



Clinical Experience from a Hereditary Cancer Clinic: How Comprehensive Multigene Panels and PARP Inhibitor Responses Shape the Indian Hereditary Breast Cancer Profile

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Abstract

Background: Breast Cancer (BC) is a leading cause of cancer mortality in women worldwide, with significant clinical, and genetic heterogeneity. While BRCA1 and BRCA2 are the most well-studied genes in hereditary breast and ovarian cancer (HBOC), non-BRCA genes, including CHEK2, TP53, ATM, PALB2, and RAD51C, also contribute to BC predisposition [1]. Moreover, the complexity of genetic risk assessment is further highlighted by the fact that many patients with no discernible family history also develop the disease [2]. To improve precision in diagnostics, genetic counselling and focus on targeted treatments, it is essential to comprehend both regional patterns in India and global trends.

Methods: We performed a retrospective analysis of clinical and genetic data from 382 BC patients who were tested between 2022 and 2025 at a tertiary care center in India. Variables included age at diagnosis, family history, molecular subtype, and germline variants across HBOC-related cancer predisposition genes. Retrospective analysis was performed using descriptive statistics. Comparative analysis was conducted with published cohorts from Asian, Caucasian, and American populations. Disease-free survival (DFS) was evaluated in a subset of BRCA1- and BRCA2-mutated patients treated with the PARP inhibitor olaparib.

Results: Pathogenic, likely pathogenic and variant of unknown significance - damaging (VUS-D) were identified in 17.3% (n=66), variant of uncertain significance (VUS) in 30.1% (n=115) and no detectable variants in 52.6% (n=201). Of the positive cases BRCA1 & 2 were most frequently altered genes 78% (n=51). Among the non-BRCA pathogenic variants concluded to 22% (n=15) of the total positive cases. 66 % of patients reported a family history of breast or ovarian cancer. Peak incidence occurred in the 40-to-60-year age group. Among

olaparib-treated patients, BRCA2-mutated individuals demonstrated prolonged DFS with fewer recurrence events compared to BRCA1-mutated patients.

Conclusions: *In addition to characterizing the genetic landscape of breast cancer in this cohort, family and with no family history correlation was found to be significant. Genomic molecular subtype distribution reflects international patterns, but non-BRCA mutations in patients with no family history highlights the need for broad panel testing. The prolonged DFS outcomes observed in BRCA2 mutated patients support the clinical utility of PARP inhibitors in real-world Indian settings and guide the development of mutation-specific therapeutic benefit and reinforces the importance of integrating germline genetic findings into individualized treatment planning. Such efforts are essential in advancing precision oncology in underrepresented populations.*

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Introduction

Breast Cancer (BC) is the most commonly diagnosed malignancy in women worldwide, accounting for one in four of all female cancer cases and a leading cause of cancer related mortality [3]. It is a genetically heterogeneous disease influenced by a combination of environmental, hormonal, and genetic factors [4]. Among the genetic contributors, germline mutations in high penetrance genes such as BRCA1 and BRCA2 play a pivotal role in significantly increasing the lifetime risk of BC. These tumor suppressor genes are essential for maintaining genomic stability through homologous recombination, and their inactivation predisposes individuals to both breast and ovarian cancers [5].

Besides BRCA1/2, several moderate and low penetrance genes have been implicated in hereditary BC, including PALB2, CHEK2, ATM, RAD51, etc. Mutations in these genes are associated with varying degrees of risk and can inform clinical management strategies when interpreted appropriately [6]. The advent of multigene panel testing and next generation sequencing (NGS) has revolutionized the identification of hereditary cancer syndromes, enabling the detection of a broader spectrum of germline mutations. However, it has also led to a growing number of VUS, genetic changes with unclear pathogenicity that complicate

clinical interpretation [7].

BC is a molecularly diverse disease and is classified into distinct subtypes based on receptor expression: luminal A, luminal B, HER2 enriched, and Triple negative breast cancer (TNBC) [8]. TNBC is often associated with BRCA1 mutations and tends to exhibit more aggressive clinical behavior [9]. On the other hand, luminal A and B subtypes, have been associated with CHEK2, ATM, and some BRCA2 alterations, while HER2 enriched are associated with TP53 genes [10,11]. These distinctions are not only relevant to prognosis but also guide targeted treatment strategies.

