.109	1.84 (0.76-4.24)	.158	.204
Stage IV vs stage I at diagnosis	3.69 (1.55-8.40)	**.002**	4.35 (1.72-10.42)	.001	**.002**
Bilateral breast cancer yes vs no	2.95 (1.50-5.49)	**<.001**	3.83 (1.80-7.82)	<.001	**.001**
TNBC yes vs no	2.33 (1.47-3.66)	**<.001**	2.41 (1.43-4.02)	<.001	**.002**

Statistically significant results are highlighted in bold ($p < 0.05$). OR: odds ratio, CI: confidence of interval, PV: (likely) pathogenic variant, TNBC: Triple Negative Breast Cancer, Adj
High-risk PV: *BRCA1, BRCA2, PALB2, PTEN*, and *TP53*
^ Multivariate analysis were adjusted with age, sex, family history, personal cancer history, stage at diagnosis, bilateral breast cancer and TNBC subtype
^^Adjusted p-value were calculated using the false discovery rate (Benjamini-Hochberg) correction method.
* Age as a continuous variable

eTable 7: Differences in clinical characteristics between negative patients and Moderate-risk PV and Lynch syndrome-related genes (*ATM, BARD1, CHEK2, MSH2* and *RAD51C*) based on germline testing

	No. (%)		
Clinical Variable	**PV-Negative (n=783)**	**Moderate-risk PV (n=37)**	**p value**
Sex			
Female	774 (98.9%)	35 (94.6%)	.084
Male	9 (1.1%)	2 (5.4%)	
Age at diagnosis (median)	48.16 (24.44-88.55)	47.30 (33.07-74.13)	.633
Age at diagnosis (groups)			.870
≤ 50 years	445 (56.8%)	22 (59.5%)	
>50 years	338 (43.2%)	15 (40.5%)	
Menopausal status			.290
Premenopausal	431 (56.90%)	23 (67.6%)	
Postmenopausal	326 (43.10%)	11 (32.4%)	
Gene			
ATM		15 (40.5%)	
BARD1		2 (5.4%)	
CHEK2		17 (46.0%)	
MSH2		2 (5.4%)	
RAD51C		1 (2.7%)	
Race and ethnicity:			.850
White or European	738 (94.3%)	37 (100.0%)	
Hispanic or South or Central America	20 (2.6%)	0	
Asian or Southeast Asian	12 (1.5%)	0	
Middle Eastern or North Africa	3 (0.4%)	0	
Black, African, or Caribbean	1 (0.1%)	0	
Ashkenazi Jews	1 (0.1%)	0	
Criteria for genetic testing:			.071
Family aggregation	315 (40.2%)	14 (37.8%)	
Breast cancer ≤ 40 years	163 (20.8%)	10 (27.0%)	
TNBC < = 60 years	136 (17.4%)	0	
Therapeutic Benefit	56 (7.2%)	4 (10.8%)	
Related tumors in the same patient	38 (4.8%)	3 (8.1%)	
≥o 2 breast cancer	31 (4.0%)	2 (5.4%)	
Not meet clinical criteria	16 (2.0%)	2 (5.4%)	
Family no informative	15 (1.9%)	0	
Male Breast cancer	8 (1.0%)	2 (5.4%)	
Ashkenazi Jews	5 (0.6%)	0	
Family History*			.506
No	173 (22.3%)	6 (16.2%)	

Yes	604 (77.7%)	31 (83.8%)	
Personal Cancer History**			
No	689 (88.2%)	30 (81.1%)	.195
Yes	92 (11.8%)	7 (18.9%)	
Histology			
Invasive Ductal carcinoma	642 (85.9%)	31 (86.1%)	.418
Invasive Lobular carcinoma	52 (7.0%)	4 (11.1%)	
Others	53 (7.1%)	1 (2.8%)	
Grade of differentiation			.089
Low-grade (1 and 2)	425 (69.8%)	24 (85.7%)	
High grade (3)	184 (30.2%)	4 (14.3%)	
Stage at diagnosis			.209
I	286 (39.4%)	12 (35.3%)	
II	315 (43.4%)	15 (44.1%)	
III	82 (11.3%)	2 (5.9%)	
IV*	43 (5.9%)	5 (14.7%)	
cT			.559
≤ 2 cm	323 (45.0%)	17 (50.0%)	
2-5 cm	278 (38.7%)	14 (41.2%)	
> 5 cm	117 (16.3%)	3 (8.8%)	
cN			.161
Negative	472 (65.6%)	18 (52.9%)	
Positive	248 (34.4%)	16 (47.1%)	
IHC subtype*			**.027**
HR+/HER2-	489 (63.3%)	26 (70.3%)	
HR+/HER2+	83 (10.7%)	8 (21.6%)	
HR-/HER2+	40 (5.2%)	1 (2.7%)	
TNBC	161 (20.8%)	2 (5.4%)	
Bilateral Breast Cancer			.479
No	729 (94.2%)	34 (91.9%)	
Yes	45 (5.8%)	3 (8.1%)	
TNBC			**.019**
No	612 (79.2%)	35 (94.6%)	
Yes	161 (20.8%)	2 (5.4%)	
Intrinsic subtypes (PAM50)			.661
LumA	148 (43.9%)	10 (47.6%)	
LumB	85 (25.2%)	6 (28.6%)	
HER2 enriched	53 (15.7%)	4 (19.0%)	
Basal	51 (15.1%)	1 (4.8%)	
Neoadjuvant chemotherapy			.848
No	397 (54.8%)	18 (58.1%)	
Yes	327 (45.2%)	13 (41.9%)	
pCR			.384
No	192 (61.9%)	10 (70.6%)	
Yes	118 (38.1%)	3 (23.1%)	

Type of surgery			
Lumpectomy	419 (58.4%)	20 (64.5%)	.158
Mastectomy	281 (39.2%)	9 (29.0%)	
Bilateral Mastectomy	17 (2.4%)	2 (6.5%)	
Adjuvant chemotherapy			
No	442 (61.9%)	18 (58.1%)	.723
Yes	272 (38.1%)	13 (41.9%)	
Adjuvant hormonotherapy			
No	201 (27.5%)	3 (9.7%)	**.036**
Yes	531 (72.5%)	**28 (90.3%)**	
Carboplatin NA/A			
No	618 (88.4%)	31 (93.9%)	.574
Yes	81 (11.6%)	2 (6.1%)	
Radiotherapy			
No	149 (21.0%)	9 (27.3%)	.512
Yes	560 (79.0%)	24 (72.7%)	
Risk Reduction Mastectomy (RRM)			**<.001**
No	687 (95.2%)	29 (78.4%)	
Yes	35 (4.8%)	**8 (21.6%)**	
Risk Reduction Salpingo-oophorectomy (RRSO)			**.015**
No	707 (97.7%)	33 (89.2%)	
Yes	17 (2.3%)	**4 (10.8%)**	
RRM+ RRSO			1.00
No	719 (99.6%)	37 (100.0%)	
Yes	3 (0.4%)		

Statistically significant results are highlighted in bold ($p < 0.05$).
PV: (likely) pathogenic variant, cT: tumor size at diagnosis, cN: node involvement at diagnosis, ER: estrogen receptor, TNBC: triple negative breast cancer, N/A: not available (not calculable)
*Family cancer history: 1º, 2º and 3º degree relatives
**Personal Cancer History included the diagnosis of other cancer except a second breast cancer
***Family aggregation: Multiple relatives on the same side of the family with the same or related types of cancer (e.g., breast, ovarian, prostate, pancreatic cancer, colorectal cancer, melanoma, endometrial, and others).
****IHC: immunohistochemical subtype, HR: hormone receptor, HR +: estrogen receptor >= 1%, HR -: estrogen receptor <1%, HER2: Human Epidermal growth factor Receptor 2;
HER2 +: positive (IHC 3+ or FISH positive), TNBC: Triple Negative Breast Cancer (HR 1-10%)
High-risk PV: *BRCA1, BRCA2, PALB2, PTEN*, and *TP53*

eTable 8. Association of clinical variables with moderate-risk PVs-carriers

Variables	Univariable analysis (OR, 95% CI)	p value	Multivariable analysis (OR, 95% CI) ^	p value	Adj p value^^
Age*	0.99 (0.96-1.02)	.613	0.98 (0.95-1.01)	.273	.409
Sex:male vs female	4.91 (0.73-19.97)	**.047**	5.46 (0.69-29.88)	.066	.149
Personal Cancer History yes vs no	1.75 (0.69-3.88)	.198	1.99 (0.73-4.82)	.148	.267
Stage vs stage I at diagnosis II	1.13 (0.52-2.51)	.749	1.26 (0.57-2.85)	.570	.649
Stage III vs stage I at diagnosis	0.58 (0.09-2.19)	.483	0.65 (0.10-2.47)	.577	.649
Stage IV vs stage I at diagnosis	2.77 (0.85-7.88)	.067	3.11 (0.92-9.11)	**.048**	.149
Bilateral Breast Cancer yes vs no	1.43 (0.34-4.18)	.565	0.87 (0.14-3.06)	.849	.848
TNBC yes vs no	0.22 (0.04-0.72)	**.037**	0.25 (0.04-0.84)	.060	.149

Statistically significant results are highlighted in bold ($p < 0.05$). OR: odds ratio, CI: confidence of interval, PV: (likely) pathogenic variant

Moderate-risk: *ATM, CHEK2, BARD1, RAD51C, RAD51D*, and *MSH2*.

^ Multivariate analysis were adjusted with age, sex, family history, personal cancer history, stage at diagnosis, bilateral breast cancer and TNBC subtype

^^Adjusted p-value were calculated using the false discovery rate (Benjamini-Hochberg) correction method.

* Age as a continuous variable

TNBC: Triple Negative Breast Cancer

eTable 9: Multinomial analysis of the association between immunohistochemical (IHC) subtypes and PVs

IHC Subtype	Gene	Number	Multinomial analysis (OR, CI 95%)	p value
HER2 positive	Negative (Ref)	123	0.25 (0.21-0.31)	<.001
HER2 positive	*ATM*	4	1.45 (0.45-4.62)	.534
HER2 positive	*BRCA1*	N/A	N/A	N/A
HER2 positive	*BRCA2*	6	0.82 (0.33-2.03)	.671

HER2 positive	*CHEK2*	3	0.92 (0.26-3.27)	.894
HER2 positive	*PALB2*	N/A	N/A	N/A
HER2 positive	*TP53*	3	11.92(1.23-115.58)	**.032**
TNBC	Negative (Ref)	161	0.33 (0.28-0.39)	<.001
TNBC	*ATM*	N/A	N/A	N/A
TNBC	*BRCA1*	24	10.41 (4.40-24.62)	**<.001**
TNBC	*BRCA2*	6	0.63 (0.26-1.54)	.310
TNBC	*CHEK2*	1	0.23(0.03-1.80)	.163
TNBC	*PALB2*	4	1.35 (0.41-4.44)	.622
TNBC	*TP53*	1	3.04 (0.19-48.81)	.433

PVs: (likely) pathogenic variants, HR (Hormone Receptor), OR: odds ratio, CI: confidence of interval, Ref: reference

***BARD1, MSH2, PTEN* and *BARD1* were removed from the analysis due to the low number of patients (≤2)

***HR+/HER2- (Reference)

N/A: not available (not calculable)

eTable 10: Patients with early breast cancer (eBC) and candidates to adjuvant PARP inhibitor.

No (%)		
Eligibility criteria	**All patients eBC (n=841)**	**Candidates to PARPi (*BRCA1/2* PV)**
Triple Negative Breast Cancer	177 (21.0%)	26 (3.1%)
Following neoadjuvant chemotherapy, residual disease	44 (5.2%)	6 (0.7%)
Primary surgery, at least pT2N0 or pN1	32 (3.8%)	4 (0.4%)
ER+ HER2 - Breast cancer	484 (57.6%)	25 (3.0%)
Neoadjuvant chemotherapy with CPS + EG score >=3	6 (0.7%)	2 (0.2%)
Primary surgery, pN2	17 (2.0%)	1 (0.1%)
Total	99 (11.8%)	13 (1.6%)

eBC: early breast cancer; PARPi: PARP inhibitor. CPS + EG

Unpublished data: ***"Impact of BRCA2 pathogenic variants on outcomes to first-line CDK4/6 inhibitors plus endocrine therapy in HR+/HER2– metastatic breast cancer"***

Abstract:

Background: Currently, three cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) are approved in combination with endocrine therapy (ET) as first-line treatment for patients with hormone receptor–positive/HER2-negative (HR+/HER2-) metastatic breast cancer (MBC). The impact of homologous recombination repair (HRR) pathogenic variants (PV) on outcomes with first-line CDK4/6i plus ET in HR+/HER2–MBC remains uncertain.

Patients and methods: We conducted a multicenter, real-world, case–control study including 233 patients with HR+/HER2– MBC treated with first-line CDK4/6i and ET 116 with HRR PVs and 117 matched controls with negative germline testing). The primary objective was to compare progression-free survival (PFS) and overall survival (OS) among *BRCA2* PV carriers, other-HRR PV carriers, and controls. To minimize baseline differences in prognostic factors between PV carriers and controls, inverse probability of treatment weighting was applied. Molecular analyses in pre-CDK4/6i samples among patients with *BRCA2* PV were performed, including RAD51-foci, PAM50 intrinsic subtype, and RB1 loss of heterozygosity (LOH).

Results: The cohort included 231 females (99%); median age at diagnosis was 45 years (IQR 39–56) and 33% had de novo metastatic disease. Primary resistance to adjuvant ET according to ABC7 definition was present in 9.4% and secondary resistance in 24.5%. After a median follow-up of 44 months, patients with *BRCA2* PVs (n=74) had significantly shorter PFS (12 vs 27 months; adjusted hazard ratio (HR)2.11;95%CI1.42–3.12, p<0.001) compared with controls. Among patients with endocrine-sensitive disease, *BRCA2* PV carriers had markedly shorter PFS (median PFS 12 vs 38 months; HR 4.24;95% CI 2.54–7.07, p<0.001). Exploratory analyses revealed *RB1* LOH before CDK4/6i-treatment in most evaluable *BRCA2* tumors.

Conclusions: *BRCA2* PVs were independently associated with poorer outcomes to first-line CDK4/6i plus ET in HR+/HER2– MBC compared to controls, especially relevant among patients with endocrine-sensitive disease. These findings suggest that patients with a PV in *BRCA2* may require alternative first-line strategies.

Impact of *BRCA2* pathogenic variants on outcomes to first-line CDK4/6 inhibitors plus endocrine therapy in HR+/HER2– metastatic breast cancer

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Running Title: CDK4/6i in HRR Pathogenic Variant Carriers

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Tables: 1
Figures: 3

Highlights (3 to 5 highlights, 125 characters each one)

1. Patients with MBC and a *BRCA2* PV showed significantly poorer PFS on first-line CDK4/6i + ET than wild-type patients.
2. Patients with endocrine-sensitive BC and a *BRCA2* PV experienced reduced benefit from CDK4/6i+ET compared with wild-type patients
3. *BRCA2* germline/tumoral status may refine first-line treatment selection in HR+/HER2– metastatic breast cancer.

Abstract (300)

Background: Currently, three cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i) are approved in combination with endocrine therapy (ET) as first-line treatment for patients with hormone receptor–positive/HER2-negative (HR+/HER2-) metastatic breast cancer (MBC). The impact of homologous recombination repair (HRR) pathogenic variants (PV) on outcomes with first-line CDK4/6i plus ET in HR+/HER2–MBC remains uncertain.

Patients and methods: We conducted a multicenter, real-world, case–control study including 233 patients with HR+/HER2– MBC treated with first-line CDK4/6i and ET 116 with HRR PVs and 117 matched controls with negative germline testing). The primary objective was to compare progression-free survival (PFS) and overall survival (OS) among *BRCA2* PV carriers, other-HRR PV carriers, and controls. To minimize baseline differences in prognostic factors between PV carriers and controls, inverse probability of treatment weighting was applied. Molecular analyses in pre-CDK4/6i samples among patients with *BRCA2* PV were performed, including RAD51-foci, PAM50 intrinsic subtype, and *RB1* loss of heterozygosity (LOH).

Results: The cohort included 231 females (99%); median age at diagnosis was 45 years (IQR 39–56) and 33% had de novo metastatic disease. Primary resistance to adjuvant ET according to ABC7 definition was present in 9.4% and secondary resistance in 24.5%. After a median follow-up of 44 months, patients with *BRCA2* PVs (n=74) had significantly shorter PFS (12 vs 27 months; adjusted hazard ratio (HR)2.11;95%CI1.42–3.12,p<0.001) compared with controls. Among patients with endocrine-sensitive disease, *BRCA2* PV carriers had markedly shorter PFS (median PFS 12 vs 38 months; HR 4.24;95% CI 2.54–7.07,p<0.001). Exploratory analyses revealed *RB1* LOH before CDK4/6i-treatment in most evaluable *BRCA2* tumors.

