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Mutational landscape of BRCA gene mutations in Indian breast cancer patients: retrospective insights from a diagnostic lab

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Abstract

Background Presence of Germline mutations in the *BRCA*1 and *BRCA*2 genes is the most significant epidemiological factor for breast cancer (BC), where germline BRCA1 (g*BRCA* 1) mutation increases the risk for BC by 59–87% and g*BRCA* 2 mutation increases the risk by 38–80%. In this retrospective study, we have analyzed NGS-based genetic data for samples received at our laboratory for genetic testing over a three-year period to understand the prevalence and pattern if any of *BRCA*1 and *BRCA*2 mutations in Indian breast cancer patients.

Results BRCA gene sequencing using NGS was performed in 395 consecutive cases of BC referred for testing to our lab between 2021 and 2023. Genetic analysis of mutations *BRCA* 1 and *BRCA* 2 genes resulted in 115 (29%) positive patients. Out of 115 patients, 79 reported *BRCA*1 mutations, whereas 36 had *BRCA*2 mutations. Exon 10 (57.3%) of *BRCA*1 and exon 11 (52%) of *BRCA*2 were the most mutated exons observed in this study. The c.1961delA (26.4%) variant, followed by the c.68_69delAG (22.7%) variant in *BRCA*1, and the c.6373delA (20.5%) variant in *BRCA*2, were the most common mutations found in our study. Our data shows positive correlation of younger age group (20–45 years) with BRCA positive status (Chi-square *p* value = 0.001).

Conclusion *BRCA* mutation prevalence was 29.1% in our data which is higher than Western countries. Based on our findings BRCA screening looks imperative for women with BC especially younger women (< 50 years), as family history based BRCA testing would miss out many *BRCA* positive candidates which could benefit from PARP therapy options.

Keywords Breast cancer, BRCA1/2, Next generation sequencing, Mutations, Indian population

Introduction

Incidences of Breast Cancer (BC) are on rapid rise (by 39% over past two decades) making it the most common malignancy among Indian women, accounting for 28.2% of all female cancers [1–3]. Also, as compared to western countries, the burden of avoidable deaths from BC disproportionately affects low-income and middle income countries, where more than 70% of breast cancer deaths occur in people younger than 70 years of age. This is because 70% of BC cases are reported in the advanced stages [4, 5]. The epidemiology of BC, incidence rate, clinical outcomes and mortality rate differ significantly in Indian women when compared with the Western population [2] but limited data availability hinders objective comparison [2, 6, and 7]. Furthermore, genetic epidemiology data for BC in Indian patients is even scarce.

Presence of Germline mutations in the *BRCA*1 and *BRCA*2 genes is the most significant epidemiological factor for BC, where germline BRCA1 (g*BRCA* 1) mutation increases the risk for BC by 59–87% and g*BRCA* 2 mutation increases the risk by 38–80%. [8, 9]. *BRCA* 1/2 gene test not only helps in risk prediction for BC, but

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also helps in treatment regime planning with Poly-Adp Ribose Polymerase inhibitors (PARPi) [10, 11]. Various studies both clinical and preclinical, showed that *BRCA* is an important factor affecting chemotherapy response and treatment toxicity in breast cancer patients [12, 13]. Therefore, screening for *BRCA* mutations almost becomes a prerequisite for early intervention in women with a family history of breast cancer or among young BC patients. However, for the management of those with *BRCA*-mutated BC, it is essential that healthcare providers understand the burden of *BRCA*-mutated disease and the prevalence in the given population, as it is noted that the frequency of mutations in these genes is higher in certain populations [9].

To date, the knowledge about the prevalence of *BRCA* 1/2 mutations in BC patients belonging to some regions of India is poor. Compilation and understanding of genetic data along with correlation of treatment follow-up could help in reducing time and cost ensuring better reach to mass Indian population.

Therefore, in this retrospective study, we have analyzed NGS-based (Next Generation Sequencing) genetic data for samples received at our laboratory for genetic testing over a three-year period to understand the prevalence and pattern if any of BRCA1 and BRCA2 mutations in Indian breast cancer patients.

Materials and methods

Study population: Informed consent was obtained for a total of 395 patients diagnosed with breast cancer, and subsequently referred for germline BRCA 1/ 2 gene testing to our laboratory between January 2021 and December 2023. Patients were not selected for clinical characteristics/ cancer subtype or family history. All patients were females in the age ranging from 22 to 87 years.