In the Indian context, the prevalence of BRCA and non BRCA mutations, as well as the frequency of VUS, varies considerably due to population specific variants and the underrepresentation of Indian genomes in global databases [12].

This study evaluates genetic results in a cohort of 382 BC patients. The goal is to contribute to the growing body of evidence in the clinical setup of hereditary care hospitals of western India supporting the integration of multigene panel testing and genetic counseling.

Materials and Methodology

Data Collection

This study involved 382 cases diagnosed with BC that underwent Germline Cancer panel testing, over a period of four years at the Department of Molecular Pathology and Genomics, Kokilaben Dhirubhai Ambani Hospital and Medical Research Institute, Mumbai, India. The data included 363 female patients and 2 male patients with ages between 24 to 85 years.

Inclusion Criteria

- All cases above 18 years of age with a clinical history.
- Both male and female patients were included.
- Patients underwent germline cancer panel testing for 113 genes by NGS and Multiple Ligand Probe Amplification (MLPA).

Exclusion Criteria

- Patients other than BC were excluded from the study.

Germline Testing Protocol

Patient Recruitment and Pre-test Genetic Counseling

All patients underwent comprehensive pre-test genetic counseling conducted by certified genetic counselors. Detailed personal and three generation family histories were obtained to assess hereditary cancer risk. Counseling sessions adhered to established ethical principles, ensuring that patients were fully informed and empowered to make autonomous decisions. Only patients who provided written informed consent proceeded to genetic testing.

Sample Collection and Processing

For consenting individuals, two peripheral blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tubes. Clotted, jaundiced or hemolysed samples were excluded from analysis.

DNA Extraction

Genomic DNA was isolated from 200 μ L of peripheral blood using the Qiagen DNA Mini Kit, following the manufacturer's protocol with minor modifications. Briefly, 200 μ L of blood was mixed with 200 μ L of AL buffer and 20 μ L of Proteinase K, pulse vortexed using a vortex for 10–15 seconds, followed with a brief spin and incubated at 56°C for 10 minutes. Subsequently, 200 μ L of absolute ethanol was added and vortexed & spin briefly. The lysate was loaded onto a silica

membrane spin column centrifuged and sequentially washed with 500 μ L of AW1 and AW2 buffers. A final dry spin was performed before eluting the DNA in 150 μ L of AE buffer. DNA concentration and purity were assessed via spectrophotometry, and samples were stored at –20°C until use [13].

NGS and Detection of Large Genomic Rearrangements (LGRs)

All patient samples underwent multigene panel testing using NGS targeting 113 genes associated with hereditary cancer predisposition. The panel included high and moderate penetrance genes such as BRCA1, BRCA2, TP53, CHEK2, ATM, PALB2, RAD51C, BRIP1, MLH1, among others. The assay was designed to capture all coding exons, flanking intronic regions, canonical splice sites, and known mutation hotspots, enabling detection of single nucleotide variants (SNVs), small insertions and deletions (indels), and splice site alterations [14].

To ensure comprehensive variant detection, MLPA was performed in parallel for selected genes, particularly BRCA1 and BRCA2, to identify large genomic rearrangements (LGRs), including exon level deletions or duplications that may not be detected by standard NGS workflows. MLPA was conducted using SALSA kits (MRC Holland). The integration of NGS with MLPA enhanced diagnostic sensitivity by enabling the detection of both small sequence variants and structural alterations across BRCA1 & 2 hereditary cancer genes [15].

Library Preparation and Sequencing

Library preparation was performed using Illumina's Germline Cancer Panel Kit, employing either hybrid capture or amplicon-based enrichment strategies, depending on the sample batch. Prepared libraries were subjected to paired end sequencing on the Illumina MiSeq platform, generating high depth sequence data [16].

Bioinformatics Pipeline and Variant Classification

Raw sequencing data were processed using a standardized bioinformatics pipeline. Reads were aligned to the human reference genome (GRCh37/hg19) using BWA MEM, followed by duplicate marking, base quality recalibration, and realignment using GATK tools. Variant calling was performed

using HaplotypeCaller (GATK), and annotation was conducted using ANNOVAR and Ensembl VEP, incorporating population databases (gnomAD, 1000 Genomes), disease databases (ClinVar, HGMD), and computational prediction tools (e.g., SIFT, PolyPhen 2, MutationTaster, CADD) [17].