Conclusions: *BRCA2* PVs were independently associated with poorer outcomes to first-line CDK4/6i plus ET in HR+/HER2– MBC compared to controls, especially relevant among patients with endocrine-sensitive disease. These findings suggest that patients with a PV in *BRCA2* may require alternative first-line strategies.

Background

The complexity in MBC therapeutic strategies has markedly increased in recent years[1,2]. Concurrently, the criteria for germline genetic testing in patients with BC have expanded substantially, driven by their implications for both prevention and treatment, particularly given the growing availability of targeted therapies such as poly (ADP-ribose) polymerase inhibitors (PARPi)[3-7]. Pathogenic or likely pathogenic variants (PV) in genes such as *BRCA1, BRCA2*, or *PALB2* are identified in approximately 4-5% of BC patients[3,8]. The prognostic and predictive impact of germline PV in these genes for targeted therapies (other than PARPi) among patients with hormone receptor–positive and human epidermal growth factor receptor 2–negative (HR+/HER2-) BC remains controversial, and data with the most recent therapies in the metastatic setting are scarce.

Currently, three cyclin-dependent kinase 4 and 6 inhibitors (CDK4/6i)—palbociclib, ribociclib, and abemaciclib— are approved by both the FDA and EMA in combination with endocrine therapy (ET) as first-line treatment for patients with HR+/HER2- metastatic BC (MBC). All three agents have demonstrated clinical benefit compared with ET monotherapy[9-15]. Nevertheless, the registration trials of CDK4/6i in the advanced setting did not assess the germline status of the enrolled population, precluding any subsequent evaluation of its potential influence on treatment outcomes.

Clinical outcomes of patients with MBC harboring germline or somatic PV in homologous recombination repair (HRR) genes and treated with first-line CDK4/6i plus ET have been evaluated in prior retrospective studies[16-20]. These studies have reported shorter progression-free survival (PFS) and overall survival (OS) in patients with a germline or somatic PV in *BRCA2* or other HRR genes treated with CDK4/6i in the metastatic setting[16-20]. These findings might be partially explained by the frequent development of *RB1* biallelic loss in *BRCA2*-

mutated tumors, a known mechanism of resistance to CDK4/6i[20], due to the close chromosomal proximity of the two genes and the selective pressure of treatment[20]. Conversely, a subanalysis of an invasive disease-free survival-events-enriched subpopulation within the monarchE trial demonstrated that patients with germline *BRCA1/2* PVs (n=41) derived greater benefit from adjuvant CDK4/6i plus ET compared with ET alone[21]. Considering the availability of other targeted options, such as PARPi, which have demonstrated significant PFS benefit in advanced BC, further clarification of this issue is warranted[22-25].

Given that CDK4/6i in combination with ET remains the standard first-line treatment for HR+/HER2- advanced BC[26], this study aims to evaluate, using real-world data, if patients harboring a germline or tumoral PV in an HRR-related gene experience worse outcomes compared with those without a germline PV in any HRR gene. Additionally, we investigate the resistance mechanisms by tumor genomics and gene expression.

Methods

This was a retrospective, multicenter, real-world case-control study that included patients diagnosed with advanced HR+/HER2- BC who received first-line CDK4/6i plus ET between January 2016 and July 2024. Patients who fulfilled the inclusion criteria outlined in **Supplementary Table 1** were eligible. Cases were defined as patients with a germline PV in an HRR gene (*ATM*, *BARD1*, *BRCA1*, *BRCA2*, *BRIP1*, *CHEK2*, *PALB2*, *RAD51C*, *RAD51D*) or a tumor PV in *BRCA1*, *BRCA2* or *PALB2* (germline testing results of these cases could be negative or unknown). Controls (wild-type group, WT) were defined as patients with a negative germline genetic test, including the analysis of the predefined HRR genes. Cases were recruited from 11 participating centers and the SOLTI-1903 HOPE BC trial (NCT04497285)[27], while controls were enrolled at Vall d'Hebron Hospital and Hospital Clinic of Barcelona. The study was approved by the institutional review board (IRB) at the coordinating center (Vall d'Hebron Hospital, IRB study code: PR(AG)130/2023) and by each participating center IRB.

The primary objective of the analysis was to compare PFS and OS between patients with a *BRCA2* PV to those with a PV in other HRR genes and to the wild-type (WT) group. Secondary objectives were to evaluate PFS and OS between patients with a PV in any HRR-related gene and the WT cohort. We also assessed the likelihood of identifying a *BRCA2* PV among endocrine-sensitive patients with short PFS (≤12 months), using previously published prevalence data as the reference **(Supplementary Table 2).**

Exploratory outcomes included the assessment of median PFS in patients treated with second-line PARPi following prior CDK4/6i + ET, and the characterization of pre- and post-treatment resistance mechanisms to CDK4/6i + ET through tumor sample analysis.

Clinical data were obtained from electronic medical records. Germline testing criteria were based on current guidelines[6]. Genetic testing and pathologic examination were performed locally. In this study, pathogenic and likely pathogenic variants were collectively referred to as PV. Variant classification was performed according to the guidelines of the American College of Medical Genetics and Genomics (ACMG) and the Association for Molecular Pathology (AMP)[28]. Hormone receptor status was assessed via immunohistochemistry (IHC), and HER2 status via IHC and fluorescence in situ hybridization (FISH). The definition of luminal not otherwise specified (NOS), luminal A-like and luminal B-like subtypes are based on established criteria from previous publications[29]. Primary and secondary endocrine resistance to adjuvant ET were defined according to the international consensus guidelines for the management of advanced breast cancer[30] (**Supplementary *Methods Section* 1.2**).

Molecular analysis of biomarkers of sensitivity and resistance were focused on: HRR status by RAD51 foci assay[31, 32], intrinsic subtype by PAM50 gene expression profiling[1, 33, 34], and loss of heterozygosity (LOH) of tumor *RB1* by sequencing[35]. These analyses were performed on pre-CDK4/6i samples with a *BRCA2* PV, with additional genomic sequencing and RAD51 foci

performed on post-treatment tissue to assess mechanisms of resistance and potential second-hit alterations. Further details regarding molecular data collection and sample processing are provided in **Supplementary *Methods Section 1.1*.**

Statistical Analysis

To minimize baseline differences in prognostic factors between PV carriers and controls, inverse probability of treatment weighting (IPTW) was applied using the Average Treatment effect on the Treated (ATT) approach, outcomes were estimated in the resulting weighted cohort[36]. Weighted covariate balance diagnostics are reported in **Table S3** (**Supplementary *Methods Section 1.2*).**

The Kaplan–Meier method was used to estimate PFS and OS. Hazard ratios (HR) were calculated using the Cox proportional hazards model and compared using the log-rank test. Missing data were handled using multiple imputations with the *mice* package in R. A sensitivity and robustness analysis was performed to compare the multivariable Cox models derived from the imputed and complete-case datasets (**Supplementary *Methods Section 1.3***). Statistical significance was set at $p<0.05$, using two-sided p-values. To estimate the probability that patients who experienced a PFS event carried a germline PV, an adjustment was applied to reflect the real-world prevalence of germline PV (**Supplementary *Methods Section 1.4***). All statistical analyses were conducted using R version 4.5.1

Results

Overall, 233 patients were included in the study (116 harboring a germline or tumor PV [cases] and 117 controls). The main characteristics of the study population are summarized in **Table 1.**

Within the cases group, 99 (84.6%) harbored a germline PV in an HRR-related gene. The most frequently mutated gene was *BRCA2* (n=63), followed by *BRCA1* (n=11), *PALB2* (n=8), *ATM*

(n=7), *CHEK2* (n=7), *RAD51C* (n=2) and *BRIP1* (n=1) (Table 1). Additionally, 17 patients were included based on the presence of a tumor PV (6 with confirmed negative germline testing and 11 with unknown germline status). Of these 17 patients, 11 patients had a tumor *BRCA2* PV, 3 had a tumor *BRCA1* PV and 3 had a tumor *PALB2* PV. Clinical characteristics of overall population, cases and controls are summarized in **Table 1** and clinical characteristics of patients with *BRCA2* PVs and controls are shown in **Supplementary Table 4.** After a median follow-up of 44 months, the median PFS was 12 months for patients with a *BRCA2* PV, 24 months for those with a PV in other HRR-related genes, and 27 months for controls. The HR for PFS was 2.46 (95% CI 1.67–3.62; p<0.001) for patients with a *BRCA2* PV, and1.04 (95% CI 0.68–1.60; p=0.800) for patients with a PV in other HRR-related genes compared with the control group (**Figure 1A).** Similarly, the median OS was shorter for patients with a *BRCA2* PV (44 months), compared with 55 months for patients with a PV in other HRR-related genes and not reached (NR) for controls. The HR for OS was 1.81 (95% CI 1.04–3.16; p=0.035) for patients with a *BRCA2* PV, and 1.42 (95% CI 0.71–2.83; p=0.300) for patients with a PV in other HRR-related genes compared with the control group **(Figure 1B).**

The univariable Cox analysis is provided in the **Supplementary Figure 1A and 1B**. The multivariable Cox analysis showed significantly worse PFS in patients with a PV in *BRCA2* (adjusted HR 2.11; 95% CI 1.42–3.12; p<0.001) and in those with endocrine-resistant disease to adjuvant therapy (adjusted HR 3.00; 95% CI 1.90–4.73; p<0.001) **(Figure 2A).** Additionally, a numerically worse OS was observed in patients harboring a *BRCA2* mutation (adjusted HR 1.73; 95% CI 0.95–3.15; p = 0.074). Independent predictors of shorter OS were endocrine-resistant disease to adjuvant therapy (HR 3.36; 95% CI 1.60–7.08; p = 0.001) and the presence of three or more metastatic sites (HR 2.16; 95% CI 1.18–3.95; p = 0.012), while premenopausal status was associated with a more favorable OS (HR 0.52; 95% CI 0.28–0.95; p = 0.035) **(Figure 2B).** We also included the multivariate PFS and OS-Cox Model between *BRCA2* PV

patients, other HRR-PV patients and controls without imputation data **(Figure S2A and S2B).** In the analysis comparing PFS and OS between patients with a HRR PV and the controls, patients with a HRR PV experienced shorter PFS (median PFS: 16 vs 27 months; HR 1.70; 95% CI 1.22-2.30), although OS was not statistically significantly different (median OS: NR vs 47 months; HR 1.66; 95% CI 0.98-2.80) **(Figure S3A and S3B**). The univariate and multivariate analysis of PFS and OS between patients with a HRR PV and controls are shown in the **Supplementary Figure S4.** In an exploratory analysis limited to patients carrying a germline PV (in *BRCA2* or in another HRR-related genes), we observed significantly worse PFS and OS among carriers of a germline PV in *BRCA2* **(Figure S5A and S5B)** The univariate and multivariate analysis of PFS and OS between germline *BRCA2* PV patients, other HRR PV patients and controls are shown in the **Supplementary Figure S6.**

We explored differences in median PFS across the three main groups (*BRCA2* PV, germline/ tumoral (g/t) PV in other HRR-related genes, and controls) stratifying by endocrine-sensitive disease to prior adjuvant therapy. Patients with endocrine-resistant disease showed poor PFS in all groups **(Figure 3A)**. However, among endocrine-sensitive patients, those with a *BRCA2* PV had significantly shorter PFS than controls (12 months vs 38 months; HR 4.24; 95% CI 2.54–7.07; $p<0.001$) **(Figure 3B).**

We then focused on assessing the impact of endocrine sensitivity among patients with a *BRCA2* PV. Interestingly, no differences in median PFS nor OS were observed according to endocrine sensitivity in this subgroup **(Figure S7A, S7B),** whereas a statistically significant difference was observed among patients without a *BRCA2* PV **(Figure S7C, S7D).**

To further explore treatment outcomes beyond first-line therapy, we analyzed the median PFS of patients who received PARPi as second-line treatment (n=26). The median PFS was 11

months (95% CI 6.9–NR), and at 36 months of follow-up, 15% of the patients remained on PARPi **(Figure S8).**

The estimated probability of carrying a *BRCA2* PV among endocrine-sensitive patients experiencing disease progression within ≤12 months was 2.3% **(Table S5).** The detection of a *BRCA2* PV in endocrine-sensitive patients with disease progression within ≤6 months had a sensitivity of 14% and a specificity of 82.5% **(Table S6).** The ROC curve yielded an AUC of 0.57 **(Figure S9).**

We identified 22 patients with a *BRCA2* PV (19 germline, 3 tumoral) and metastatic BC with either a pre-CDK4/6i archival tumor sample (n=19), a post-CDK4/6i (n=8), or both (n=5) **(Table S7).** Clinical characteristics are described in **Table S8.** Due to the heterogeneity in tissue availability, not all pre-planned analyses were performed across all samples.

Among the 13 pretreatment samples from *BRCA2* PV carriers with sufficient tissue for RAD51 assay, five were non-evaluable due to technical constraints. Of the eight evaluable cases, we identified five samples with HR deficiency (HRD), and three with HR proficiency (HRP). RAD51 foci values ranged from 0% to 7% in the HRD group and from 15% to 43% in the HRP group. Median PFS did not differ significantly between these groups: 10 months (95% CI 9.0–NR) in HRD and 9 months (95% CI 8.1–NR) in HRP (HR 1.85; 95% CI 0.37–9.28; p=0.500). Only one patient had RAD51 data available at both pre- and post-treatment timepoints; this patient was HRP at baseline and remained HRP post-CDK4/6i, with a PFS of 11 months **(Figure S10).**

PAM50 intrinsic subtype analysis from *BRCA2* PV carriers was performed on 15 available pre-CDK4/6i samples; one was non-evaluable. Among the other 14, the subtype distribution included one luminal A tumor (PFS 11 months), three luminal B (median PFS 13 months; 95% CI 10–NR), two HER2-enriched (PFS 20 months; 95% CI 9.0–NR), three basal-like (PFS 12

401 months; 95% CI 9.0–NR) and five normal (median PFS 22 months; 95% CI 13–NR). No
402 statistically significant association was found between intrinsic subtype and PFS (p>0.05)
403 **(Figure S10).**

404 *RB1* LOH was evaluable in pre-CDK4/6i samples from 6 patients with *BRCA2* PV. Four
405 patients had LOH in *RB1* (median PFS 11 months, 95% CI). One patient had an equivocal *RB1*
406 status (PFS 30 months), and one had no *RB1* LOH (PFS 47 months). No significant association
407 was observed between *RB1* LOH status and PFS in this cohort (p>0.05) **(Figure S11);**
408 however, this result may be limited by the small sample size and lack of statistical power.

409 Post-CDK4/6i tumor sequencing was performed in 2 samples. Both cases had a tumoral *BRCA2*
410 PV with a variant allele fraction over 50% and concordant with their germline status, and both
411 had a mutation in *GATA3*. No definite *RB1* second-hit mutations were identified, but the small
412 number of cases precludes definitive conclusions.

413 **Discussion**

414 We conducted a multicenter, real-world case–control study including 116 patients with MBC
415 harboring HRR PVs and treated with first-line CDK4/6i and ET. Notably, we observed
416 differences in survival outcomes, both PFS and OS, among carriers of a *BRCA2* PV. After a
417 median follow-up of 44 months, patients with a *BRCA2* PV had significantly shorter PFS in
418 the multivariable analysis (HR 2.11; p<0.001). No significant differences in outcomes were
419 observed between patients with non-*BRCA2* HRR PV and controls. Sensitivity analyses
420 restricted to patients with germline PVs yielded similar results, further supporting the adverse
421 impact of *BRCA2* PVs.

422 Our study reinforces previous findings reporting that patients with a *BRCA2* PV and HR+,
423 HER2- MBC have worse outcomes in first-line treatment with CDK4/6i plus ET. This
424 observation aligns with several retrospective cohorts and two recent meta-analyses[16-20, 37, 38].

To our knowledge, our study is one of the largest multicenter series restricted to the first-line setting, thereby minimizing confounding from prior therapies and treatment heterogeneity. We also incorporated a matched control group with confirmed negative germline testing, reducing the risk of misclassification bias that may have affected earlier reports. Unlike other previous studies that pooled HRR-related genes, our analysis isolates *BRCA2*, demonstrating that the adverse outcomes are specifically driven by *BRCA2* PVs. Additional evidence supporting this finding has recently been reported. In a comprehensive analysis, Safonov et al. examined outcomes of 533 patients treated with first-line CDK4/6i and ET, including 105 *BRCA1* and 127 *BRCA2* germline PV carriers. *BRCA2* carriers experienced significantly shorter PFS (HR 2.28)[20]. These poor outcomes were consistent across therapy lines[20]. Similarly, a recently published meta-analysis including data from 14 studies with a total of 618 patients with a germline *BRCA1/2* PV reached similar conclusions to our findings, reporting a HR for PFS of 1.68 and a HR for OS of 1.73 comparing germline *BRCA1/2* PV carriers with wild-type counterparts. Only one study within the meta-analysis evaluated *BRCA1* and *BRCA2* carriers separately; in that study, *BRCA1* carriers did not show statistically significant differences in clinical outcomes[17]. Additionally, Azim et al. reported similarly poor clinical outcomes in both the adjuvant and metastatic settings among patients with germline *BRCA1/2* PVs treated with CDK4/6i[16].