DNA extraction and high-throughput sequencing

3 ml peripheral blood was obtained from each patient and genomic DNA was extracted using a DNA Blood Mini kit (Qiagen). The target regions in the *BRCA*1 and *BRCA*2 genes were amplified using the Ion AmpliSeq Library kit plus and Oncomine *BRCA* Research Assay panel (ThermoFisher Scientific) according to manufacturer's protocol (Detailed methods has been provided in supplementary data). Next generation sequencing was performed on Ion Torrent S5 NGS platform (ThermoFisher Scientific).

Bioinformatics analysis and variant interpretation

The sequence reads were aligned to hg19/GRCh37 using Torrent suite software v.50. Variant calling and annotation was performed using Ion Reporter Software 5.12 (*BRCA* Oncomine 5.12, Thermo Fisher Scientific). Only exonic and splice site variants (Indels/SNVs) found in the Oncomine *BRCA* assay were used for clinical interpretation. Variants were classified according to the American College of Medical Genetics and Genomics (ACMG) recommendations [14, 15]. Statistical analyses were performed in R programming language and Vassar stats [16].

Results

A total of 395 female breast cancer patients were considered for the study. The women belonged to different age groups, with youngest patient being tested at 18 years and the oldest patient at 76 years. Among the studied 395 patients, majority belonged to premenopausal age group; $n=218$ (55.1%) and 177 (44.8%) women were in post-menopausal stage. Overall mean age at diagnosis was observed to be 45 years. The mean age at diagnosis for the premenopausal women was 40 years and 60 years in the post-menopausal group.

The NGS sequencing of these 395 patients had an average of 0.25million reads per patient, with the mean read length being 106 bp. The average read depth was ~500X, with the mean percentage of reads on target being >98.6%. Genetic analysis of mutations *BRCA* 1 and *BRCA* 2 genes resulted in 115 (29%) positive patients. *BRCA* positive patients were defined as patients who were identified with Pathogenic or Likely Pathogenic variant in one of the *BRCA* genes. Current analyses have not considered VUS (variants of uncertain significance) due to their unknown effects at this point of time. Among the 115 positive patients, a total of 34 SNVs and 82 Indels (short insertions/deletions) in the exonic region *BRCA*1 and *BRCA*2 genes were identified.

Out of 115 patients, 79 reported *BRCA*1 mutations, whereas 36 had *BRCA*2 mutations. Among the 79 positive *BRCA*1 patients, 76 (96.2%) variants were classified as pathogenic and 3 (3.79%) were likely pathogenic. Similarly. Out of 36 positive *BRCA*2 mutations, 34 (94.7%) Pathogenic and 2 (5.71%) Likely pathogenic variants were detected. Interestingly, the co-presence of two different *BRCA*2 variants; c.475+1G>A and c.476-2A>G was observed in one patient. Some patients showed presence of an additional VUS along with a pathogenic BRCA mutation.

Exon 10 (57.3%) of *BRCA*1 and exon 11 (52%) of *BRCA*2 were the most mutated exons observed in this study. The c.1961delA (26.4%) variant, followed by the c.68_69delAG (22.7%) variant in *BRCA*1, and the c.6373delA (20.5%) variant in *BRCA*2, were the most common mutations found in our study. The most common types of mutation are attributed to small insertion/deletion frameshift (66.7%), nonsense mutations (27.8%) and missense mutations (5.2%). Mutational