Variants were interpreted and classified according to the 2015 ACMG guidelines, incorporating data from ClinVar, Varsome, and internal curation systems. Classification categories included pathogenic, likely pathogenic, VUS, likely benign, and benign. Variants were curated manually by a multidisciplinary team including clinical geneticists, molecular pathologists, and bioinformaticians [18].

Post-test Genetic Counseling

All patients received post-test genetic counseling tailored to their test results. Individuals with pathogenic or likely pathogenic variants were counseled regarding cancer risk, implications for family members, and potential surveillance or risk reduction strategies. Psychological support and appropriate clinical referrals were provided where needed.

Statistical Analysis

A retrospective analysis was performed using descriptive statistics to summarise the cohort. We studied categorical variables, including age group, family history, histopathological and molecular subtype, gene mutations, and variant classifications, were summarized by frequencies and percentages. Kaplan-Meier survival analysis is a statistical method used to measure the time it takes for an event recurrence and disease-free survival (DFS) analysis, the Kaplan-Meier method tracks the time from the start of a treatment until the disease returns or a patient dies from any cause [19].

Comparative Literature Search

A thorough literature review was conducted using PubMed, Google Scholar, and ScienceDirect. The SEARCH STRATEGY COMBINED MEDICAL SUBJECT HEADINGS (MeSH) using keywords like “BC,” “age distribution,” “BRCA1,” “gene mutation,” and population labels (“Indian,” “Asian,” “Caucasian,” etc.).

Variant Reclassification Protocol

All reported VUS were systematically re-evaluated using updated databases, like Clinvar, in silico prediction tools, and current ACMG guidelines every six months. This reclassification process was undertaken to assess potential clinical implications, including eligibility for targeted therapies, consideration of risk reducing interventions, and the need for familial cascade testing [19].

Results

Patient Characteristics

The cohort comprises 382 BC patients and serves as a representative sample for investigating clinical and molecular patterns within the local population.

Age Distribution

Out of a cohort of 382 patients, 10.67 % were aged between 20-30 years; 20.79 % were aged between 30-40 years, 25.56% were aged between 40-50 years, 28.37% were aged between 50-60 years, 10.95% were aged between 60-70 years, and 3.65% over the age of 70 years. This age distribution parallels patterns seen in Asian cohorts [2].

Family History

Analysis of family history revealed that 66% of patients had known history of breast and ovarian cancer in the family. Notably, of which 57% of these were diagnosed at a younger age (under 50 years). These findings support the importance of genetic testing, particularly in first degree relatives of affected individuals [20]. Moreover, patients with the absence of a family history in rest of the cases (34%), pathogenic variants were seen in non- BRCA genes like FANCA, MLH1, RAD51D, MUTYH, etc. highlighting the need for broader genetic screening beyond family history criteria.

Molecular Subtypes

TNBC was most commonly observed across young age (24 to 50) patients tested positive for BRCA1 mutations [21]. BRCA2, and other non BRCA variants were seen commonly in Luminal A & B across a broader age range (35-70) whereas HER2 enriched tumors were prevalent in older age patients (>50) with ATM mutations. This molecular subtype highlights the molecular heterogeneity of BC in the cohort and provides context for both clinical management and genetic counselling.

Germline Variant Distribution

Germline genetic testing revealed that pathogenic, likely pathogenic and VUS-D variants were identified in 17.3 % of the cohort. VUS constituted a substantial proportion, accounting for 30.1 % and no detectable variants were found in 52.7% of the cases.

Among the identified mutations, BRCA1 and BRCA2 emerged as the most frequently altered genes, with mutation frequencies of 54% (n=36) and 22.7 % (n=15) of the total positive cases, respectively. Within BRCA1, Intron 16 and Exon 2 were the most commonly affected regions among BRCA1 mutations. Similarly, within BRCA2, exon 11 was most common mutation site for BRCA2 mutations.

In addition to BRCA mutations, pathogenic variants were also detected in non BRCA genes like FANCA, CDH1, MUTYH, PALB2, PTEN, RAD51D, MLH1, ATM, and RET genes contributing to the 22.7% (n=15). Moreover, a variant of uncertain clinical significance with a damaging (VUSD) was reported in PALB2, further highlighting the genomic complexity.