In our cohort, the presence of a *BRCA2* PV and endocrine resistance to adjuvant therapy were both independently associated with shorter PFS, consistent with previous reports[39]. Surprisingly, among *BRCA2* carriers, PFS did not differ significantly between the endocrine-sensitive and endocrine-resistant subgroups. Importantly, within the endocrine-sensitive subset, patients with a *BRCA2* PV experienced significantly shorter PFS compared with controls (12 vs 38 months; $p<0.001$), reinforcing the adverse impact of *BRCA2* PVs regardless

of endocrine sensitivity. These results support the concept of an intrinsic resistance to CDK4/6i among *BRCA2* PV carriers.

Of note, among cases, 26 patients received subsequent PARPi, with a median PFS of 11 months, which is comparable to the 10 months reported in the OlympiAD trial for HR+/HER2− tumors[40,41]. This suggests that PARPi may remain effective after CDK4/6i, likely due to distinct resistance mechanisms.

Regarding mechanisms of resistance to CDK4/6i, biallelic *RB1* loss—a known CDK4/6i resistance pathway—has been reported in tumors from *BRCA2* carriers[16,20]. This may reflect a shared vulnerability, as *BRCA2*-mutated tumors are more likely to harbor *RB1* LOH, likely due to their genomic proximity[16,20]. In addition, HR-deficient tumors are predisposed to acquiring *RB1* loss-of-function mutations, which may further contribute to resistance to CDK4/6 inhibition[26]. In our cohort, *RB1* LOH was observed in 4 out of 5 evaluable samples. Although *RB1* LOH was not statistically associated with PFS, likely due to the small sample size, the median PFS was 11 months in patients with LOH compared with 47 months in the single patient without *RB1* LOH. Similarly, no statistically significant association was found between intrinsic subtypes and PFS. However, patients with basal-like and HER2-enriched subtypes tended to have numerically shorter PFS, consistent with prior observations of CDK4/6i resistance in these subtypes[33, 34, 42]. However, the small cohort size, heterogeneous tissue availability, and incomplete assay coverage across samples limit statistical power and the interpretability of these exploratory analyses.

Our findings indicate that early progression on first-line CDK4/6i therapy among endocrine-sensitive patients may be associated with the presence of a germline *BRCA2* PV. Therefore, early progression on CDK4/6i despite apparent endocrine sensitivity may serve as a practical clinical indicator to prompt germline genetic testing in patients with HR+/HER2– MBC.

Identifying these variants has potential implications for subsequent targeted therapies and familial risk assessment.

Despite the strengths of this study, including relatively large, clinically well-annotated cohorts, several limitations should be acknowledged. The retrospective design and selection bias, with patients referred for testing based on clinical criteria, may have influenced some of the observed associations. The younger age of cases compared with controls likely reflects referral and selection bias, as younger patients are more often tested for germline variants[43,44]; however, this approach was preferred over including individuals with unknown genetic status, which could have introduced misclassification bias. The limited number of PVs in genes other than *BRCA2* restricted our ability to draw firm conclusions regarding their predictive impact; however, *BRCA2* PVs represent the most frequent germline HRR alterations in HR+/HER2– tumors, which underscores the clinical relevance of the observed associations[45,46]. Germline testing status and family history were missing in 11 cases with tumoral PVs, precluding the origin of the PV. Similarly, tumoral testing excluding a somatic PV in *BRCA1/2* was not required for control selection. Finally, mechanistic exploration of resistance was limited by the small number and high heterogeneity of tumor samples, precluding correlation analyses.

Currently, patients with ER+ MBC are treated with first-line CDK4/6i and ET[2,26,47], but there are no data from phase III randomized trials specifically addressing outcomes in carriers of a HRR gene PV.

Our findings are in line with prior evidence [16-20] and suggest that in *BRCA2* carriers, the most appropriate treatment and sequencing strategy in the metastatic setting may need to be further evaluated. One potential approach would be initiating treatment with a PARPi and reserving ET+CDK4/6i for second line, particularly in patients who acquire reversion mutations and restore *BRCA2* function, therefore turning into HRP. One may hypothesize that in these cases,

the presence of *RB1* LOH might be less prevalent. Prospective studies evaluating the optimal sequencing of PARPi and CDK4/6i, particularly in patients with *BRCA2* PVs and HR+, HER2- MBC, are essential to refine treatment strategies for this population. In this regard, an ongoing phase III clinical trial (NCT06380751) is assessing in first-line the combination of a second-generation SERD with CDK4/6i, versus SERD with PARPi, versus standard ET with CDK4/6i, in patients with a *BRCA1/2*, or *PALB2* gene PV.

To conclude, our study provides real-world evidence that *BRCA2* PV, whether germline or tumoral, are independently associated with poor clinical outcomes in patients with HR+/HER2− MBC treated with first-line CDK4/6i plus ET. These findings highlight the need to develop novel therapeutic approaches and to refine treatment sequencing for this subgroup and reinforce the importance of comprehensive genetic characterization to guide personalized treatment decisions.

Data availability statement: The data supporting the findings of this study are not publicly available due to patient confidentiality and institutional restrictions. De-identified data may be made available upon reasonable request to the corresponding author, subject to institutional approval and applicable data-sharing agreements.

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Table

Table 1. Clinical characteristics of cases, controls, and the overall study cohort.

Table 1.			
Characteristic	**Overall**	**Cases**	**Controls**
	N = 233[1]	n= 116[1]	n = 117[1]
Sex assigned at birth			
Female	231 (99%)	114 (98%)	0 (0%)
Male	2 (1%)	2 (2%)	117 (100%)
Age at BC diagnosis			
Mean (SD)	49 (12)	46 (12)	51 (12)
Median (Q1, Q3)	45 (39, 56)	45 (38, 52)	48 (41, 60)
Min, Max	26, 79	26, 78	27, 79
Pre/postmenopausal at diagnosis			
Premenopausal	125 (55%)	79 (70%)	46 (40%)
Postmenopausal	103 (45%)	34 (30%)	69 (60%)

Not applicable	2	2	0
Missing	3	1	2
Endocrine resistance[3]			
No	154 (66%)	74 (64%)	80 (68%)
Primary	22 (10%)	8 (7%)	14 (12%)
Secondary	57 (24%)	34 (29%)	23 (20%)
Histological grade			
1	15 (11%)	5 (8%)	10 (13%)
2	97 (69%)	42 (66%)	55 (71%)
3	29 (20%)	17 (26%)	12 (16%)
Missing	92	52	40
Subtype according to IHQ			
Luminal A	57 (25%)	20 (17%)	37 (35%)
Luminal B	154 (70%)	89 (77%)	65 (62%)
Luminal NOS	10 (5%)	7 (6%)	3 (3%)
Missing	12	0	12
Metastatic de novo			
No	155 (67%)	76 (66%)	79 (68%)
Yes	78 (33%)	40 (34%)	38 (32%)
Only bone metastasis			
No	149 (66%)	70 (64%)	79 (68%)
Yes	77 (34%)	39 (36%)	38 (32%)
Missing	7	7	0
Visceral metastasis[4]			
No	131 (56%)	64 (55%)	67 (57%)
Yes	102 (44%)	52 (45%)	50 (43%)
3 or more metastatic sites			
No	153 (70%)	63 (62%)	90 (77%)
Yes	66 (30%)	39 (38%)	27 (23%)
Missing	14	14	0
Type of CDK4/6i			
Abemaciclib	34 (15%)	16 (14%)	18 (15%)
Palbociclib	86 (37%)	50 (43%)	36 (31%)
Ribociclib	113 (48%)	50 (43%)	63 (54%)
PARPi after CDK4/6i			
No	143 (76%)	52 (54%)	91 (99%)
Yes	46 (24%)	45 (46%)	1 (1%)
Missing	44	19	25
Germline genetic test result			
Negative	123 (55%)	6 (6%)	117 (100%)
Positive	99 (45%)	99 (94%)	0 (0%)
Missing	11	11	0
Year germline genetic test			
2020	24 (10%)	16 (14%)	8 (6%)

2021	48 (21%)	19 (16%)	29 (25%)
2022	31 (13%)	14 (12%)	17 (15%)
2023	32 (14%)	9 (8%)	23 (20%)
2024	16 (7%)	5 (4%)	11 (9%)
before 2019	82 (35%)	53 (46%)	29 (25%)
gen_germline			
ATM	7 (7%)	7 (7%)	NA
BRCA1	11 (11%)	11 (11%)	NA
BRCA2	63 (64%)	63 (64%)	NA
BRIP1	1 (1%)	1 (1%)	NA
CHEK2	7 (7%)	7 (7%)	NA
PALB2	8 (8%)	8 (8%)	NA
RAD51C	2 (2%)	2 (2%)	NA
gen_tumor			
BRCA1	3 (18%)	3 (18%)	NA
BRCA2	11 (64%)	11 (64%)	NA
PALB2	3 (18%)	3 (18%)	NA
[1] n (%)			
[2] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test			
[3] Endocrine resistant to adjuvant therapy is defined as disease progression occurring during or within 12 months after completing adjuvant endocrine therapy based on ABC7 guidelines (primary if disease progression occurring within first two years and secondary occurring after two years of adjuvant therapy but within 12 months of completion).			
[4] Visceral metastasis including Central Nervous System involvement (N=1)			
BC: breast cancer; IHC: immunohistochemistry; PARPi: PARP inhibitor treatment; CDK4/6i: CDK4/6 inhibitor treatment; gen_germline: gen detected in the germline testing; gen_tumor: gen detected tumor DNA analysis			

Figures Legends:

Figure 1. (A) Progression-free survival and (B) overall survival analysis between patients with a g/t *BRCA2* PV, patients with a PV in other HRR-related genes and controls.

PV: pathogenic or likely pathogenic variant; HRR: Homologous Recombination Repair; HR: Hazard ratio; NR: Not reach; other mut: other germline or tumoral (g/t) pathogenic variants in other HRR genes; W at risk: weighted sample size at risk.

Figure 2. Multivariate PFS Cox model (A) and multivariate OS Cox model (B) of overall population and all subgroup analysis.

PFS: progression free survival; OS: overall survival

Figure 3. Kaplan-Meier estimates of progression-free survival comparing those with a g/t *BRCA2* PV, those with a PV in another HRR-related gene, and controls in (A) patients with endocrine-resistant disease and (B) patients with endocrine-sensitive disease

PV: pathogenic or likely pathogenic variant; HRR: Homologous Recombination Repair; HR: Hazard ratio; NR: Not reach; other mut: other germline or tumoral (g/t) pathogenic variants in other HRR genes; W at risk: weighted sample size at risk.

Supplementary methods

1.1 Clinical and Molecular Data Collection

Tumor purity, defined as the proportion of tumor cells relative to the total number of cells in a sample, was evaluated to assess sample quality. According to institutional standards, a minimum cellularity of 30% was required. Tumor sequencing was performed using the VHIO-300 panel on DNA extracted from formalin-fixed, paraffin-embedded (FFPE) samples (reference pending). DNA was fragmented using a Covaris M220 ultrasonicator, followed by hybrid capture–based targeted sequencing with the ISO-accredited VHIO-300 panel, which includes 435 exonic genes. Libraries were prepared with the SureSelect XT Human system (Agilent) and sequenced on an Illumina HiSeq2500 platform (2 × 100 bp paired-end). This approach also enabled assessment of RB1 loss of heterozygosity (LOH). RAD51 nuclear foci and γH2AX were manually stained and quantified in 14 pretreatment samples using an immunofluorescence assay, as previously described. Functional HRD was predefined as a RAD51 score ≤10% (RAD51-low)[1, 2]. All RAD51 nuclear foci were evaluated blinded and centrally at VHIO. RNA was extracted from FFPE tumor samples using the High Pure FFPET RNA Isolation Kit (Roche). PAM50 subtype classification was determined in 20 pretreatment patient samples from individuals receiving CDK4/6 inhibitors + ET using the nCounter platform at the Translational Genomics and Targeted Therapies in Solid Tumors laboratory (Institut d'Investigacions Biomèdiques August Pi i Sunyer). Intrinsic subtypes were assigned based on the PAM50 predictor algorithm described by Parker et al[3].

1.2 Statistical Analysis

Inverse probability of treatment weighting (IPTW) accounts for potential biases in observational studies. Weights were estimated using the average treatment effect on the treated (ATT) method,

incorporating the following variables: type of CDK4/6 inhibitor (abemaciclib, palbociclib, or ribociclib), year of germline testing, presence of visceral metastases (including central nervous system involvement), de novo metastatic disease, and endocrine resistance to adjuvant therapy. Endocrine resistance was defined as primary endocrine resistance, when disease progression occurred within the first two years of adjuvant ET, and secondary endocrine resistance, when progression occurred while on adjuvant ET but after the first two years or within 12 months of completing adjuvant ET[4, 5]. Age at breast cancer diagnosis was also included in the model.

1.3 Handling of missing data

Missing data were handled using the *mice* (Multiple Imputation by Chained Equations) package in R. This method generates multiple complete datasets by imputing missing values through chained regression models, where each variable with missing data is imputed conditionally on the others. The imputation process preserves the multivariable relationships among variables and reduces potential bias associated with complete-case analyses. For the main analysis, one of the imputed datasets was used to fit the multivariable Cox proportional hazards models. A complete-case analysis was also performed as a sensitivity and robustness assessment, showing consistent results with the imputed models **(Figure S8A and Figure S8B)**.

1.4 Predictive analysis

To estimate the probability that patients who experienced a PFS event carried a germline PV, an adjustment was made to reflect the real-world prevalence of germline PV. First, the probability of experiencing a PFS event was calculated separately for germline PV-carriers and non-carriers using survival estimates. This was done by subtracting the survival probability from one for each group. To account for the lower prevalence of germline PV in the general population compared to

this enriched cohort, weighted probabilities were applied, multiplying the event probabilities by the estimated real-world prevalence of *BRCA2* PVs in women with breast cancer (1.5%)[6, 7]. The same approach was used for the control group, adjusting for the complementary prevalence. The total adjusted probability was obtained by summing the weighted probabilities for both groups. Finally, the probability of a patient with a PFS event being a germline PV carrier was derived by dividing the adjusted probability for mutation carriers by the total adjusted probability. This method provides a more accurate estimate of the likelihood of germline PV or *BRCA2* PV among patients with disease progression while correcting for their lower prevalence in the broader breast cancer population.

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Supplementary figures legends:

Figure S1 (S1A and S1B). Univariate Cox analysis of PFS (A) and OS (B) between *BRCA2* PV patients, other HRR-PV patients and WT patients.

PFS: progression free survival; OS: overall survival; PV: pathogenic variant, WT: wild-type

Figure S2 (S2A and S2B). Comparison of Multivariable Cox Models without imputation data for PFS (A) and OS (B)

PFS: progression free survival; OS: overall survival; PV: pathogenic variant, WT: wild-type

Figure S3 (S3A and S3B). Kaplan–Meier estimates of PFS (A) and OS (B) between HRR-PV patients (without distinguishing *BRCA2*) named as cases and controls.

PFS: progression free survival; OS: overall survival; HRR: homologous recombination repair; PV: pathogenic variant

Figure S4 (S4A, S4B, S4C and S4D). Univariate and Multivariate Cox analysis of PFS (A and C) and OS (B and D) between HRR-PV patients and WT patients.

PFS: progression free survival; OS: overall survival; HRR: homologous recombination repair; PV: pathogenic variant; WT: wild-type

Figure S5 (S5A and S5B). Exploratory analysis Kaplan–Meier estimates of PFS (A) and OS (B) between germline *BRCA2* PV patients, germline PV patients in other genes, and controls.

PFS: progression free survival; OS: overall survival; PV: pathogenic variant

Figure S6 (S6A, S6B, S6C and S6D). Univariate and multivariate Cox analysis of PFS (A and C) and OS (B and D) between germline *BRCA2* PV patients, patients with germline PV in other HRR genes, and controls.

PFS: progression free survival; OS: overall survival; HRR: homologous recombination repair; PV: pathogenic variant

Figure S7 (S7A, S7B, S7C and S7D). Kaplan–Meier estimates of PFS and OS stratified by endocrine-sensitive versus endocrine-resistant disease to adjuvant endocrine therapy: patients with germline or tumoral *BRCA2* PV (A and B) and patients without germline or tumoral *BRCA2* PV (C and D).

PFS: progression free survival; OS: overall survival; PV: pathogenic variant

Figure S8. PFS with second-line PARP inhibitor treatment in patients carrying a germline PV in *BRCA1* or *BRCA2*.