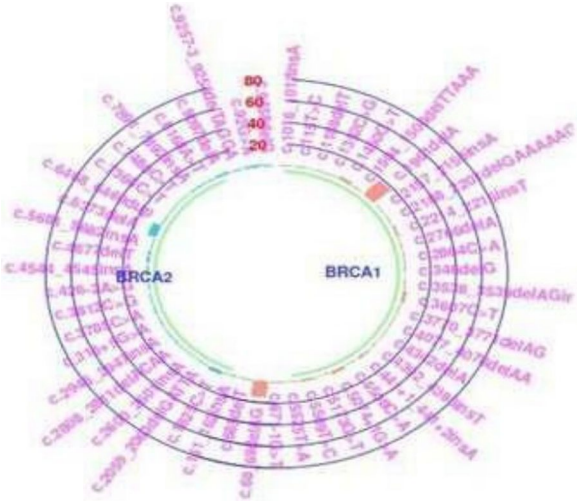

Fig. 1 Mutational landscape for Pathogenic variants in BRCA1/ 2 genes

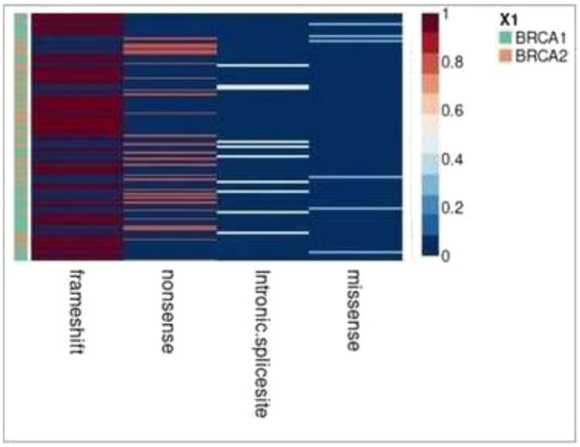

Fig. 2 Heatmap representing commonly found mutations

landscape details of pathogenic mutations in *BRCA* 1/2 genes is shown in Fig. 1 (Mutational landscape of pathogenic variants in *BRCA*1/2 genes). Figure 2 (Heatmap representing commonly found mutations) shows a heatmap of mutations in each positive patient.

Chi-square test was performed to determine correlation between age categories and *BRCA* mutation status in the *BRCA* positive patients (Table 1). We stratified patients into three groups based on age at diagnosis. Our data shows positive correlation of younger age group (20–45 years) with *BRCA* positive status (Chi-square p-value ≥ 0.0001) Fig. 3. Of the 395 patients, 218 (55.1%) women were premenopausal and 177 (44.8%) women were in post-menopausal stage. The mean age at diagnosis for the premenopausal women was 45 years and 60 years for the post-menopausal group.

Discussion

Several studies have reported how the prevalence of germline mutations and gene specific risk evaluations depend, not only on factors such as family history of cancer or tumor molecular subtype, but also on factors like age, race/ ethnicity and geographical location [17, 18].

In this retrospective study, we report our observations on the prevalence of *BRCA* gene mutations in BC patients from different geographical regions of India tested in our laboratory. However, the patients for this study were referred by clinicians based on their BC diagnosis, irrespective of age and geographical location. However, we noticed, most patients were from Northern part of the country (North India ~57.9%, South India ~25.5%, East 10.3% and West 6.07%). Since, the patients from other Indian locations (Southern/Eastern/Western or Central) were not enough for appropriate representation we did not proceed with any location specific analyses. Only patients with clinically diagnosed Breast cancer ($n=395$) were included in the study. Our data for the course of 2 years (2021–2023) shows, 29.1% patients were detected as *BRCA* positive. Chedda et al. [19] reported prevalence (31.9%) of pathogenic variants in *BRCA1*/ 2 genes in 160 HBOC Indian patients. Previous study conducted by Mittal et al. [20] reported a prevalence (18.64%) of P/LP mutations in 236 BC patients from the northern part of India [20]. Kulkarni et al. [21] with 94 BC patients showed prevalence of 25.5%, whereas Ajay Gogia et al. [22] reported a 16.6% prevalence of *BRCA*1 and *BRCA*2 mutations in 210 BC patients.

Even with larger sample size as compared to previous Indian studies our data too aligns with presence of higher prevalence rate (29.1%) of BRCA mutations. This also holds up an overall indication that the prevalence rate of *BRCA* gene mutations in Indian BC patients is much higher than the Western population which is reported in range of (5–10%) [21]. Study by Singh et al. [23] with 1010 Breast and ovarian cancer patients reported prevalence of 30.1% but this study was not exclusive for BRCA gene mutations.

Indian scenario is characterized by a younger median age of onset (<50 years) and a higher prevalence of triple-negative breast cancer (TNBC) [20]. In our study, we observed the mean age at diagnosis of the premenopausal women was 45 years and among these 60.8% were *BRCA* positive. Similarly, for the post-menopausal women the mean age at diagnosis was 60 years and 39.1% were *BRCA* positive. The correlation analyses also throws light on presence of BRCA gene mutation and age at diagnosis. Our data shows positive correlation of younger age group (20–45 years) with BRCA positive status (Chi-square p-value $=0.001$). Our observations align with other Indian studies to show peculiarity of Indian BC data in