Importantly, the majority of pathogenic and likely pathogenic variants identified in this cohort were predicted to result in loss-of-function (LOF) effects, including frameshift, nonsense, canonical splice-site, and complete exon deletions. Such variants are expected to produce truncated or absent protein due to nonsense-mediated mRNA decay (NMD) or disrupt essential functional domains, contributing to high penetrance cancer risk [22]. This mechanistic insight provides biological context for the observed early-onset and aggressive tumor phenotypes and draws attention to the clinical relevance of characterizing predicted protein consequences alongside variant classification (Table 2, 3). Missense variants are annotated based on established functional or clinical evidence.

The predominance of VUS across multiple genes, including ATM, BARD1, BLM, BMPR1A, BRIP1, CHEK2, DICER1, FANCA, FANCC, FANCI, FANCM, MRE11, MSH2, MSH6, POLD1, POLE, PTCH1, PMS2, RAD50, RAD51B, RAD51C, RAD51D, STK11, SUFU and others, accentuates the ongoing challenge in clinical classification. This highlights the necessity for continued functional studies, robust variant curation, and the development of population specific genomic databases. These findings emphasize the molecular heterogeneity of germline alterations in BC and reinforce the importance of multigene panel testing in facilitating precision oncology and informed genetic counseling.

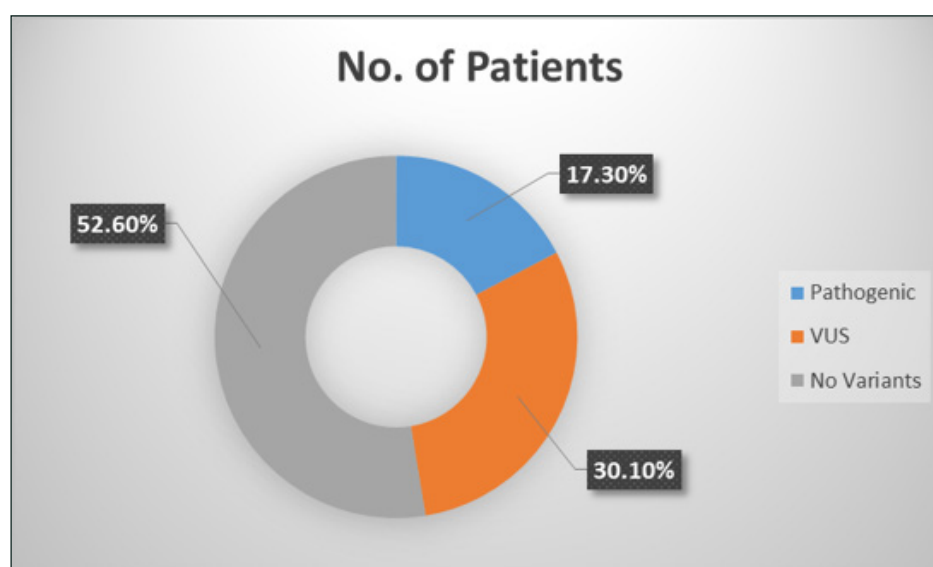


Figure 1: Distribuion of Genetic variants among Breast Cancer patients.

The donut chart illustrates the distribution of genetic testing results among the study cohort. Variants were

classified as Pathogenic, VUS, and no variants. The chart highlights the predominance of VUS and the significant proportion of patients with no detectable variants.