PFS: progression free survival; PV: pathogenic variant

Figure S9. The estimated probability of carrying a PV in *BRCA2* among endocrine-sensitive patients according to PFS

PV: pathogenic variant; PFS: progression free survival

Figure S10. PFS (in months) according to intrinsic subtype (PAM50) and HRD by RAD51foci in patients with *BRCA2* PV

PFS: progression free survival; HRD: homologous recombination deficiency; PV: pathogenic variant

Figure S11. PFS according to LOH of *Rb1* in patients with *BRCA2* PV

PFS: progression free survival; LOH: loss of heterozygosity; PV: pathogenic variant

Supplementary tables

Table S1. Inclusion and exclusion criteria

Table S2. Prevalence of germline *BRCA2* PV across breast cancer populations

PV: pathogenic variant

Table S3. Weighted covariate balance diagnostics

Table S4. Clinical characteristics of patients with *BRCA2* PV, patients with PV in other HRR genes and controls

PV: pathogenic variant; HRR: homologous recombination repair

Table S5. The estimated probability of carrying a *BRCA2* PV among endocrine-sensitive patients experiencing disease progression within ≤6 months and ≤12 months

PV: pathogenic variant

Table S6. Sensitivity and specificity metrics for *BRCA2* PV detection in patients progressing ≤6 months

PV: pathogenic variant

Table S7. Overview of analyzed tissue samples (n=22) and corresponding assays performed

Table S8. Clinical characteristics of patients included in the tissue analysis

A

Univariate PFS-Cox Model

Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Mutation				
Control	115		Reference	
BRCA2	71		2.46 (1.67, 3.63)	**<0.001**
Other mut	42		1.07 (0.69, 1.64)	0.771
Menopausal status				
Postmenopausal	103		Reference	
Premenopausal	125		1.18 (0.84, 1.65)	0.343
Endocrine resistance				
No	151		Reference	
Yes	77		2.35 (1.66, 3.34)	**<0.001**
Subtype				
Lumil A	58		Reference	
Lumil B HER2 negative	158		1.77 (1.12, 2.80)	**0.015**
Lumil NOS	12		0.76 (0.39, 1.48)	0.419
Metastasis novo				
No	151		Reference	
Yes	77		0.86 (0.62, 1.20)	0.383
Visceral metastasis				
No	129		Reference	
Yes	99		1.24 (0.88, 1.74)	0.217
Only bone metastaisis				
No	146		Reference	
Yes	82		0.91 (0.63, 1.32)	0.633
Age				
0–40	67		Reference	
41–60	119		1.14 (0.77, 1.70)	0.510
over 60	42		0.84 (0.48, 1.48)	0.555
Site metastasis >3				
No	160		Reference	
Yes	68		1.25 (0.88, 1.79)	0.211

B

Univariate OS-Cox Model

Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Mutation				
Control	110		Reference	
BRCA2	65		1.92 (1.10, 3.35)	**0.021**
Other mut	41		1.36 (0.67, 2.77)	0.390
Menopausal status				
Postmenopausal	97		Reference	
Premenopausal	119		0.74 (0.44, 1.25)	0.263
Endocrine resistance				
No	141		Reference	
Yes	75		2.49 (1.49, 4.16)	**<0.001**
Subtype				
Lumil A	58		Reference	
Lumil B HER2 negative	158		1.27 (0.67, 2.42)	0.460
Metastasis novo				
No	140		Reference	
Yes	76		0.47 (0.26, 0.84)	**0.011**
Visceral metastasis				
No	123		Reference	
Yes	93		1.62 (0.96, 2.72)	**0.071**
Only bone metastaisis				
No	136		Reference	
Yes	80		0.51 (0.29, 0.90)	**0.021**
Age				
0–40	63		Reference	
41–60	112		1.40 (0.76, 2.57)	0.287
over 60	41		1.45 (0.66, 3.18)	0.351
Site metastasis >3				
No	154		Reference	
Yes	62		2.06 (1.23, 3.46)	**0.006**

A

Multivariate PFS-Cox Model without imputation

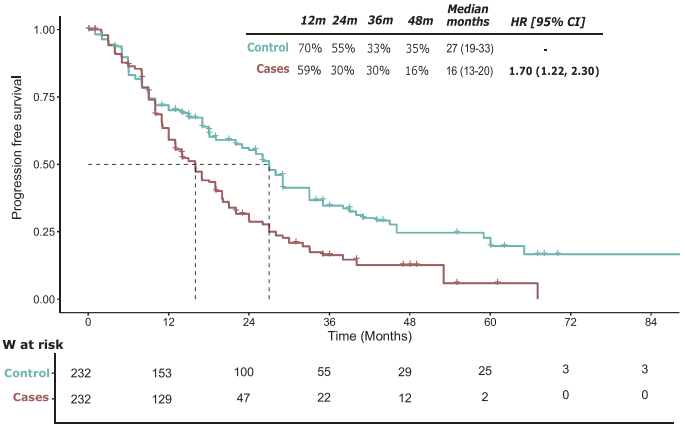
Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Mutations				
Control	103		Reference	
BRCA2	62		2.04 (1.32, 3.16)	**0.001**
Other mut	37		0.89 (0.54, 1.47)	0.659
Menopausal status				
Postmenopausal	94		Reference	
Premenopausal	108		0.76 (0.49, 1.17)	0.211
Endocrine resistance				
No	133		Reference	
Yes	69		3.42 (2.09, 5.60)	**<0.001**
Subtype				
Lumil A	54		Reference	
Lumil B HER2 negative	143		1.35 (0.82, 2.23)	0.244
Lumil NOS	5		0.50 (0.17, 1.48)	0.213
Metastasis novo				
No	129		Reference	
Yes	73		1.65 (1.01, 2.70)	0.047
Visceral metastasis				
No	117		Reference	
Yes	85		0.84 (0.53, 1.35)	0.478
Bone metastasis				
No	126		Reference	
Yes	76		0.82 (0.51, 1.32)	0.421
Age				
0–40	57		Reference	
41–60	104		0.98 (0.65, 1.47)	0.907
over 60	41		0.66 (0.33, 1.33)	0.245
Sites metastasis >3				
No	145		Reference	
Yes	57		1.44 (0.93, 2.23)	0.105

B

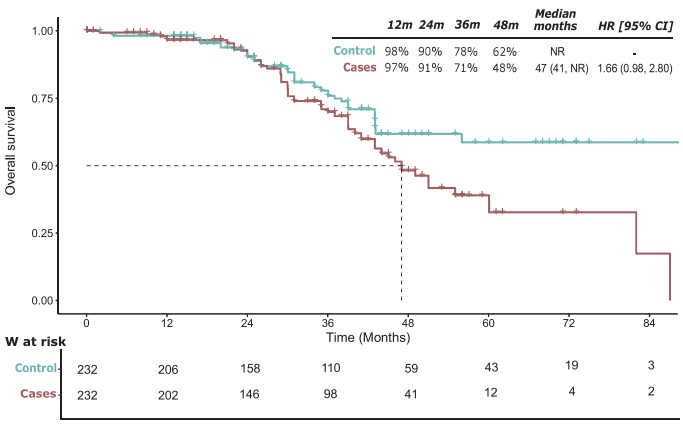
Multivariate OS-Cox Model without imputation

Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Mutations				
Control	100		Reference	
BRCA2	61		1.70 (0.88, 3.31)	0.115
Other mut	36		1.29 (0.61, 2.74)	0.511
Menopausal status				
Postmenopausal	90		Reference	
Premenopausal	107		0.55 (0.30, 1.00)	**0.049**
Endocrine resitance				
No	129		Reference	
Yes	68		3.45 (1.55, 7.68)	**0.002**
Subtype				
Lumil A	54		Reference	
Lumil B HER2 negative	143		0.90 (0.43, 1.90)	0.789
Metastasis novo				
No	124		Reference	
Yes	73		1.06 (0.46, 2.44)	0.899
Visceral metastasis				
No	115		Reference	
Yes	82		0.74 (0.39, 1.38)	0.343
Bone metastasis				
No	122		Reference	
Yes	75		0.47 (0.23, 0.96)	**0.038**
Age				
0–40	56		Reference	
41–60	101		1.18 (0.59, 2.38)	0.643
over 60	40		0.79 (0.26, 2.39)	0.677
Sites metastasis >3				
No	142		Reference	
Yes	55		1.56 (0.84, 2.89)	0.157

A. **PFS in the cohort (Cases vs Controls).**



B. **OS in the cohort (Cases vs Controls).**



A

Univariate PFS-Cox Model

Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Arm				
Control	115		Reference	
Cases	113		1.71 (1.22, 2.40)	**0.002**
Menopausal status				
Postmenopausal	103		Reference	
Premenopausal	125		1.18 (0.84, 1.65)	0.343
Endocrine resitance				
No	151		Reference	
Yes	77		2.35 (1.66, 3.34)	**<0.001**
Subtype				
Lumil A	58		Reference	
Lumil B HER2 negative	158		1.77 (1.12, 2.80)	**0.015**
Lumil NOS	12		0.76 (0.39, 1.48)	0.419
Metastasis novo				
No	151		Reference	
Yes	77		0.86 (0.62, 1.20)	0.383
Visceral metastasis				
No	129		Reference	
Yes	99		1.24 (0.88, 1.74)	0.217
Only bone metastasis				
No	146		Reference	
Yes	82		0.91 (0.63, 1.32)	0.633
Age				
0–40	67		Reference	
41–60	119		1.14 (0.77, 1.70)	0.510
over 60	42		0.84 (0.48, 1.48)	0.555
Site metastasis >3				
No	160		Reference	
Yes	68		1.25 (0.88, 1.79)	0.211

B

Univariate OS-Cox Model

Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Arm				
Control	110		Reference	
Cases	106		1.69 (1.00, 2.87)	**0.051**
Menopausal status				
Postmenopausal	97		Reference	
Premenopausal	119		0.74 (0.44, 1.25)	0.263
Endocrine resitance				
No	141		Reference	
Yes	75		2.49 (1.49, 4.16)	**<0.001**
Subtype				
Lumil A	58		Reference	
Lumil B HER2 negative	158		1.27 (0.67, 2.42)	0.460
Metastasis novo				
No	140		Reference	
Yes	76		0.47 (0.26, 0.84)	**0.011**
Visceral metastasis				
No	123		Reference	
Yes	93		1.62 (0.96, 2.72)	**0.071**
Only bone metastasis				
No	136		Reference	
Yes	80		0.51 (0.29, 0.90)	**0.021**
Age				
0–40	63		Reference	
41–60	112		1.40 (0.76, 2.57)	0.287
over 60	41		1.45 (0.66, 3.18)	0.351
Site metastasis >3				
No	154		Reference	
Yes	62		2.06 (1.23, 3.46)	**0.006**

C

Multivariate PFS-Cox Model

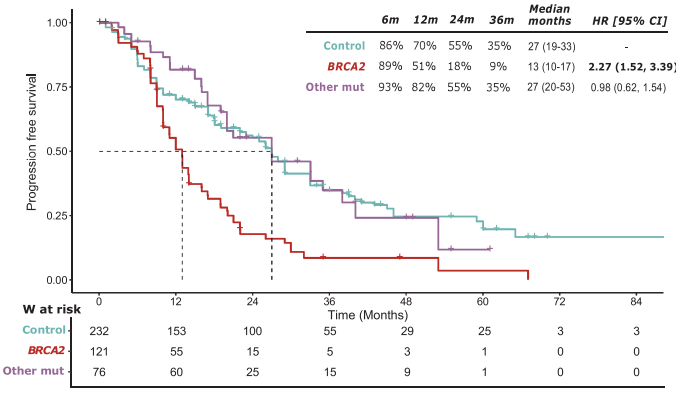
Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Arm				
Control	115		Reference	
Cases	113		1.49 (1.04, 2.12)	**0.030**
Menopausal status				
Postmenopausal	103		Reference	
Premenopausal	125		0.76 (0.50, 1.15)	0.189
Endocrine resistance				
No	151		Reference	
Yes	77		2.93 (1.86, 4.60)	**<0.001**
Subtype				
Lumil A	58		Reference	
Lumil B HER2 negative	158		1.53 (0.96, 2.44)	0.072
Lumil NOS	12		0.71 (0.32, 1.56)	0.389
Metastasis novo				
No	151		Reference	
Yes	77		1.43 (0.92, 2.22)	0.112
Visceral metastasis				
No	129		Reference	
Yes	99		0.89 (0.57, 1.38)	0.597
Only bone metastaisis				
No	146		Reference	
Yes	82		1.01 (0.63, 1.60)	0.975
Age				
0–40	67		Reference	
41–60	119		1.03 (0.70, 1.53)	0.881
over 60	42		0.67 (0.35, 1.29)	0.228
Site metastasis >3				
No	160		Reference	
Yes	68		1.51 (1.01, 2.28)	**0.047**

D

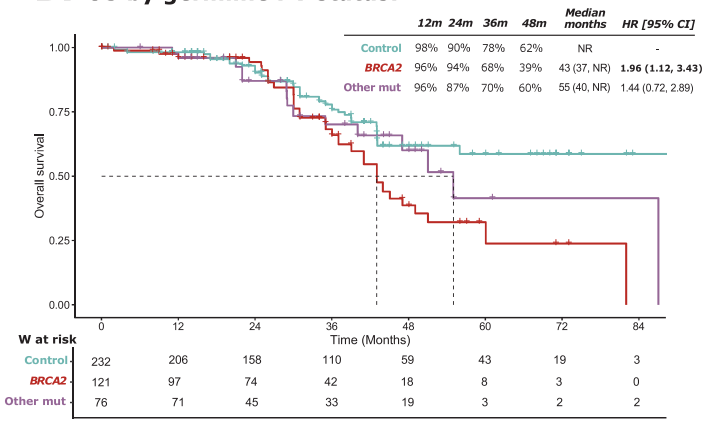
Multivariate OS-Cox Model

Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Arm				
Control	110		Reference	
Cases	106		1.53 (0.88, 2.64)	0.131
Menopausal status				
Postmenopausal	97		Reference	
Premenopausal	119		0.51 (0.28, 0.95)	**0.034**
Endocrine resistance				
No	141		Reference	
Yes	75		3.27 (1.58, 6.79)	**0.001**
Subtype				
Lumil A	58		Reference	
Lumil B HER2 negative	158		0.90 (0.47, 1.70)	0.736
Metastasis novo				
No	140		Reference	
Yes	76		1.01 (0.47, 2.16)	0.986
Visceral metastasis				
No	123		Reference	
Yes	93		0.64 (0.34, 1.19)	0.156
Only bone metastaisis				
No	136		Reference	
Yes	80		0.53 (0.27, 1.05)	**0.067**
Age				
0–40	63		Reference	
41–60	112		1.09 (0.57, 2.10)	0.790
over 60	41		0.75 (0.27, 2.06)	0.579
Site metastasis >3				
No	154		Reference	
Yes	62		2.23 (1.21, 4.11)	**0.010**

A. **PFS by germline PV status.**



B. **OS by germline PV status.**



A

Univariate PFS-Cox Model (Germline PV)

Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Mutation				
Control	115		Reference	
BRCA2	60		2.25 (1.50, 3.38)	**<0.001**
Other mut	36		1.00 (0.64, 1.57)	0.996
Menopausal status				
Postmenopausal	101		Reference	
Premenopausal	110		1.07 (0.75, 1.52)	0.718
Endocrine resistance				
No	141		Reference	
Yes	70		2.32 (1.61, 3.34)	**<0.001**
Subtype				
Lumil A	57		Reference	
Lumil B HER2 negative	149		1.71 (1.06, 2.74)	**0.026**
Lumil NOS	5		0.59 (0.29, 1.22)	0.155
Metastasis novo				
No	137		Reference	
Yes	74		0.95 (0.67, 1.35)	0.794
Visceral metastasis				
No	118		Reference	
Yes	93		1.29 (0.90, 1.85)	0.164
Only bone metastaisis				
No	136		Reference	
Yes	75		0.91 (0.62, 1.34)	0.637
Age				
0–40	61		Reference	
41–60	109		1.11 (0.73, 1.69)	0.626
over 60	41		0.95 (0.53, 1.68)	0.849
Site metastasis >3				
No	148		Reference	
Yes	63		1.22 (0.84, 1.78)	0.302

B

Univariate OS-Cox Model (Germline PV)

Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Mutation				
Control	112		Reference	
BRCA2	59		1.97 (1.12, 3.46)	**0.019**
Other mut	35		1.48 (0.74, 2.96)	0.264
Menopausal status				
Postmenopausal	97		Reference	
Premenopausal	109		0.80 (0.47, 1.35)	0.405
Endocrine resistance				
No	137		Reference	
Yes	69		2.84 (1.68, 4.81)	**<0.001**
Subtype				
Lumil A	57		Reference	
Lumil B HER2 negative	149		1.11 (0.60, 2.04)	0.742
Metastasis novo				
No	132		Reference	
Yes	74		0.53 (0.30, 0.94)	**0.030**
Visceral metastasis				
No	116		Reference	
Yes	90		1.63 (0.95, 2.79)	0.076
Only bone metastaisis				
No	132		Reference	
Yes	74		0.62 (0.35, 1.10)	0.102
Age				
0–40	60		Reference	
41–60	106		1.27 (0.68, 2.36)	0.455
over 60	40		1.47 (0.67, 3.21)	0.337
Site metastasis >3				
No	145		Reference	
Yes	61		1.66 (0.97, 2.85)	0.063

C

Multivariate PFS-Cox Model (Germline PV)

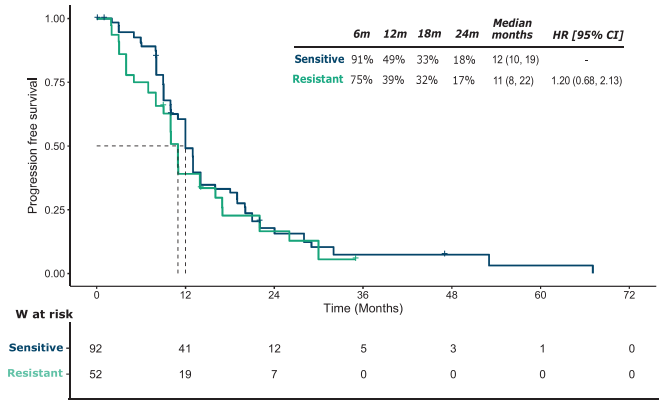
Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Mutation				
Control	115		Reference	
BRCA2	60		1.89 (1.25, 2.86)	**0.002**
Other mut	36		0.89 (0.54, 1.47)	0.655
Menopausal status				
Postmenopausal	101		Reference	
Premenopausal	110		0.73 (0.47, 1.13)	0.156
Endocrine resistance				
No	141		Reference	
Yes	70		3.12 (1.91, 5.10)	**<0.001**
Subtype				
Lumil A	57		Reference	
Lumil B HER2 negative	149		1.40 (0.86, 2.27)	0.174
Lumil NOS	5		0.54 (0.18, 1.59)	0.260
Metastasis novo				
No	137		Reference	
Yes	74		1.61 (1.00, 2.59)	0.049
Visceral metastasis				
No	118		Reference	
Yes	93		1.01 (0.61, 1.66)	0.968
Only bone metastaisis				
No	136		Reference	
Yes	75		0.98 (0.61, 1.60)	0.948
Age				
0–40	61		Reference	
41–60	109		0.99 (0.65, 1.50)	0.965
over 60	41		0.68 (0.34, 1.34)	0.262
Site metastasis >3				
No	148		Reference	
Yes	63		1.28 (0.82, 1.99)	0.280

D

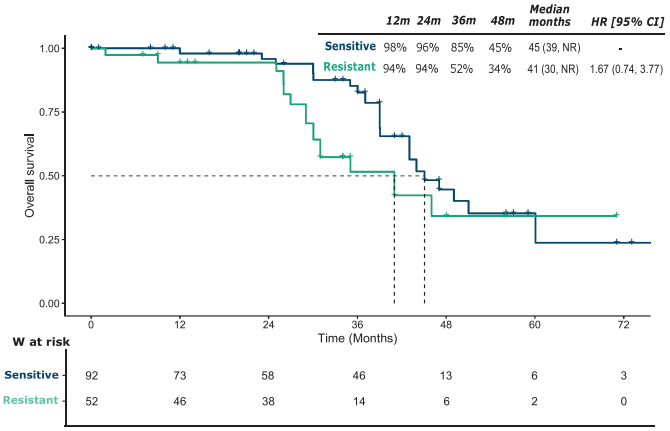
Multivariate OS-Cox Model (Germline PV)

Variable	N	Hazard ratio	HR [95% CI]	*p-value*
Mutation				
Control	112		Reference	
BRCA2	59		2.00 (1.00, 4.00)	**0.050**
Other mut	35		1.38 (0.66, 2.90)	0.394
Menopausal status				
Postmenopausal	97		Reference	
Premenopausal	109		0.55 (0.30, 1.00)	**0.050**
Endocrine resistance				
No	137		Reference	
Yes	69		3.63 (1.73, 7.61)	**<0.001**
Subtype				
Lumil A	57		Reference	
Lumil B HER2 negative	149		0.84 (0.42, 1.68)	0.615
Metastasis novo				
No	132		Reference	
Yes	74		1.14 (0.52, 2.54)	0.741
Visceral metastasis				
No	116		Reference	
Yes	90		0.75 (0.37, 1.55)	0.440
Only bone metastaisis				
No	132		Reference	
Yes	74		0.63 (0.30, 1.32)	0.222
Age				
0–40	60		Reference	
41–60	106		1.02 (0.53, 1.95)	0.953
over 60	40		0.73 (0.26, 1.99)	0.534
Site metastasis >3				
No	145		Reference	
Yes	61		1.75 (0.89, 3.45)	0.106

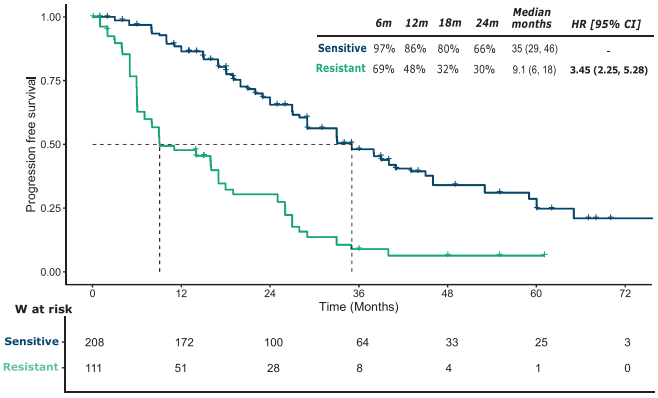
A. PFS among patients with g/t *BRCA2* PV.



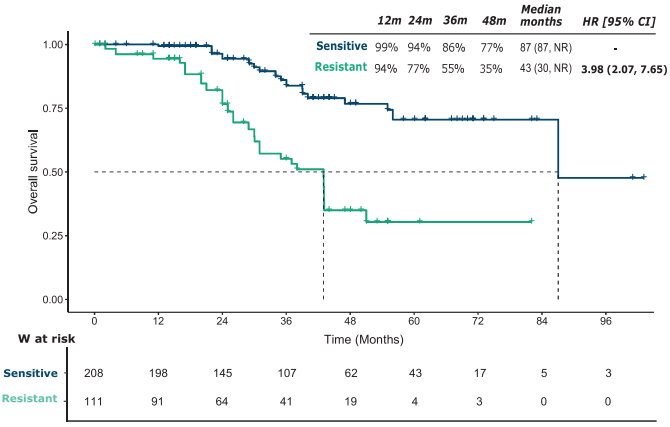
B. OS among patients with g/t *BRCA2* PV.

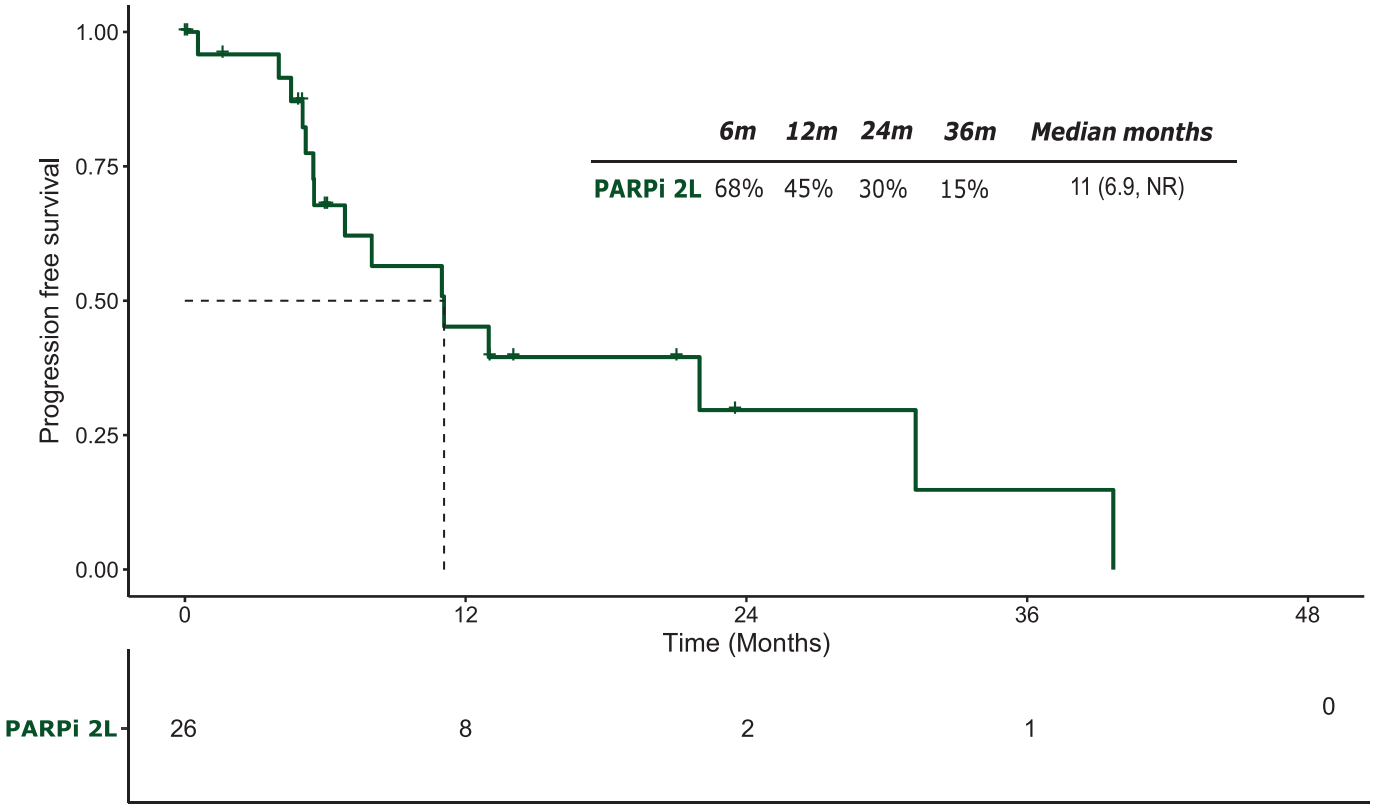


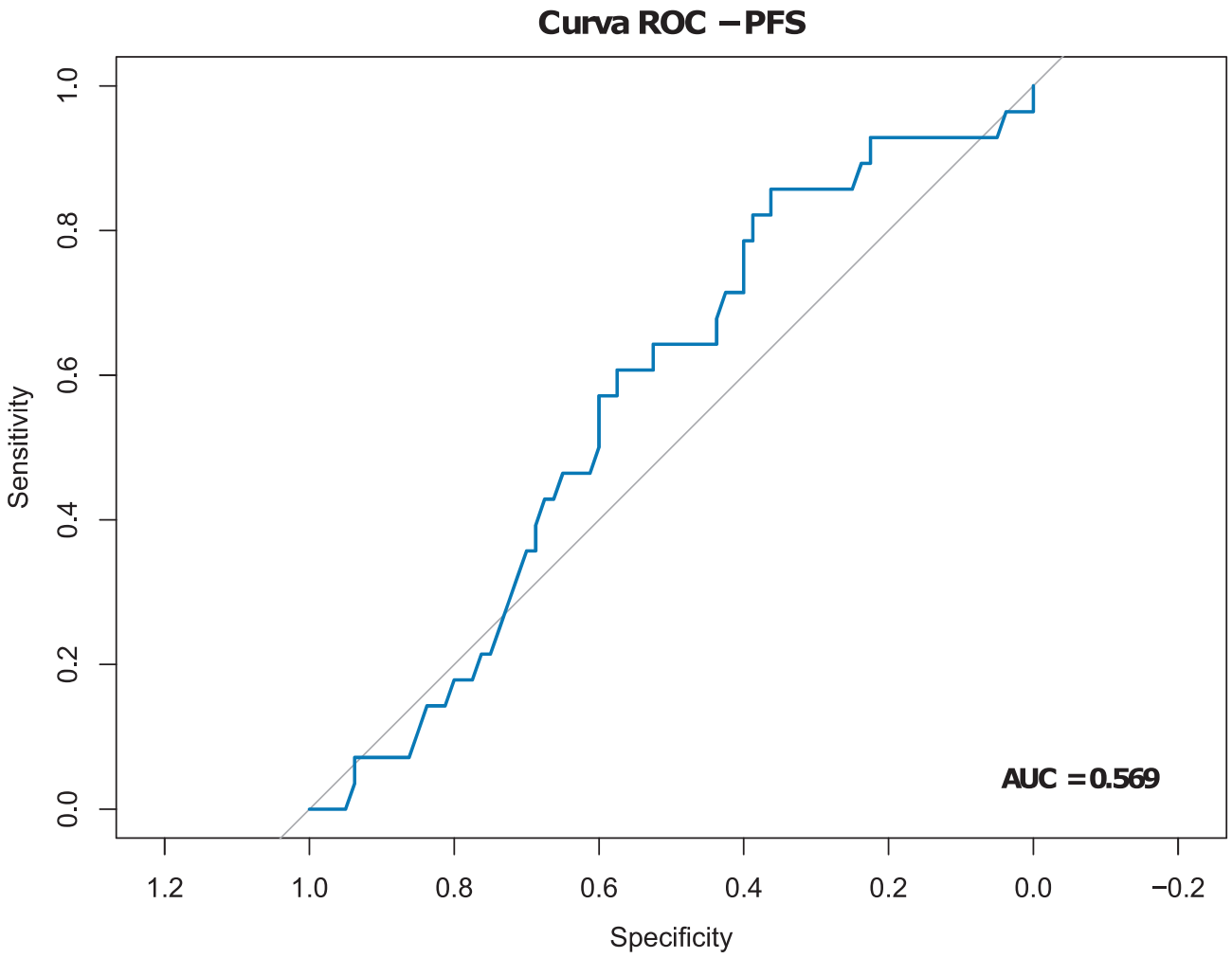
C. PFS among patients without a g/t *BRCA2* PV.

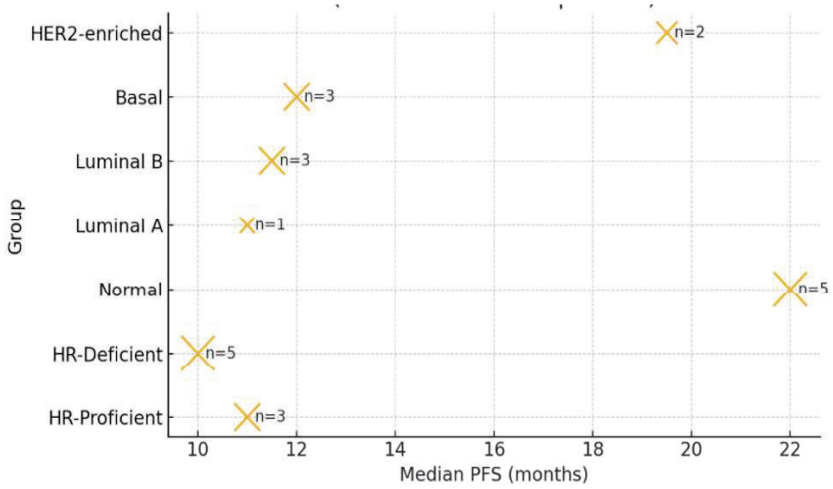


D. OS among patients without a g/t *BRCA2* PV.




1.00
0.75
0.50
0.25
0.00
Progression free survival
6m 12m 24m 36m Median months
PARPi 2L 68% 45% 30% 15% 11 (6.9, NR)
0
12
24
36
48
Time (Months)
PARPi 2L
26
8
2
1
0


Curva ROC −PFS
Sensitivity
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.0
0.8
0.6
0.4
0.2
0.0
−0.2
Specificity
AUC = 0.569


HER2-enriched
Basal
Luminal B
Luminal A
Normal
HR-Deficient
HR-Proficient
Group
n=2
n=3
n=3
n=1
n=5
n=5
n=3
10
12
14
16
18
20
22
Median PFS (months)

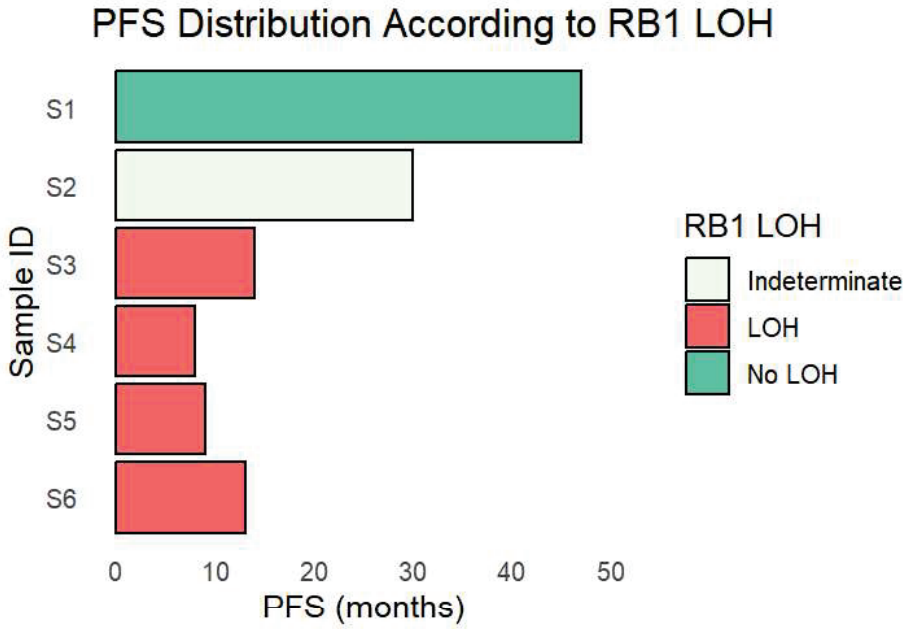

PFS Distribution According to RB1 LOH
Sample ID
S1
S2
S3
S4
S5
S6
0
10
20
30
40
50
PFS (months)
RB1 LOH
Indeterminate
LOH
No LOH

Table S1. Inclusion and exclusion criteria
Inclusion criteria
Individuals (women and men) with HR +,HER2- advanced BC (metastatic or unresectable disease)
Treatment with endocrine therapy and CDK4/6i as first line for advance disease
Germline HRR-PV cohort: Carriers of a germline PV in any HRR-related gene
Tumoral HRR-PV cohort: Individuals with a PV in *BRCA1, BRCA2* or *PALB2* identified through tumor genetic testing
Genetic-negative control cohort: Patients with documented negative germline genetic testing for HRR-related genes
Exclusion criteria
Patients harboring a germline variant of unknown significance in an HRR-related gene
Patients with metastatic BC with a synchronic HER2+ or triple negative BC
Patients with missing clinical information

HR+: Hormone receptor positive; BC: Breast cancer; CDK4/6i: Cyclin-dependent kinase 4 and 6 inhibitors; HRR: Homologous recombination repair; PV: Pathogenic variant

Table S2.