Table 1 Distribution of BRCA positive patients based on age and menstrual status

***BRCA* mutations**				
Characteristics	**Total**	**Carriers**	**Non-carriers**	***P* value**
	n	***n* (%)**	***n* (%)**	
Total	395	115 (29.1%)	280 (70.8%)	$P \leq 0.0001$
Age				
20-45 years	156	56 (35.8%)	100 (64.1%)	$P \geq 0.0001$
45-65 years	200	51 (25.5%)	149 (74.5%)	$P = 0.329X10^{-12}$
> 65 years	39	8 (20.5%)	31 (79.4%)	$P = 0.003$
Menstrual status				
Premenopausal < 50 years	218	70 (60.8%)	148 (67.8%)	$P = 0.372X10^{-7}$
Postmenopausal > 50 years	177	45 (39.1%)	132 (74.5%)	$P = 0.617 \times 10^{-11}$
Tumor localization				
Right	202			
Left	177			
Bilateral	3			
Unknown	13			

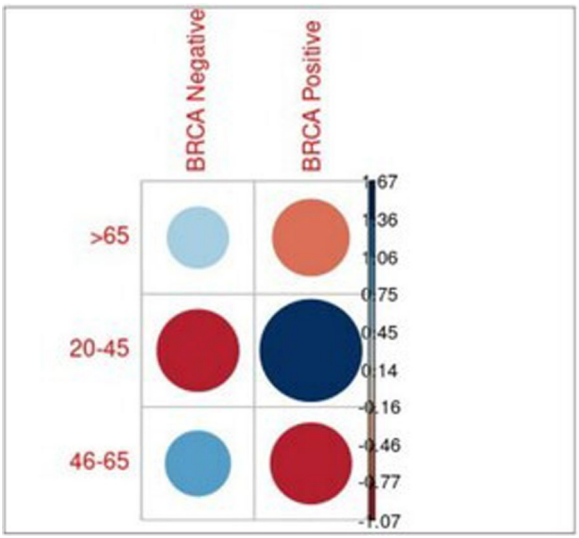

Fig. 3 Plot showing 20-45 years age category is highly associated with BRCA positive status (p value = 0.0001) using Chi square test

terms of peak age. This observation is also in line with the fact that germline mutations drive early onset and most often an aggressive cancer.

The most frequent BRCA1 gene mutations were c.1961delA and c.68_69delAG found in our study. The c.1961delA mutation, also known as 185delAG was seen in 22.7% cases ($n=18$) in this study. This is also a common mutation reported in the Central and Southern European populations [8], but it has also been reported among several other Peruvian, Russian, Egyptian, Korean, Iranian [24, 25], and some of the Indian populations [26]. Another frequent recurrent mutation in *BRCA*1 was c.68_69delAG, seen in 21.5% cases ($n=17$), which was also reported in two different studies conducted in India [27, 28]. Also, the c.68_69del *BRCA*1 mutation was first reported in Ashkenazi Jews [28, 29], consequently in Argentina, Brazil (0.3%), Chilean (0.6%), Peru (2.6%) and Russian populations. In addition, this c.68_69del mutation is described as a founder mutation in Egyptian and Hungarian BC patients [30]. Generally, the *BRCA*1 c.68_69delAG mutation is also found more commonly in Asian, Arabic, African, European, and American populations than the c.1961delA mutation [31]. Mannan et al. [32] reported that c.68_69delAG mutation is associated mainly with Northern and Southern parts of India [28, 33–35]. A previous study suggested that the origin of the c.68_69delAG mutation in Indian population is independent of that of Ashkenazi Jews based on haplotype analysis [36]. Further studies will be helpful in determining the frequency of the c.68_69delAG mutation and its origin in Indian population.

Six known *BRCA*1 pathogenic mutations were identified in twelve patients. Among them: (1) c.5074 + 1G > A variant of exon 16 was $n=4$ (3.47%) (2) Both c.3607C > T and c.1352C > G variants of exon 10 in $n=3$ (2.6%) each, (3) c.5509 T > C, c.2214_2215insT and c.441 + 1_441 + 2insA in exon 23, 10 and 6, respectively in n = 2 (1.7%) each. The c.5074 + 1G > A which was earlier reported as an Icelandic founder mutation was found in $n=4$ (3.47%) [37]. Saxena et al. [35] and Mannan et al. [32] from India reported the same mutation in three patients suggesting that this could be due to common ancestry or they migrated from the same place. The c.3607C > T variant which produces the amino

acid change p.Arg1203Ter has been associated with an increased risk of breast and ovarian cancer in earlier studies. It was reported previously in Greece, Italy, Turkey and Israel and also mentioned as dominant variant of North Western Romania [32, 38, and 39]. Among all mutations which were found in our BC cases, exon 10 seems to be more influenced by single base pair change, multiple insertions and deletions and by frameshift mutations in *BRCA*1 gene.