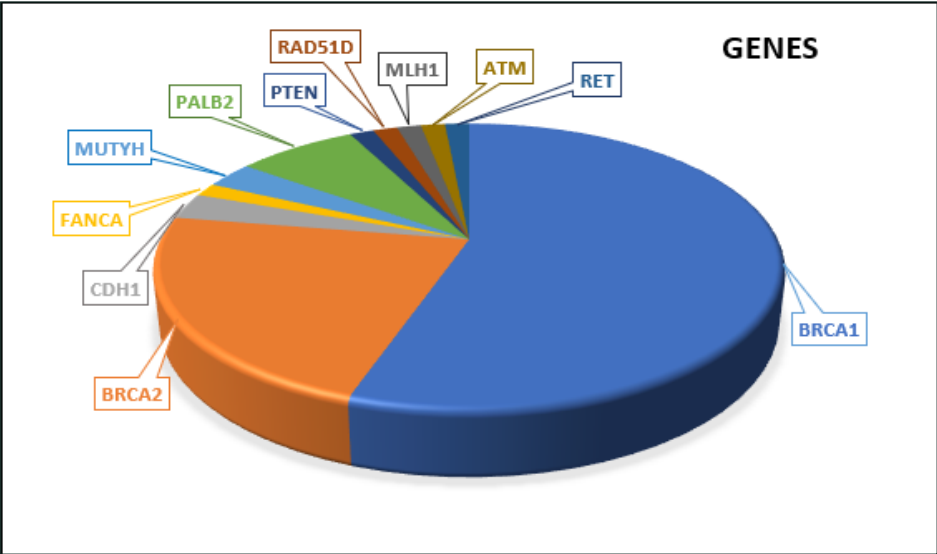


Figure 2: Distribution of pathogenic variants across genes.

The pie chart illustrates the proportion of pathogenic variants, likely pathogenic variants and variants of uncertain significance with damaging effects detected in each gene, including BRCA1, BRCA2, CDH1, FANCA, MUTYH, PALB2, and PTEN, RAD51D, MLH1, ATM, RET. The chart highlights the relative contribution of each gene to the overall mutational landscape observed.

Table 1: Distribution and Molecular Consequences of Pathogenic and Likely Pathogenic BRCA1 and BRCA2 Germline Variants Identified in Breast Cancer Patients

This table summarizes the distribution and molecular consequences of pathogenic and likely pathogenic germline variants identified in the BRCA1 and BRCA2 genes among breast cancer patients. Single-nucleotide variants (SNVs), insertions, deletions, duplications, indels, or copy number variants (CNVs). The number of patients indicates the count of unrelated individuals harboring each variant.

Gene location	No. of Patients	Type of mutation	Mutation Nomenclature	Functional Consequence
BRCA1 Intron 16	9	SNV	c.5074+1G>A, p.?	Exon skipping
BRCA1 Intron 17	3	SNV	c.5153-2 A>G, p.?	Exon skipping
BRCA1 Exon 2	7	Deletion	c.68_69del, p.Glu23ValfsTer17	Frameshift
BRCA1 Exon 3	2	SNV	c.81T>A, p.Cys27Ter	Nonsense
BRCA1 Exon 4	1	SNV	c.212+2T>C, p.?	Exon skipping
BRCA1 Exon 6	1	Deletion	c.431delA, p.Asn144IlefsTer19	Frameshift
BRCA1 Exon 7	2	Duplication	c.512dupT, p.Gln172ThrfsTer10	Frameshift
BRCA1 Exon 10	1	Deletion	c.2679_2682del, p.Lys893AsnfsTer106	Frameshift
	1	Deletion	c.2981_2982del, p.Cys994Ter	Nonsense
	1	Duplication	c.1961dupA, p.Tyr655ValfsTer18	Frameshift
	1	Deletion	c.2188_2201del, p.Glu730ThrfsTer5	Frameshift
	1	Deletion	c.2966_2967delTT, p.Phe989CysfsTer2	Frameshift

BRCA2 Exon 11	1	Deletion	c.4251_4252delGT, p.Leu1418ArgfsTer9	Frameshift
	1	SNV	c.4370C>G, p.Ser1457Ter	Nonsense
	1	Deletion	c.(4484+1_4485-1)_(4675+1_4676-1)del	Haplo-insufficient
	3	Deletion	c.5035del, p.Leu1679Ter	Nonsense
	1	SNV	c.5444G>A, p.Trp1815Ter	Nonsense
	1	SNV	c.682-2A>G, p.?	Exon skipping
	1	Deletion	c.394_398delTGTCC, p.Cys132ThrfsTer7	Frameshift
	1	Deletion	c.1773_1776delTTAT, p.Ile591MetfsTer22	frameshift
BRCA2 Exon 11	3	Deletion	c.5851_5854del, p.Ser1951TrpfsTer11	frameshift
	1	Deletion	c.6601del, p.Ser2201LeufsTer5	frameshift
	1	Deletion	c.3599_3600delGT, p.Cys1200Ter	Nonsense
	1	Deletion	c.2808_2811delACAA, p.Ala938ProfsTer21	Missense
	1	Deletion	c.2092delC, p.Leu698TyrfsTer32	frameshift
	1	Frameshift	c.2677delC, p.Gln893LysfsTer2	frameshift
	1	Deletion	c.4570_4573delTTTC, p.Phe1524IlefsTer18	frameshift
BRCA2 Exon 14	1	Indel	c.7219_7221delinsA, p.Val2407ThrfsTer4	frameshift
BRCA2 Exon 16	1	SNV	c.7795G>T, p.Glu2599Ter	nonsense
BRCA2 Exon 22	1	Insertion	c.8915_8916insTC, p.Leu2972PhefsTer5	frameshift