Population	Prevalence of *BRCA1/BRCA2* germline pathogenic variant
Unselected breast cancer population	*BRCA1*: 0.85%[1]-1.05%[2]; *BRCA2*: 1.29%[1]-1.54[2]
Metastatic HER2 negative BC	*BRCA2*: 5%[3] ; European population *BRCA2*: 4%[3]
ER positive HER2 negative early BC	*BRCA1* + *BRCA2*: 3.2%[4]
Early high-risk HER2 negative	*BRCA1* + *BRCA2*: 12.76%[5]
Triple negative early BC aged 40 years or younger	*BRCA1* + *BRCA2*: 2.7%[6]

[1]Hu C, Hart SN, Gnanaolivu R, Huang H, Lee KY, Na J, Gao C et al. A Population-Based Study of Genes Previously Implicated in Breast Cancer. N Engl J Med. 2021 Feb 4;384(5):440-451.

[2]Dorling L, Carvalho S, Allen J, González-Neira A, Luccarini C, Wahlström C, reast Cancer Risk Genes - Association Analysis in More than 113,000 Women. N Engl J Med. 2021 Feb 4;384(5):428-439.

[3]O'Shaughnessy J, Brezden-Masley C, Cazzaniga M, Dalvi T, Walker G, Bennett J, Ohsumi S. Prevalence of germline BRCA mutations in HER2-negative metastatic breast cancer: global results from the real-world, observational BREAKOUT study. Breast Cancer Res. 2020 Oct 27;22(1):114.

[4]Morganti S, Khan RA, Berrocal-Almanza LC, Miranda M, Luo L, Xu X, Partridge AH, Lynce F. Oncotype DX Recurrence Score and Germline BRCA Variants in Patients with HR-Positive/HER2-Negative Early Breast Cancer: A Retrospective Observational Study. Oncol Ther. 2025 Jun;13(2):363-379.

[5]Tutt ANJ, Garber JE, Kaufman B, Viale G, Fumagalli D, Rastogi P et al; OlympiA Clinical Trial Steering Committee and Investigators. Adjuvant Olaparib for Patients with *BRCA1*- or *BRCA2*-Mutated Breast Cancer. N Engl J Med. 2021 Jun 24;384(25):2394-2405.

[6]Couch FJ, Hart SN, Sharma P, Toland AE, Wang X, Miron P et al. Inherited mutations in 17 breast cancer susceptibility genes among a large triple-negative breast cancer cohort unselected for family history of breast cancer. J Clin Oncol. 2015 Feb 1;33(4):304-11.

Table S3.

	UB-cohort			B-cohort		
	Control N = 117	**Cases** N = 116	**SMD**	**Control** N = 232	**Cases** N = 232	**SMD**
Age at BC diagnosis	48 (41, 60)	45 (38, 52)	0.33	45 (39, 57)	45 (39, 54)	0.00
Endocrine resistance	37 (32%)	42 (36%)	0.10	83 (36%)	81 (35%)	0.02
Metastatic de novo	38 (32%)	40 (34%)	0.04	71 (31%)	75 (32%)	0.03
Visceral metastatic	50 (43%)	52 (45%)	0.04	101 (44%)	102 (44%)	0.01
Year Germline study			0.62			0.03
2020	8 (6%)	16 (14%)		23 (10%)	24 (11%)	
2021	29 (25%)	19 (16%)		48 (21%)	48 (21%)	
2022	17 (15%)	14 (12%)		29 (13%)	29 (13%)	
2023	23 (20%)	9 (8%)		32 (14%)	31 (13%)	
2024	11 (9%)	5 (4%)		16 (6.9%)	16 (7.1%)	
before 2019	29 (25%)	53 (46%)		84 (36%)	84 (36%)	
CDK4/6i treatment			0.26			0.04
Abemaciclib	18 (15%)	16 (14%)		34 (15%)	33 (14%)	
Palbociclib	36 (31%)	50 (43%)		84 (36%)	89 (39%)	
Ribociclib	63 (54%)	50 (43%)		114 (49%)	110 (47%)	

[1] n (%)

[2] UB, unbalanced; B, balanced; SMD, standardized mean difference; BC, breast cancer; CDK4/6i: CDK4/6 inhibitor treatment.

Table S4.

Characteristic	Overall N = 233[1]	*BRCA2* N = 74[1]	Control N = 117[1]	p-value[2]
Sex assigned at birth				0.148
Female	231 (99%)	72 (97%)	117 (100%)	
Male	2 (1%)	2 (3%)	0 (0%)	
Age at BC diagnosis				0.047
Mean (SD)	49 (12)	46 (12)	51 (12)	
Median (Q1, Q3)	45 (39, 56)	45 (38, 51)	48 (41, 60)	
Min, Max	26, 79	26, 78	27, 79	
Pre/postmenopausal at diagnosis				<0.001
Premenopausal	125 (55%)	49 (69%)	46 (40%)	
Postmenopausal	103 (45%)	22 (31%)	69 (60%)	
Not appicable	2	2	0	
Missing	3	1	2	
Endocrine resistance primary/secondary				0.327
No	154 (66%)	48 (65%)	80 (68%)	
Primary	22 (10%)	4 (5%)	14 (12%)	
Secondary	57 (24%)	22 (30%)	23 (20%)	
Histological grade				0.742
1	15 (12%)	4 (11%)	10 (13%)	
2	97 (68%)	26 (68%)	55 (71%)	
3	29 (20%)	8 (21%)	12 (16%)	
Missing	92	36	40	
Subtype according to IHQ				0.002
Luminal A	57 (26%)	8 (11%)	37 (35%)	
Luminal B	154 (70%)	60 (81%)	65 (62%)	
Luminal NOS	10 (4%)	6 (8%)	3 (3%)	
Missing	12	0	12	
Metastatic de novo				0.789
No	155 (67%)	47 (64%)	79 (68%)	
Yes	78 (33%)	27 (36%)	38 (32%)	
Only bone metastasis				0.837
No	149 (66%)	45 (65%)	79 (68%)	
Yes	77 (34%)	24 (35%)	38 (32%)	
Missing	7	5	0	
Visceral metastatic[4]				0.901
No	131 (56%)	40 (54%)	67 (57%)	
Yes	102 (44%)	34 (46%)	50 (43%)	
3 or more metastatic sites				0.019
No	153 (70%)	37 (57%)	90 (77%)	
Yes	66 (30%)	28 (43%)	27 (23%)	
Missing	14	9	0	
Type of CDK4/6i treatment				0.133
Abemaciclib	34 (15%)	7 (9%)	18 (15%)	
Palbociclib	86 (37%)	34 (46%)	36 (31%)	
Ribociclib	113 (48%)	33 (45%)	63 (54%)	

PARPi after CDK4/6i				<0.001
No	143 (76%)	28 (43%)	91 (99%)	
Yes	46 (24%)	37 (57%)	1 (1%)	
Missing	44	9	25	
Test result in germline genetic test:				<0.001
Negative	123 (55%)	4 (6%)	117 (100%)	
Positive	99 (45%)	63 (94%)	0 (0%)	
Missing	11	7	0	
Year Germline study				
2020	24 (10%)	10 (14%)	8 (7%)	
2021	48 (21%)	14 (19%)	29 (25%)	
2022	31 (13%)	7 (9%)	17 (15%)	
2023	32 (14%)	5 (5%)	23 (20%)	
2024	16 (7%)	2 (2%)	11 (9.4%)	
before 2019	82 (35%)	36 (49%)	29 (25%)	

[1] n (%)

[2] Wilcoxon rank sum test; Pearson's Chi-squared test; Fisher's exact test

[3] Endocrine resistant to adjuvant therapy defined as disease progression occurring during or within 12 months after completing adjuvant endocrine therapy based on ABC7 guidelines.

[4] Visceral metastasis including Central Nervous System involvement (N=1)

BC: breast cancer; IHC: immunohistochemistry; PARPi: PARP inhibitor treatment; CDK4/6i: CDK4/6 inhibitor treatment; gen_germline: gen detected in the germline testing; gen_somatic: gen detected in the tumor; gen_bio_liquid: gen detected in liquid biopsy (circulating tumor DNA (ctDNA)

Table S5.

	Estimated probability			
Group	***PPV 6 months***	***PPV 12 months***	***PPV 18 months***	***PPV 24 months***
BRCA2 PV mutated and endocrine-sensitive patients	0.96	2.31	2.40	2.49
Others	99.04	97.69	97.60	97.51

PPV: Positive predicted value.

Table S6.

Youden for PFS-Cutoff

Cutoff (Months)	TP	FP	TN	Sensitivity	Specificity	Youden
2	0	2	78	0.0%	97.5%	−2.5%
4	2	8	72	7.1%	90.0%	−2.9%
6	4	14	66	14.3%	82.5%	−3.2%
8	6	20	60	21.4%	75.0%	−3.6%
10	12	26	54	42.9%	67.5%	10.4%
12	14	32	48	50.0%	60.0%	10.0%
14	16	33	47	57.1%	58.8%	15.9%
16	17	38	42	60.7%	52.5%	13.2%

PFS: Progression free survival; TP: true positive; FP: false positive; TN: true negative; FN:false negative.

Table S7.

Gen affected	Type of PV	PV orgin (germline or tumoral)	CDK4/6 inhibitor	Pre/postmenopausal	Endocrine resistance to adjuvant ET	Breast cancer subtype by IHC	Visceral M1	Sample pre CDK4/6 inhibitor	HRD status by RAD51 foci	PAM50	*Rb1* loss	Sample post CDK4/6 inhibitor	HRD status by RAD51 foci
BRCA2	Truncate	Germline	Palbociclib	Pre	No	Luminal A	No	Yes	NE	Normal	NE	Yes	NA
BRCA2	Truncate	Germline	Palbociclib	Pre	No	inal B HER2 neg	No	Yes	NE	NE	NE	Yes	HRD (2%)
BRCA2	Truncate	Germline	Palbociclib	Pre	Yes	Luminal A	Yes	Yes	HRD (0%)	NE	NE	No	NA
BRCA2	Truncate	Germline	Palbociclib	Post	No	inal B HER2 neg	Yes	Yes	HRD (7%)	NE	NE	No	NA
BRCA2	Truncate	Germline	Palbociclib	Post	No	inal B HER2 neg	No	Yes	NE	NE	NE	Yes	HRD (2%)
BRCA2	Truncate	Tumoral	Abemaciclib	Pre	Yes	inal B HER2 neg	No	Yes	HRD (1%)	Normal	NE	No	NA
BRCA2	Truncate	Germline	Palbociclib	Pre	No	inal B HER2 neg	No	Yes	NE	NE	NE	No	NA
BRCA2	Missense	Germline	Palbociclib	Pre	No	inal B HER2 neg	Yes	Yes	NE	Basal	NE	Yes	HRD 3%
BRCA2	Truncate	Germline	Palbociclib	Pre	Yes	inal B HER2 neg	No	Yes	NE	Her2	Indeterminate	Yes	NE
BRCA2	Truncate	Tumoral	Ribociclib	Pre	Yes	inal B HER2 neg	No	Yes	HRP (15%)	Luminal A	NE	Yes	HRP (11%)
BRCA2	Truncate	Germline	Palbociclib	Pre	No	inal B HER2 neg	No	Yes	NE	NE	NE	Yes	HRD (3%)
BRCA2	Truncate	Germline	Ribociclib	Pre	No	inal B HER2 neg	Yes	Yes	HRP (15%) (sample with fibrosis)	NE	Yes	Yes	NA
BRCA2	Truncate	Germline	Ribociclib	Pre	No	inal B HER2 neg	Yes	Yes	HRD (2%)	Luminal B	Yes	No	NA
BRCA2	Truncate	Germline	Palbociclib	Pre	No	inal B HER2 neg	No	Yes	NE	Normal	NE	No	NA
BRCA2	Truncate	Germline	Ribociclib	Pre	No	inal B HER2 neg	No	Yes	NE	Basal	NE	No	NA
BRCA2	Truncate	Germline	Palbociclib	NA	No	inal B HER2 neg	Yes	Yes	NE	Her2	NE	No	NA
BRCA2	Missense	Germline	Palbociclib	Post	Yes	inal B HER2 neg	Yes	Yes	NE	Normal	NE	No	NA
BRCA2	Truncate	Tumoral	Ribociclib	Post	No	inal B HER2 neg	No	Yes	NE	Basal	NE	No	NA
BRCA2	Truncate	Germline	Ribociclib	Pre	No	inal B HER2 neg	No	Yes	NE	Luminal B	NE	No	NA
BRCA2	Truncate	Germline	Ribociclib	Post	No	inal B HER2 neg	No	Yes	NE	Normal	No	No	NA
BRCA2	Truncate	Germline	Ribociclib	Pre	Yes	inal B HER2 neg	Yes	Yes	HRD (3%)	Luminal B	Yes	No	NA
BRCA2	Truncate	Germline	Ribociclib	Post	No	inal B HER2 neg	No	Yes	HRP (43%)	NE	Yes	No	NA

PV: pathogenic variant; ET: endocrine therapy, IHC: immunohistochemistry; M1: metastasis; HRD: homologous recombination deficiency; HRP: homologous recombination ; NE: Non evaluable; NA: Not

Table S8.	
Characteristic	**N = 22**[1]
Age at BC diagnosis	
Mean (SD)	48 (11)
Median (Q1, Q3)	47 (43, 52)
Min, Max	30, 72
Premenopausal or postmenopausal at diagnosis	
Premenopausal	15 (71%)
Postmenopausal	6 (29%)
Missing	1
Endocrine resistance primary/secondary	
No	16 (73%)
Secondary	6 (27%)
Histological grade	
1	1 (6.7%)
2	12 (80%)
3	2 (13%)
Missing	7
Subtype according to IHQ	
Luminal A	2 (9.1%)
Luminal B HER2 negative	20 (91%)
Metastatic de novo	
No	13 (59%)
Yes	9 (41%)
Only bone metastasis	
No	15 (68%)
Yes	7 (32%)
Visceral metastatic[3]	
No	14 (64%)
Yes	8 (36%)
3 or more metastatic locations	
No	13 (59%)
Yes	9 (41%)
CDK4/6i treatment	
Abemaciclib	1 (4.5%)
Palbociclib	12 (55%)
Ribociclib	9 (41%)
PARPi after CDK4/6i	
No	12 (57%)
Yes	9 (43%)
Missing	1
Test result in germline genetic test	
Negative	3 (14%)
Positive	19 (86%)
Year Germline study	
2020	3 (14%)

2021	7 (32%)
2022	3 (14%)
2023	3 (14%)
2024	1 (4.5%)
before 2019	5 (23%)
gen_germline	
BRCA2	19 (86%)
gen_tumor	
BRCA2	3 (14%)
Intrinsic subtype by PAM50	
Basal-like	3 (21%)
Her2-enriched	2 (15%)
Luminal A	1 (7%)
Luminal B	3 (21%)
Normal	5 (36%)
Non evaluable	8
HRD status by RAD51 foci	
Homologous recombination deficiency	5 (23%)
Homologous recombination proficiency	3 (14%)
Non evaluable	14 (64%)

[1] n (%)

[2] Endocrine resistant to adjuvant therapy defined as disease progression occurring during or within 12

[3] Visceral metastasis including Central Nervous System involvement (N=1)

BC: breast cancer; IHC: immunohistochemistry; PARPi: PARP inhibitor treatment; CDK4/6i: CDK4/6 inhibitor treatment; gen_germline: gen detected in the germline testing; gen_tumor: gen detected tumor DNA analysis; HRD: Homologous recombination deficiency

DISCUSSION

Key results and contextualization

This thesis addresses the process of identifying, characterizing, and treating patients with breast cancer who undergo genetic germline testing to identify pathogenic or likely pathogenic variants (gPV). Specifically, it examines the difficulty of the interpretation of the genetic testing results, the clinical and biological characteristics of breast tumors arising in patients with a gPV in genes involved in the HRR pathway, and the implications of these alterations for treatment responses and outcomes. Together, this work provides a comprehensive view of how germline information affects clinical decision-making, and treatment strategies for this group of patients.