In *BRCA*2 gene, we identified the highest recurrence variant as c.6373delA, which represented 11.1% cases ($n=4$) among other mutations. This mutation has been reported rarely in Indian studies but is a common or founder mutation in Danish population [28, 40]. In addition, Karami et al. [8] reported that, it is not prevalent in the USA or other European countries. The c.1855C > T mutation in exon 10 and c.92G > A in exon 3 were seen in two patients (5.5%) each.

Several studies have reported that the contribution of *BRCA*2 mutations in familial BC seems to be rather low in India, which supports our data [35, 37]. We have shown some *BRCA*1 and *BRCA*2 novel pathogenic variants (Tables 2 and 3, respectively). To our knowledge, our study is the first to report such mutations in Indian women with familial breast cancer.

Altogether, these findings may suggest genetic and ethnic associations among distinct populations or that these mutations occur in mutational hotspots. Nevertheless, various novel and specific mutations in *BRCA*1/2 genes occurring at a high frequency in different populations have been reported to date. So, knowledge of most recurrent mutations in *BRCA*1/2 according to ethnicity and population, treatment and diagnosis options for BC patients become better through efficient genetic testing methods.

Our study suggests a high prevalence rate (29.1%) of BRCA gene mutations in an Indian BC cohort. We believe with higher prevalence rate like this, *BRCA* sequence variant screening is imperative for Indian women with BC especially in younger women (< 50 years), as *BRCA* testing recommendations based on family history alone would miss out potential *BRCA* positive patients. Hence, reducing the mortality rate of BC in Indian women requires a multidisciplinary approach that includes education campaigns, preventive measures, early detection screening programs including BRCA genetic screen. Identification of a BRCA mutation is of paramount importance not only for providing appropriate genetic counseling and discussing risk-reducing interventions, but also for determining treatment options in patients with metastatic disease. A BRCA positive patient is eligible for PARP inhibitor therapy, leading to better survival rates/outcomes. At the same time, detection of BRCA mutation in a patient also, alerts testing of close relatives due to autosomal dominant mode of inheritance of this gene. This is in turn, inevitably encourages early screen and management of potential at risk individuals.

Our analysis includes female BC patients from across India, not selected for age, histologic subtypes or family history, thus presenting a more generalized results of this patient population. This adds concurring information on higher prevalence rate of BRCA gene variants and positive correlation between onsets of age-BRCA mutation status in Indian BC patients. We strongly believe, burden of BRCA gene variants in Indian BC patients may be different from Western BC patients and hence the need for designing the population specific testing criteria and protocols for *BRCA1*/2 and other cancer predisposing genes.

Study limitations

Limited access to clinical data: The study uses data generated in a referral diagnostic laboratory. Very often data like clinical details, family history, patient ethnicity or follow-up on treatment responses is not available to us as we depend on the physician for collection of this data. Due to lack of such data, the study is unable to correlate current genetic findings with above mentioned factors as a result of design constraint. Future studies will strive to achieve this study design. However, these limitations do not impact the gross findings of the study which focuses on peculiarity of overall Indian BC patients with regards to higher BRCA mutation burden, and correlation of young age with BRCA mutation status.

Conclusion

Our results highlight

- *BRCA* mutation prevalence was 29.1% in our data, which is higher than Western countries.
- Median age of women with cancer was 49 years, whereas median age for *BRCA* positive patients was 45 years. Young age category (20–45 Years) was found to be significantly associated with *BRCA* positive status.
- Most common mutations were c.1961delA and c.68_69delAG in *BRCA* 1 and c.6373delA in *BRCA* 2 gene. Most common exon was exon 10 in *BRCA1* and exon 11 in *BRCA* 2 gene.
- Based on above BRCA screening, it is imperative for women with BC especially younger women (< 50 Years) as family history based BRCA testing would miss out many *BRCA* positive candidates.