Male Breast Cancer

Table 2: Distribution and Molecular Consequences of Pathogenic and Likely Pathogenic Variants Identified in Non-BRCA Cancer Susceptibility Genes

This table presents pathogenic and likely pathogenic germline variants identified in non-BRCA cancer susceptibility genes, including CDH1, FANCA, MUTYH, PALB2, PTEN, RAD51D, MLH1, ATM, and RET, among breast cancer patients. Variant type denotes the underlying genomic alteration (SNV, insertion, deletion, duplication, indel, or CNV). The number of patients represents the count of unrelated individuals carrying each variant.

Gene location	No. of Patients	Type of mutation	Mutation Nomenclature	Functional Consequence
CDH1 Exon 11	2	c.1679C>G, p.Thr560Arg	SNV	missense
FANCA Exon 27	1	c.(2504+1_2505-1)_(2601+1_2602-1)del	Deletion	frameshift
MUTYH Exon 13	1	c.1156C>T, p.Gln386Ter	SNV	Nonsense
MUTYH Exon 14	1	c.1354G>T, p.Glu452Ter	SNV	Nonsense
PALB2 Exon 4	1	c.758dupT, p.Ser254IlefsTer3	Duplication	frameshift
PALB2 Exon 5	1	c.2390_2396del, p.Gln797ProfsTer52	Deletion	frameshift
PALB2 Exon 7	1	c.2713C>T, p.Gln905Ter	SNV	nonsense
PALB2 Exon 13	1	c.3494dupC, p.Thr1167Tyrfs	Duplication	frameshift
PALB2 Intron 9	1	c.2997-1G>A, p.?	SNV	Exon skipping
PTEN Exon 5	1	c.388C>T, p.Arg130Ter	SNV	Nonsense
RAD51D Exon 5	1	c.363delA, p.Ala122GlnfsTer14	Deletion	Frameshift
MLH1 Exon 8	1	c.676C>T, p.Arg226Ter	SNV	Nonsense

ATM Exon 18 *	1	c.2672C>G, p.Ser891Ter	SNV	Nonsense
RET Exon 14	1	c.2410G>A, p.Val804Met	SNV	Missense

Male Breast Cancer

Disease-Free Survival Outcomes

In this retrospective study of patients with germline BRCA-mutated breast cancer treated with olaparib in either the adjuvant or maintenance setting, we evaluated for disease-free survival (DFS) across two cohorts, predominantly in maintenance settings.

BRCA1 Cohort (n = 25/37)

In a separate cohort of patients with BRCA1-mutated breast cancer, DFS ranged from 10 to 120 months, with 2 recurrence events, one of which occurred prior to initiation of olaparib. Kaplan–Meier analysis of DFS for the BRCA1 cohort is presented in Figure 2, with patient-level outcomes detailed in Table 5.1. Median DFS for the BRCA1 cohort, as reported in Table 4, was approximately 15–18 months.

BRCA2 Cohort (n = 11/15)

Patients with BRCA2-mutated breast cancer (Table 5.1) received olaparib either in the adjuvant or maintenance setting. DFS ranged from 10 to 22 months, with 2 recurrence events observed (at 10 and 12 months). Patients with high-risk clinical features, including bilateral disease, prior contralateral breast cancer, and exposure to neoadjuvant chemotherapy, maintained DFS following olaparib initiation. No disease-related deaths were reported. The Kaplan-Meier estimate of DFS is shown in Figure 1, and patient-level outcomes are summarized in Table 5.2. Median DFS for this cohort, as summarized in Table 4, was approximately 36 months. Overall, olaparib treatment was well tolerated, with no disease-related mortality observed in either cohort during follow-up. Patients demonstrated durable disease control, with censored observations indicated in the Kaplan-Meier curves representing individuals who remained disease-free at last assessment. These findings are summarized in Table 4, which provides a comprehensive overview of patient characteristics, molecular subtype, olaparib treatment setting, median DFS, DFS range, and recurrence events.