Breast cancer is a leading cause of health problems and death for females worldwide. Early diagnosis and using MRI for high-risk patients have helped improve clinical outcomes. Identifying individuals at increased risk is crucial, as it allows intensified surveillance and risk-reducing surgeries, such as bilateral mastectomy and salpingo-oophorectomy, which can reduce breast and ovarian cancer risk by up to 90% and 80%, respectively, while also offering important economic implications for healthcare systems by preventing advanced-stage disease(18, 22, 98, 101, 121).

The evolution of hereditary cancer research, from the identification of *TP53, BRCA1*, and *BRCA2* to more recent discoveries like *PALB2* and other HRR genes, has defined contemporary genetic testing strategies(236). However, while historical research focused primarily on penetrance and risk assessment of *BRCA1/2(237)*, the goal of our work is to show how using this genetic information can impact and modify current clinical decisions and treatment strategies.

In clinical practice, patients diagnosed with breast cancer in Spain and other developed countries undergo germline genetic testing based on their personal cancer history and the potential implications for their clinical management. Germline testing may influence surgical decision-making, neoadjuvant or adjuvant treatment selection, and first-line therapy choices in the advanced setting(21). Additional factors, including family history of breast or related cancers, tumor subtype, age at diagnosis, and personal history of other malignancies, may also prompt genetic evaluation. Historically, patients with triple-negative breast cancer (TNBC) diagnosed before the age of 60, a group with a higher gPV prevalence (10–30%), as well as patients with hormone receptor–positive (HR+), HER2-negative breast cancer diagnosed at a young age, have been prioritized for testing(18).

Additionally, the identification of a gPV in a patient with breast cancer will also affect her surveillance related to the risk of a recurrence, a second breast cancer or other malignancy(19). Importantly, germline testing affects not only the patient but also their relatives, with implications for their psychological well-being, long-term surveillance strategies, and preventive interventions(12, 18, 28, 29). Criteria for germline genetic testing

and access to testing vary across countries and healthcare systems, often influenced by economic considerations and resource availability. However, testing criteria have evolved over time and continue to be refined as evidence accumulates regarding the clinical relevance of gPV across all breast cancer subtypes(12, 18, 28, 29).

Although genetic testing has been available for many years, the clinical implications of detecting gPV in genes such as *BRCA1*, *BRCA2*, *PALB2*, *ATM*, or *CHEK2* with respect to tumor biology, treatment sensitivity, and prognosis are not yet fully characterized. This uncertainty reflects both biological heterogeneity across genes and variants, as well as limitations in the available clinical evidence.

In parallel, the therapeutic landscape of both early and advanced breast cancer has expanded substantially over the past decade, resulting in meaningful improvements in survival outcomes(20, 24, 219). Despite these advances, a subset of patients continues to present with aggressive disease, unfavorable prognosis, or limited therapeutic options, underscoring the need for improved risk stratification and more individualized approaches to prevention and treatment.

The significant shift toward earlier age at diagnosis and higher rates of bilateral disease or stage IV de novo among patients with gPV in our cohort, compared to non-carriers, reinforces the need for mainstreaming genetic testing. This distinct clinical profile justifies a more aggressive screening approach, and a higher clinical suspicion when evaluating the metastatic setting. These patients also showed a higher prevalence of TNBC, mainly among *BRCA1* carriers, as well as a greater personal history of other cancer diagnoses. Although several of these clinical features have been reported previously, our findings in a real-world clinical cohort from a national publicly funded healthcare system reinforce the feasibility and the existence of a distinct clinical profile in this population and may facilitate earlier identification, prioritization for germline testing, and more personalized clinical management(238).

Access to germline genetic testing and the technologies required to perform it is now widely available and straightforward in developed countries. A blood draw, buccal swab, or saliva sample is sufficient to analyze DNA from white blood cells and identify gPV. However, despite the increasing accessibility of testing, greater technological sensitivity has introduced additional complexity in patient selection criteria, type of testing (including the number of genes analyzed), and result interpretation. This complexity has increased the need for specialized, multidisciplinary teams, typically involving medical oncologists, clinical geneticists, genetic counselors, surgeons, pathologists, and other relevant specialists, to appropriately identify candidates for testing, provide pre-test counseling, interpret results, and deliver post-test counseling. Such teams are essential to guide surveillance strategies, inform cancer management, and address the implications for carriers' relatives(12, 28, 46, 61, 62).

The prevalent adoption of multigene panel testing in patients with breast cancer or other malignancies has increased interpretative complexity by incrementing the number of genes

analyzed and improving the sensitivity of variant allele frequency (VAF) detection in blood and other tissues, including saliva and skin(31, 46, 47).

Germline testing is often perceived by both clinicians and patients as a binary diagnostic tool, expected to result in a clear positive or negative result. In the absence of appropriate pre- and post-test counseling, this simplified view may lead to misunderstanding of test results and their clinical implications.

The psychological dimension of germline genetic testing further complicates this decision-making process. Receiving information about inherited cancer risk can generate significant anxiety, guilt, and decisional conflict, not only for patients but also for their relatives(239, 240).

In practice, germline testing may identify clearly variants with allele frequencies consistent with heterozygous germline events, typically above 30–50% depending on the testing platform. These variants may be inherited or arise de novo; however, their clinical consequences for affected individuals and their offspring are generally comparable(50).

Germline testing frequently identifies variants of uncertain significance (VUS), for which the clinical implications and appropriate surveillance strategies remain undefined. Interpretation and management of VUS require careful consideration of multiple factors, including the gene involved, the predicted functional impact of the variant, prior associations with cancer, and the individual's personal and family history. The identification of VUS highlights the non-binary nature of germline testing results and remains a common source of clinical uncertainty. Current guidelines recommend that individuals with VUS be managed according to their personal and family history rather than the presence of the VUS itself, with ongoing monitoring of variant reclassification through international databases such as (https://www.ncbi.nlm.nih.gov/clinvar/), or through communication with testing laboratories and expert consortia(42, 241).

Accurate interpretation and contextualization of germline findings are essential not only from a medical standpoint but also to mitigate psychological harm. As clinicians, it is essential to clearly communicate the meaning of uncertainty, reassure patients about the lack of management implications based solely on a VUS, and emphasize that most of the VUS are ultimately reclassified as likely benign or benign, not having an impact on their cancer risk(42, 241, 242).

While this thesis does not specifically address the classification or clinical management of VUS, it provides a detailed characterization of their prevalence within our cohort of nearly 1,000 patients with breast cancer. We observed that 16.2% of patients harbored at least one VUS, a rate lower than 30% or higher reported in other published series(238). Given that the misinterpretation of these variants can lead to non-indicated preventive measures or inappropriate treatment decisions, it is imperative to establish standardized guidelines and recommendations.

Ambiguous genetic results are complex to manage and communicate, underscoring the importance of clear communication and multidisciplinary support. An additional example of these ambiguous or uncertainty germline results is when variants are detected at LAFVs, generally below 30%. In such cases, multiple potential biological and technical origins must be considered, including sequencing artifacts, CHIP, constitutional mosaicism, or true gPV present at lower than expected levels. Although the overall detection rate of LAFVs is low, typically below 1% in big published cohorts— (0.8% in our cohort(243))—their identification poses significant interpretative challenges with important clinical consequences(50).

LAFVs detected in blood-based germline testing may result from technical limitations related to sequencing depth, alignment, or platform-specific artifacts. Despite advances in next-generation sequencing technologies, such issues cannot be entirely eliminated. Distinguishing technical noise from true biological events is therefore essential, particularly when results may influence cancer risk assessment and clinical management decisions(50, 61, 62).

Beyond technical considerations, CHIP represents an increasingly recognized source of LAFVs. CHIP becomes more prevalent with age, personal history of tobacco, cancer or chemotherapy or radiotherapy treatments and may involve genes included in hereditary cancer panels, such as *TP53*, *ATM*, or *BRCA1/2(54, 55, 58)*. When detected incidentally through germline testing, these variants may be misclassified as inherited alterations, leading to erroneous conclusions regarding cancer risk and surveillance management. This issue is particularly relevant in oncology populations, where prior exposure to chemotherapy or radiotherapy may increase the prevalence of CHIP(52).

Additionally, constitutional mosaicism represents another important biological explanation for LAFVs. In this scenario, a PV arises during early embryonic development and is present in only a subset of cells. Mosaic variants may be detectable at low allele frequency in peripheral blood while being present at higher levels in other tissues, such as skin biopsy or a tumor tissue sample. This has important implications for genetic counseling, as the management of the individual is similar to the recommendations based on the guidelines for carriers of gPV related with cancer risk. This is because you can´t biopsy all the tissues from the body and be sure about which organs/tissues are at an increased risk of cancer and need an increased surveillance, and which ones are free or in the same risk group as the rest of the population. Additionally, the risk of transmission to offspring may be substantial, as low variant allele frequency in blood does not reliably exclude germline involvement. Failure to recognize mosaicism may lead to underestimation of both individual and familial cancer risk(50, 58, 63, 64).

True gPV may also occasionally be detected at lower-than-expected allele frequencies due to biological or sampling variability, including de novo events. These rare cases underscore the importance of avoiding rigid allele-frequency thresholds in isolation and instead adopting an integrated interpretative approach that incorporates laboratory data, clinical features, and family history(12, 50, 64). The findings of this thesis demonstrate that LAFVs cannot be

dismissed as incidental but require careful evaluation within a multidisciplinary framework and expert team.

In this context, our work contributes to clarifying the clinical scenarios in which LAFVs are more likely to be detected and to refining their interpretation. We observed that LAFVs compared to heterozygous variants were more frequently identified in older individuals and in patients with a personal history of any type of cancer, and particularly breast cancer, findings that are consistent with prior reports(243).

As an illustrative example directly aligned with the aims of this thesis, to improve the clinical interpretation and management of complex germline testing results, we incorporated a diagnostic algorithm in our publication (Figure 3)(243). This algorithm was developed from one of the largest international clinical cohorts reported to date with detection of LAFVs and integrates comparative clinical analyses between individuals with LAFVs and those with heterozygous germline variants. In addition, it incorporates in-depth evaluation of 62 cases with LAFVs who underwent additional testing to clarify variant origin, including constitutional mosaicism and CHIP(243).

This framework operationalizes the central objective of the thesis by translating cohort-level observations into a structured, clinically applicable approach. For instance, when a PV in *TP53* is detected at an allele frequency of approximately 30% on germline next-generation sequencing in a 50-year-old woman with early breast cancer, the algorithm guides a stepwise evaluation. In accordance with current guideline based recommendations, the first step is to exclude a technical artifact, followed by confirmation using an orthogonal method such as Sanger sequencing to distinguish a true LAFV from a heterozygous germline variant. Once a LAFV is confirmed, interpretation relies on integration of clinical context and targeted additional testing to determine the most likely biological origin and its implications for patient management and familial risk assessment(49, 63-65, 203).

Consistent with ACMG-aligned interpretative frameworks, additional evaluation may include analysis of a non-hematopoietic tissue sample from the proband, such as skin biopsy, and/or testing of offspring when clinically appropriate. Detection of the variant in non-hematopoietic tissue supports a diagnosis of constitutional mosaicism. Identification of the variant in tumor tissue may also be compatible with constitutional mosaicism when interpreted in the appropriate clinical context(50); however, this finding must be interpreted cautiously, given the high prevalence of somatic *TP53* alterations in tumors and the possibility that the variant represents a tumor-restricted somatic event(63).

In cases in which constitutional mosaicism involving *TP53* is confirmed or cannot be reliably excluded and given the inability to accurately determine the distribution of affected tissues or germline involvement, clinical management should follow Li-Fraumeni syndrome surveillance recommendations as outlined in current NCCN guidelines(19). In this setting, genetic testing of offspring should be considered, as the presence of mosaicism does not preclude gonadal involvement and potential transmission(49, 63-65, 203).

Conversely, if the variant is not detected in additional non-hematopoietic tissues and clinical factors such as patient age, prior exposure to chemotherapy or radiotherapy, and personal cancer history are considered, CHIP may be considered the most likely explanation(63, 65). This distinction has important clinical implications, as CHIP is not associated with inherited cancer risk for offspring but is associated with an increased risk of hematologic malignancies and cardiovascular disease. Accordingly, management in this context should focus on hematologic monitoring, including periodic complete blood counts and assessment and management of cardiovascular risk factors, rather than on hereditary cancer surveillance(66).

Importantly, accurate interpretation of complex germline findings, such as LAFVs, is a prerequisite for correctly defining carrier status and enabling downstream analyses of clinical phenotypes. In this context, our observation that, compared with heterozygous variants, LAFVs were significantly more frequent among patients with breast cancer, but not among other cancer diagnoses, underscores the need for careful and structured interpretation in oncologic populations. Building on this interpretative framework, our work further characterizes the clinical and tumor features of patients with breast cancer who, following rigorous interpretation of germline testing results by an expert team and laboratory, are confirmed to carry gPV in HRR genes beyond *BRCA1* and *BRCA2*, compared with patients with negative germline testing.

Our work characterizes the clinical and tumor features of approximately 1,000 patients with breast cancer from Spain who underwent germline genetic testing, of whom 14.1% were found to carry a gPV in genes associated with breast cancer or Lynch syndrome. Compared with non-carriers, these patients exhibited distinct clinical characteristics, including younger age at diagnosis, higher rates of bilateral disease, and specific tumor subtypes, such as TNBC. Improved characterization of this population is essential to refine genetic testing strategies and to inform more individualized clinical management.

Current genetic testing criteria, while effective in identifying high-risk individuals, remain imperfect and may fail to capture a proportion of gPV carriers, particularly among patients with HR+ or HER2-positive disease or those with limited family history(78, 79). Incorporating tumor phenotypes and available clinical features into testing strategies may facilitate identification of individuals who would benefit from germline evaluation and help address gaps inherent to existing criteria.

In this context, our findings have important clinical implications, particularly for optimizing genetic testing strategies in resource-limited settings. When financial and infrastructural constraints restrict access to comprehensive multigene testing, the use of routinely collected clinical variables, such as age at diagnosis, tumor stage, bilaterality, histological subtype, and personal or family cancer history, offers a pragmatic and cost-effective approach to prioritizing patients at higher likelihood of carrying a gPV. In addition to improving access, the use of more focused gene panels targeting well-established breast cancer susceptibility and Lynch syndrome may also facilitate result interpretation by reducing the complexity associated with

broader panels. This approach supports a more rational use of limited resources while maintaining meaningful clinical impact. Furthermore, expanding access through alternative delivery models, including mainstreamed testing pathways and telemedicine-based genetic counseling, may further help overcome existing barriers to germline evaluation(156, 244, 245).

Identification of HRR gPV has implications that extend beyond cancer risk assessment. Knowledge of carrier status may influence surgical decision-making, including consideration of bilateral mastectomy or CPM, as well as timing of risk-reducing interventions(18, 22, 99, 120). Our results show that patients with gPV receive neoadjuvant and adjuvant chemotherapy more often and undergo risk-reducing or CPMs at higher rates than non-carriers. This occurs even without clear evidence of a survival benefit for some of these treatments(97, 98, 100-102, 108, 246). These findings show why identifying this group correctly is key to improving their clinical management and outcomes. Carriers may also benefit from tailored surveillance strategies for second primary cancers. Importantly, penetrance and cancer spectrum vary across genes and variants, highlighting the need for gene-specific and context-dependent risk assessment(12, 18, 19, 28).