Table 2 Variant details as observed in *BRCA*1 positive patients

S. no	Locus	Exon/intron	Variation	Aminoacid change	Variant effect	Frequency	Previously reported
1	chr17:41228596	13	c.4392_4393insT	p.Ile1465TyrfsTer11	Frameshift	1	Novel
2	chr17:41244008	10	c.3538_3539delAGinsT	p.Val1181SerfsTer29	Frameshift	1	Novel
3	chr17:41243469	10	c.4077_4078delAA	p.Ser1360HisfsTer7	Frameshift	1	Novel
4	chr17:41197762	23	c.5525 T > A	p.Val1842Glu	Missense	1	Novel*
5	chr17:41219663	16	c.5035delC	p.Leu1679Ter	Nonsense	1	Novel*
6	chr17:41246862	10	c.685delT	p.Ser229LeufsTer5	Frameshift	1	Novel*
7	chr17:41245346	10	c.2188_2201delGAA AAAGAAGAGAA	p.Glu730ThrfsTer5	Frameshift	1	Novel*
8	chr17:41215370	18	c.5173G > T	p.Glu1725Ter	Nonsense	1	Novel*
9	chr17:41234435	12	c.4342delA	p.Ser1448AlafsTer8	Frameshift	1	Novel*
10	chr17:41256137	6	c.441 + 1_441 + 2insA	–	Unknown	2	Novel*
11	chr17:41246196	10	c.1352C > G	p.Ser451Ter	Nonsense	3	Novel*
12	chr17:41244781	10	c.2766delA	p.Val923LeufsTer77	Frameshift	1	Carribean, Thailand [41, 42]
13	chr17:41256233	6	c.346delG	p.Glu116AsnfsTer3	Frameshift	1	Japanese [43]
14	chr17:41246359	10	c.1188delT	p.Asp396GlufsTer14	Frameshift	1	Indian
15	chr17:41246039	10	c.1504_1508delTTAAA	p.Leu502AlafsTer2	Frameshift	1	South Africa [8]
16	chr17:41276044	2	c.68_69delAG	p.Glu23ValfsTer17	Frameshift	17	Indian, Jewish [27–29]
17	chr17:41246098	10	c.1450G > T	p.Gly484Ter	Nonsense	3	Global
18	chr17:41245586	10	c.1961delA	p.Lys654SerfsTer47	Frameshift	18	European [8] Peruvian, Egyptian, Korean, Russian, Iranian and Indian [24–26]
19	chr17:41219624	16	c.5074 + 1G > A	–	Unknown	4	Jewish, Indian [28, 44]
20	chr17:41245333	10	c.2214_2215insT	p.Lys739Ter	Nonsense	2	Indian [27]
21	chr17:41244684	10	c.2864C > A	p.Ser955Ter	Nonsense	1	Indian[45]
22	chr17:41243941	10	c.3607C > T	p.Arg1203Ter	Nonsense	2	Romania [38]
23	chr17:41197778	23	c.5509 T > C	p.Trp1837Arg	Missense	2	Indian [20]
24	chr17:41226515	14	c.4508C > A	p.Ser1503Ter	Nonsense	1	Indian [20]
25	chr17:41246531	10	c.1016_1017insA	p.Val340GlyfsTer6	Frameshift	1	Italian [46]
26	chr17:41245210	10	c.2338C > T	p.Gln780Ter	Nonsense	1	Indian [21]
27	chr17:41246565	10	c.981_982delAT	p.Cys328Ter	Nonsense	1	Chinese
28	chr17:41276046	2	c.66_67delAG	p.Glu23ValfsTer17	Frameshift Deletion	1	Global
29	chr17:41258474	4	c.211A > G	p.Arg71Gly	Missense	1	Global
30	chr17:41267762	3	c.115 T > C	p.Cys39Arg	Missense	1	Global
31	chr17:41246098	10	c.1450G > T	p.Gly484Ter	Nonsense	1	Global
32	chr17:41245390	10	c.2157_2158insA	p.Glu720ArgfsTer6	Frameshift	1	Global
33	chr17:41243776	10	c.3770_3771delAG	p.Glu1257GlyfsTer9	Frameshift	1	Punjabi [29]
34	chr17:41246878	10	c.671-1G > T	p.?	Unknown	1	Global
35	chr17:41244318	10	c.3228_3229delAG	p.Gly1077AlafsTer8	Frameshift	1	Italian [8]
36	chr17:41246531	10	c.1016dup	p.Val340GlyfsTer6	Frameshift	1	Norway [47]