Table 3: Clinical characteristics and disease-free survival outcomes in BRCA-mutated breast cancer patients treated with olaparib.

Gene	Patients on Olaparib/Total no. of patients	Olaparib setting	Median DFS (months)	DFS range (months)	Recurrence events
BRCA1	25/37	Predominantly Maintenance	≈15-18	10-22	2
BRCA2	12/15	Adjuvant and Maintenance	≈36	13 - 120	2

*DFS = disease-free survival. Patients without documented recurrence were censored at the last follow-up.

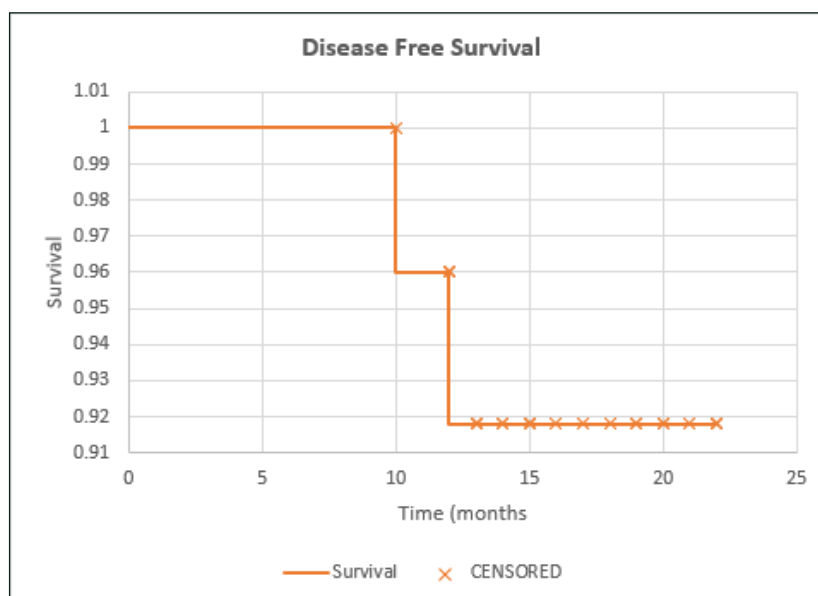


Figure 3: Kaplan–Meier Curve for Disease-Free Survival in BRCA1-Mutated Breast Cancer (25 Patients)

Kaplan–Meier estimate of disease-free survival (DFS) in a cohort of 25 patients with germline BRCA1-mutated breast cancer receiving olaparib. Two disease events occurred early during follow-up (at 10 and 12 months), after which the survival curve plateaued, indicating durable disease control. Tick marks denote censored observations. No disease-related mortality was observed during the follow-up period.

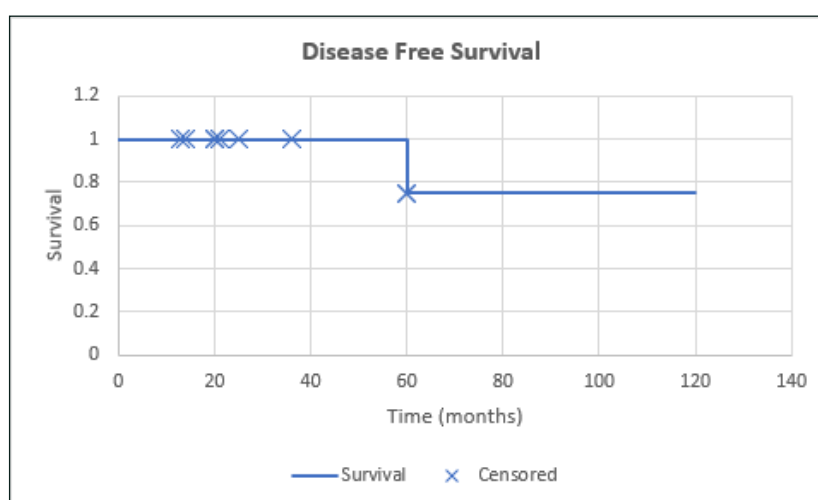


Figure 4: Kaplan–Meier Curve for Disease-Free Survival in Germline BRCA2-Mutated Breast Cancer (Cohort 1)