Clinical decision-making varies according to the gene involved. In patients with breast cancer and a gPV, surgical recommendations should be individualized based on the affected gene, patient age, breast cancer subtype, and patient preferences, including aesthetic considerations and family planning(12, 18, 19, 28). In *BRCA1/BRCA2* carriers, particularly premenopausal patients with ER–positive disease or TNBC, the risk of CBC is significantly increased(91, 201); therefore, CPM may be considered in selected cases, although no consistent OS benefit has been demonstrated(22, 98, 101, 102, 162, 246).

In contrast, CBC risk in carriers of gPV in moderate-penetrance genes such as *ATM*, *CHEK2*, or *RAD51C/D* is less clearly defined, and the role of mastectomy or CPM in this setting remains uncertain(91, 120, 201). In our cohort, the incidence of CBC was higher among carriers overall compared to non-carriers; however, in subgroup analyses, this association was statistically significant in high-risk gPV carriers but not in moderate-risk carriers. The CPM uptake was 47.3% among carriers of high-risk genes and 21.6% among carriers of moderate-risk genes. These procedures are technically more complex, require longer recovery, and may delay adjuvant treatment, potentially having an impact on clinical outcomes, factors that should be incorporated into surgical decision-making alongside absolute risk estimates(22).

As discussed above, germline testing performed before or at an early stage of breast cancer diagnosis provides clinically relevant information that can inform surgical planning, reconstruction, and adjuvant treatment decisions(22, 99). However, genetic results obtained in the context of a new cancer diagnosis and associated psychological burden may also influence treatment choices beyond what is strictly supported by evidence. Consequently, decisions regarding bilateral mastectomy or CPM should be individualized and based on

transparent discussions of both available data and existing knowledge gaps, including absolute risk and the lack of proven OS benefit in many settings(98, 99).

A recent large international cohort study of patients with breast cancer diagnosed at age 40 or younger showed a survival benefit for those who underwent CPM and RRSO, regardless of tumor stage, subtype, or the timing of genetic testing(119). However, these data have not been consistently confirmed in prospective studies or in older patient cohorts. For instance, the Prospective Outcomes in Sporadic versus Hereditary breast cancer (POSH) study, a large UK prospective cohort of women diagnosed at >40 years, reported that *BRCA1* and *BRCA2* carriers had similar OS to non-carriers at 2, 5, and 10 years. This was observed despite carriers having more aggressive features, such as a higher prevalence of TNBC among *BRCA1* carriers, although the TNBC subgroup did show a small survival benefit at the 2-year mark. Importantly, while mutation carriers in the POSH study were more likely to choose bilateral mastectomy, this higher uptake of surgery did not translate into a clear OS advantage during the follow-up period. These findings suggest that while risk-reducing surgeries effectively lower the risk of a second primary breast cancer, their impact on long-term survival in young patients remains limited and must be carefully balanced against individual risk profiles and patient preferences(162).

As mentioned above, in the lastte years, treatments in breast cancer have improved dramatically, and new drugs with new mechanisms have appeared in early and advanced stage(24, 219). Although, we know that these gPV can affect the biology and behavior of breast tumors, most of the randomized studies that get approval are not stratified on the genetics results or findings in the germline testing, and the difficulty in designing trials specifically for this population of patients(154, 190, 228). This is due to multiple reasons but mainly is because of the limitations in number of patients with these alterations detected, and the heterogenicity between genes. Although we tried to group them, trying to get better conclusions and recommendations, we are fully aware of the limitations in this strategy.

The therapeutic relevance of gPV in HRR genes represents an evolving area of breast cancer research. Defects in DNA repair pathways confer increased sensitivity to specific treatments, most notably PARPi, and may also influence response to other systemic therapies, including platinum-based chemotherapy and emerging targeted agents such as *POLQ* inhibitors(74, 185, 191, 193, 195, 213, 216, 247, 248).

In patients with breast cancer and a gPV in HRR genes, such as *BRCA1/2*, DNA damage typically starts as single-strand breaks. When PARPi are used, they "trap" the PARP enzyme on the DNA, preventing the repair of these breaks. These then convert into double strand breaks during replication. Because the cell lacks functional HRR genes, it is unable to repair these double-strand breaks, ultimately leading to synthetic lethality(30, 176, 249).

While this mechanism seems straightforward, research in recent years has described many different biomarkers of both efficacy and resistance. It is now clear that not all HRR genes are involved in the same way or even in the same repair pathways; consequently, they respond

differently to PARPi and other treatment strategies. Furthermore, prior and sequential treatments can significantly alter the biology of the tumor, creating selective pressures that impact the efficacy of subsequent lines of therapy(250).

Historically, *BRCA1* and *BRCA2* were primarily linked to TNBC(69, 148). Consequently, most early studies focused on this subtype, describing a higher sensitivity to platinum-based chemotherapy and aiming to tailor neoadjuvant regimens to achieve higher rates of pCR(185, 191, 193, 213). Similar strategies have been applied in the advanced setting. However, recent evidence has shifted this paradigm, demonstrating that patients with a PV in *BRCA2* predominantly develop HR+/HER2-negative disease. Furthermore, current screening often identifies *BRCA2* at a higher frequency than *BRCA1(69, 70)*.

In early-stage breast cancer, whether TNBC or HR+/HER2-, adjuvant Olaparib, a PARPi, for one year has demonstrated a significant benefit in iDFS and OS for *BRCA1/2* carriers(209). The therapeutic landscape has become increasingly complex with the introduction of cyclin-dependent kinase 4/6 inhibitors (CDK4/6i) in the adjuvant setting(225, 251); for instance, the MonarchE trial showed a clear benefit with two years of Abemaciclib(223, 224, 227). Consequently, current treatment strategies must carefully balance clinical criteria, potential survival benefits, toxicity profiles, and the optimal sequencing of available therapies.

In the advanced breast cancer setting, PARPi such as Olaparib and Talazoparib have demonstrated a significant benefit in PFS compared to traditional chemotherapy for both TNBC and HR +. Although a benefit in OS has not yet been established, this PFS advantage led to FDA approval for patients harboring gPV in *BRCA1/2(215-217)*. However, access to these agents in clinical practice remains challenging and varies significantly across different countries and healthcare systems.

Despite the availability of PARPi, the management of HR+/HER2- metastatic breast cancer is primarily defined by the use of CDK4/6i, which remain the cornerstone of first-line therapy.(20, 219) Nevertheless, resistance eventually develops, and predictive biomarkers for this treatment are still limited. This creates a clinical intersection: emerging evidence suggests that alterations in DNA repair pathways may directly interact with cell cycle regulation, influencing both initial sensitivity and acquired resistance to CDK4/6 inhibition. Impaired DNA repair may promote genomic instability and adaptive resistance mechanisms that affect both endocrine sensitivity and cell cycle control. In this context, germline HRR PV may contribute not only to tumor initiation but also to therapeutic evolution under selective pressure, complicating the choice of the optimal first-line agent(233).

While prior retrospective studies have suggested poorer PFS and OS for *BRCA2* and other HRR carriers treated with CDK4/6i, those analyses faced significant limitations, such as the inclusion of patients with unknown germline status or mixed lines of treatment(228, 229, 231, 233). Our research addresses these gaps by including only patients treated with first-line CDK4/6i, utilizing well-balanced cohorts, and employing a control group with confirmed negative germline testing for all relevant susceptibility genes.

Our findings demonstrate a significantly shorter PFS in patients with germline or somatic PV in *BRCA2*, consistent with previous reports. However, we did not observe a statistically significant difference in OS in the multivariable analysis. Interestingly, our data show that patients with primary or secondary endocrine resistance to adjuvant treatment experience poor outcomes regardless of *BRCA2* status. While endocrine resistance is a known predictor of poor response to first-line CDK4/6i, the specific negative impact of *BRCA2* was most evident among patients with endocrine-sensitive disease. This reduced efficacy in *BRCA2* carriers may be explained by the genomic proximity of *BRCA2* and *RB1* on chromosome 13q, as *RB1* loss is an established resistance mechanism to CDK4/6i. Furthermore, while our findings regarding *BRCA2* are robust, the low prevalence of PV in other HRR genes, such as *ATM*, *CHEK2*, or *PALB2*, limited our ability to draw definitive conclusions about their individual impact on CDK4/6i resistance.

The most prominent limitation of our study was the limited sample size available for translational analyses. Nevertheless, we observed that tumors harboring *RB1* LOH in the context of a *BRCA2* PV had a median PFS of less than 12 months. We also evaluated intrinsic molecular subtypes, building on our group's prior work(252), which reported a substantially higher proportion of non-luminal subtypes among *BRCA2*-PV tumors compared with non-*BRCA2* tumors (63% vs 12%) in patients with advanced HR+/HER2− breast cancer. This imbalance in intrinsic subtype distribution could have contributed to the previously reported association between *BRCA2* mutations and poorer prognosis in this setting. In contrast, within the limited samples available in our cohort, we did not observe a similar enrichment of non-luminal subtypes or a clear association between intrinsic subtypes and clinical outcomes.

Based on the findings of our study and Safonov et al.(233), one potential approach would be to initiate treatment with a PARPi and reserve ET plus CDK4/6 inhibition for later lines, particularly in patients who acquire *BRCA2* reversion mutations as resistance mechanisms to PARPi and regain HR proficiency. In this scenario, *RB1* LOH may be less frequent. Conversely, a recent study reported a median progression-free survival of 11.8 months with PARPi therapy administered after CDK4/6 inhibition plus ET in patient's carriers of *BRCA1/2* PV(253), underscoring the need for additional translational research and prospective clinical studies to better define the optimal sequencing of these treatments. In this context, an ongoing phase III trial (NCT06380751) is evaluating, in the first-line setting, a second-generation Selective Estrogen Receptor Degrader (SERD) combined with a CDK4/6i versus a SERD plus a PARPi, as well as standard endocrine therapy with CDK4/6 inhibition, in patients harboring *BRCA1/2* or *PALB2* germline or somatic PV.

Interpretation of associations between germline HRR PV and treatment outcomes must be cautious, as retrospective analyses are subject to confounding and selection biases. Nevertheless, our results highlight that integrating germline genetic information into treatment outcome studies helps explain differences in treatment response and resistance.

Limitations

This thesis shares several limitations common to research in hereditary cancer, most notably its retrospective design. As with similar studies, this approach is inherently associated with biases such as survival bias and ascertainment bias. However, given the specific characteristics of hereditary cancer populations, retrospective analyses remain a key starting point for hypothesis generation and for informing the design of future prospective studies.

Additional methodological limitations should be acknowledged. These include selection bias toward patients who underwent genetic testing and limited statistical power for subgroup analyses, particularly for moderate-penetrance genes such as *ATM* or *CHEK2*. These constraints are not unique to this work but reflect broader challenges in clinical cancer genetics research across breast cancer and other malignancies. Explicit recognition of these issues is essential to avoid overinterpretation of results and to appropriately frame conclusions.

Some limitations are specific to the methodologies used in individual studies. In particular, the study focused on LAFVs was not restricted to breast cancer but was based on a large commercial testing cohort that included individuals with and without a cancer diagnosis, spanning multiple tumor types. Breast cancer emerged as the only cancer type significantly associated with LAFVs compared with conventional heterozygous variants; however, it was also the most represented malignancy in the cohort, reflecting current clinical practice in which breast cancer accounts for a substantial proportion of germline genetic testing. This context should be considered when interpreting cancer-specific associations. Finally, reliance on data derived from a commercial testing cohort may be associated with incomplete clinical information.

Despite these limitations, this thesis adopts an integrated, real-world approach by combining germline genetic findings with detailed clinical and therapeutic data. This strategy allows assessment of how germline genetic information informs patient care across multiple levels, from risk assessment to treatment selection.

Future research perspectives

Future research arising from this work will focus on the longitudinal follow-up of patients with breast cancer who have undergone germline genetic testing, with the aim of addressing clinically relevant questions through large, multicenter collaborations.

A key priority will be the prospective evaluation of risk-reducing surgical strategies in patients with breast cancer who are carriers of gPV. In particular, future studies should assess the role and clinical impact of CPM/RRM and RRSO, including salpingectomy with delayed oophorectomy, in patients diagnosed with breast cancer, with special attention to women older than 40 years. Prospective data are needed to better define the benefits, timing, and patient selection criteria for these interventions beyond the context of unaffected carriers.

Another important area for future investigation is the optimization of surveillance strategies in patients with breast cancer who have undergone risk-reducing surgeries. Variability in follow-up protocols across institutions and healthcare systems remains substantial, particularly regarding imaging modalities, frequency of surveillance, and long-term monitoring after RRM or RRSO. Comparative studies across centers may help identify best practices and inform the development of more standardized, evidence-based surveillance recommendations for this growing patient population.

From a therapeutic perspective, our findings suggesting poorer outcomes in patients with *BRCA2*-associated breast cancer treated with CDK4/6i highlight the need for further investigation into optimal treatment sequencing strategies. Future studies should explore treatment designs incorporating agents currently used in routine clinical practice, including PARPi and CDK4/6i, to determine whether different sequencing approaches may improve progression-free survival beyond first progression (PFS2) and OS. Attention should be paid to whether upfront use of PARPi, delayed introduction after CDK4/6i exposure, or alternating strategies result in differential clinical benefit.

In parallel, mechanistic studies are warranted to elucidate potential resistance mechanisms associated with treatment sequencing, including the emergence of resistance-related alterations such as *BRCA2* reversion events or cell-cycle pathway adaptations. Understanding how treatment order influences resistance patterns may help guide rational sequencing strategies and inform combination or switch approaches aimed at prolonging treatment benefit.

Beyond these specific clinical questions, future research should continue to prospectively assess the impact of germline genetic findings across diverse populations and clinical settings. Standardized reporting of variant allele frequency, together with the integration of multi-tissue analyses, may improve the interpretative accuracy of LAFVs. Importantly, although germline variants are biologically stable, their clinical relevance is dynamic and may evolve over time as patients age, accumulate additional risk factors, or as new evidence informs variant interpretation. This underscores the need for longitudinal follow-up and periodic reassessment of germline findings rather than viewing genetic results as static conclusions derived at a single time point.

As germline genetic testing becomes increasingly embedded in routine oncology workflows, future efforts should also focus on refining individualized management strategies. Not all gPV confer the same magnitude of cancer risk, and extrapolating recommendations derived from high-penetrance genes to all variants may result in overtreatment. A more nuanced, patient-centered approach that integrates genetic findings with clinical, personal, and familial factors will be essential.

Finally, expanding multicenter cohorts will enable evaluation of emerging therapeutic strategies, including antibody–drug conjugates and other novel agents, within genetically defined subgroups. These data may provide the foundation for the design of prospective clinical trials aimed at improving outcomes for patients with hereditary or HRD breast cancer.

In summary, future research building on this thesis should address surgical, surveillance, and therapeutic strategies through prospective, collaborative efforts. Integrating germline genomic information into precision oncology has the potential to refine risk-reduction approaches, optimize treatment sequencing, and ultimately improve outcomes for patients with breast cancer and their families.

CONCLUSIONS

1. Germline genetic testing is essential for the clinical management of patients with breast cancer and enables personalized screening, surgical, and systemic treatment strategies.
2. The interpretation of low allele frequency variants is a major clinical challenge and requires a structured diagnostic algorithm to distinguish true germline variants from clonal hematopoiesis and constitutional mosaicism.
3. Low allele frequency variants constitute a rare but clinically relevant finding in cancer predisposition genes and are more frequently identified in older patients and in individuals with a personal history of breast cancer compared with classical heterozygous germline variants.
4. Complementary testing using additional biological samples or family studies is necessary to clarify the origin of low allele frequency variants and to guide appropriate clinical surveillance and risk management strategies.
5. Patients with breast cancer carrying germline pathogenic variants show a distinct clinical phenotype, characterized by earlier age at diagnosis, higher likelihood of bilateral disease, increased probability of de novo metastatic presentation, and a higher prevalence of aggressive tumor subtypes.
6. Germline pathogenic variants directly influence clinical management, including surgical approach, use of platinum-based chemotherapy, and treatment with poly (adenosine diphosphate–ribose) polymerase inhibitors.
7. In the metastatic setting, germline pathogenic variants in the breast cancer susceptibility gene 2 are associated with differential sensitivity and resistance to sequential systemic treatments, particularly therapies targeting cyclin-dependent kinases 4 and 6, potentially mediated by loss of heterozygosity of the retinoblastoma 1 tumor suppressor gene, with relevant implications for treatment sequencing strategies. Whether similar mechanisms may operate in carriers of other homologous recombination repair gene variants warrants further investigation.
8. The integration of germline genomic data into routine oncology practice represents a necessary step to improve risk stratification and optimize treatment selection in breast cancer.

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