Novel*- Reported first time in Indian Population in our study

Table 3 Variant details as observed in *BRCA2* positive patients

S.no	Locus	Exon/Intron	Variation	Aminoacid Change	Variant effect	Frequency	Previously reported
1	chr13:32911092	11	c.2600_2601insA	p.Thr868TyrfsTer13	Frameshift	1	Novel
2	chr13:32936745	17	c.7891_7892insA	p.Leu2631HisfsTer10	Frameshift	1	Novel
3	chr13:32911437	11	c.2946_2947insA	p.Pro983ThrfsTer5	Frameshift	1	Novel
4	chr13:32914953	11	c.6468_6469dup	p.Gln2157LeufsTer12	Frameshift	1	Novel*
5	chr13:32969024	25	c.9458delG	p.Gly3153AlafsTer10	Frameshift	1	Novel*
6	chr13:32910550	11	c.2059_2063delGATTA	p.Asp687Ter	Nonsense	1	Novel*
7	chr13:32911073	11	c.2588delA	p.Asn863IlefsTer11	Frameshift Deletion	1	Novel*
8	chr13:32907470	10	c.1855C > T	p.Gln619Ter	Nonsense	2	Novel*
9	chr13:32929356	14	c.7366C > T	p.Gln2456Ter	Nonsense	1	Novel*
10	chr13:32913165	11	c.4677delT	p.Phe1559LeufsTer9	Frameshift	1	Global
11	chr13:32907440	10	c.1825C > T	p.Gln609Ter	Nonsense	1	Japanese[29]
12	chr13:32893302	3	c.161_162insA	p.Asn54LysfsTer10	Frameshift	1	Global
13	chr13:32913165	11	c.4677delT	p.Phe1559LeufsTer9	Frameshift	1	Global
15	chr13:32912277	11	c.3785C > A	p.Ser1262Ter	Nonsense	1	Indian [48]
16	chr13:32968822	25	c.9257-3_9258delTAGGA	–	Unknown	1	Global
17	chr13:32954272	24	c.9253delA	p.Thr3085GlnfsTer19	Frameshift Deletion	1	Novel*
18	chr13:32893238	3	c.92G > A	p.Trp31Ter	Nonsense	2	Punjabi[29]
19	chr13:32893467		c.316 + 5G > A	–	Unknown	1	Global
20	chr13:32930609	15	c.7480C > T	p.Arg2494Ter	Nonsense	1	Korean [49]
21	chr13:32914172	11	c.5681_5682insA	p.Tyr1894Ter	Nonsense	1	Global
22	chr13:32912304	11	c.3812C > G	p.Ser1271Ter	Nonsense	1	Global
23	chr13:32900236	5	c.426-2A > G	–	Unknown	1	Global
24	chr13:32907420	10	c.1813delA	p.Ile605TyrfsTer9	Frameshift Deletion	3	Global
25	chr13:32911442	11	c.2957delA	p.Asn986IlefsTer5	Frameshift Deletion	1	Egyptian [50]
26	chr13:32913836	11	c.5351delA	p.Asn1784ThrfsTer7	Frameshift Deletion	1	Egyptian [51]
27	chr13:32913621	11	c.5130_5133del	p.Tyr1710Ter	Frameshift	2	Global
28	chr13:32937507	13	c.8168A > T	p.Asp2723Val	Frameshift	1	Chilean [52]
29	chr13:32914859	11	c.6373delA	p.Thr2125ProfsTer12	Frameshift	4	Danish [40]

Novel*- Reported first time in Indian Population in our study

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Author contributions
Rosy Chikkala, made substantial contributions towards literature research, and drafted the manuscript followed by editing and revision. Deepak Bhayal carried out bioinformatics and statistical analysis. Nikki Rani was involved in wet lab/ experimental work ranging from DNA extraction to NGS sequencing. Dr. Bhawna Dubey conceived the idea and design of the study, drafting the manuscript, revising it critically for important intellectual content and final submission. Rama Modali & Dr. Kishor Bhatia provided their support critical inputs and review for improvement of the manuscript.

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Availability of data and materials
Data will be available on request.

Declarations

Ethics approval and consent to participate
Written informed consent was obtained from all patients.

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All authors are aware of its submission and the paper has not been submitted elsewhere.

Competing interests
The authors declare no conflicts of interest regarding the publication of this paper.

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