Kaplan–Meier estimate of disease-free survival (DFS) in a retrospective cohort of 11 patients with germline BRCA2-mutated breast cancer treated with olaparib in the adjuvant or maintenance setting following standard multimodal therapy. At a median follow-up of approximately 18 - 24 months, DFS remained high, with only two disease events observed during follow-up. Censored observations are indicated by crosses and represent patients who remained disease-free at last assessment. No disease-related deaths occurred during the study period.

Discussion

This study provides insights into the clinical and genetic spectrum of BC patients in India and contextualizes them within global patterns. A key observation was the relatively younger age at diagnosis, with the majority of cases occurring in the fourth and fifth decades of life. This contrasts with Western cohorts, where the median age

Ser891Ter) in a case of male breast cancer. Pathogenic ATM variants are increasingly recognized as moderate to high-risk breast cancer susceptibility factors, particularly in male patients, where their relative contribution appears to be greater than in female breast cancer [36,37]. While BRCA2 remains the most frequently implicated gene in male breast cancer, accumulating evidence suggests that ATM mutations represent a significant non-BRCA genetic contributor, especially in cases lacking a strong family history [38].

The detection of an ATM-associated male breast cancer case in this Indian cohort expands the limited data on the genetic basis of male breast cancer in South Asian populations. It further supports the inclusion of ATM in multigene testing panels for male breast cancer patients and highlights the clinical relevance of identifying such variants for tailored surveillance, therapeutic decision-making, and cascade testing of at-risk relatives [39].



Figure 6: IGV visualization confirms a high-confidence heterozygous C>G variant on chromosome 11, supported by sequencing reads from both strands.

A significant finding was the identification of three patients with pathogenic PALB2 variants involving frameshift and one with a likely pathogenic variant involving a nonsense mutation. Additionally, VUSD was also observed in the PALB2 gene, which was a splice site variant. One of the most challenging findings was the high prevalence of VUS observed in our cohort, consistent with findings from other studies [40-42]. In our analysis, VUS accounted for over one-third of all reported variants. This rate is considerably higher than what has been reported in Western populations [43]. This elevated frequency may reflect the underrepresentation of Indian broader South Asian genomes in global variant reference databases, which limits the accuracy of clinical interpretation. The reclassification of a VUS to likely pathogenic during the course of the study reinforces the dynamic nature of variant interpretation and its direct clinical implications for patient management, therapeutic eligibility, and successive family testing strategies. These findings stress the urgent need to expand regional genomic databases and to invest in population-specific functional studies to reduce diagnostic uncertainty and improve precision oncology outcomes. These real-world DFS findings are concordant with evidence from pivotal clinical trials, most notably the OlympiA study, which demonstrated a significant improvement in invasive disease-free survival with adjuvant olaparib in patients with germline BRCA1- or BRCA2-mutated high-risk early breast cancer [44]. In the metastatic setting, PARP inhibitors such as olaparib have also demonstrated significant progression-free and overall survival benefits in germline BRCA-mutated breast cancer, further supporting their broad clinical utility across disease stages [45]. The durability of DFS observed in BRCA2-mutated patients in our cohort further supports the sustained benefit of PARP inhibition beyond controlled trial environments and highlights the translational relevance of integrating targeted therapies into routine clinical practice in resource-diverse settings.

Conclusions

The retrospective design, limited sample size, and relatively short follow-up for some patients represent important limitations; however, the integration of detailed genomic annotation with longitudinal clinical outcomes provides valuable real-world evidence supporting the clinical utility of olaparib in BRCA-mutated breast cancer. In addition, the identification of pathogenic truncating variants across multiple susceptibility genes, highlights therapeutic responsiveness to DNA damage-targeted agents such as PARP inhibitors [46]. A particularly challenging and critical observation was the elevated burden of VUS, which accounted for over one-third of all reported variants. This increased rate highlights the lack of adequate representation of Indian and South Asian genomes in global variant databases, which hampers accurate interpretation. The reclassification of a VUS to likely pathogenic during the study period further emphasizes the evolving nature of variant interpretation and its direct clinical consequences.

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