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ORIGINAL RESEARCH

Mutation analysis of BRCA1/2 mutations with special reference to polymorphic SNPs in Indian breast cancer patients

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Background: Germline mutations BRCA1 and BRCA2 contribute almost equally in the causation of breast cancer (BC). The type of mutations in the Indian population that cause this condition is largely unknown.

Purpose: In this cohort, 79 randomized BC patients were screened for various types of BRCA1 and BRCA2 mutations including frameshift, nonsense, missense, in-frame and splice site types.

Materials and methods: The purified extracted DNA of each referral patient was subjected to Sanger gene sequencing using Codon Code Analyzer and Mutation Surveyor and next-generation sequencing (NGS) methods with Ion torrent software, after appropriate care.

Results: The data revealed that 35 cases were positive for BRCA1 or BRCA2 (35/79: 44.3%). BRCA2 mutations were higher (52.4%) than BRCA1 mutations (47.6%). Five novel mutations detected in this study were p.pro163 frameshift, p.asn997 frameshift, p.ser148 frameshift and two splice site single-nucleotide polymorphisms (SNPs). Additionally, four nonsense and one in-frame deletion were identified, which all seemed to be pathogenic. Polymorphic SNPs contributed the highest percentage of mutations (72/82: 87.8%) and contributed to pathogenic, likely pathogenic, likely benign, benign and variant of unknown significance (VUS). Young age groups (20–60 years) had a high frequency of germline mutations (62/82;75.6%) in the Indian population.

Conclusion: This study suggested that polymorphic SNPs contributed a high percentage of mutations along with five novel types. Younger age groups are prone to having BC with a higher mutational rate. Furthermore, the SNPs detected in exons 10, 11 and 16 of BRCA1 and BRCA2 were higher than those in other exons 2, 3 and 9 polymorphic sites in two germline genes. These may be contributory for BC although missense types are known to be susceptible for cancer depending on the type of amino acid replaced in the protein and associated with pathologic events. Accordingly, appropriate counseling and treatment may be suggested.

Keywords: breast cancer, BRCA1 and BRCA2 gene mutations, polymorphic SNPs, novel types, age, NGS, Sanger gene sequencer

Introduction

The continuing global demographic and epidemiologic transitions signal an ever-increasing cancer burden over the next decades, particularly in low- and middle-income countries (LIMC), with over 20 million new cancer cases expected annually as early as 2025. Breast cancer (BC) is one of the most common and dreadful diseases affecting Indian women.[1,2] It is actually considered the main type of cancer among new cases in India.[3] It has been divided into two subtypes, early-onset and late-onset, based on age-specific cancers. Mutation in BRCA1 is thought to account for about 45% of families, whose locus is to chromosome 17p. The second one is mapped to chromosome 13q and

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responsible for the early onset of BC.[4] About 200 mutations in both genes have been reported and are widely scattered across them.[5–7] Most mutations of these genes affect the structure and function of the gene, altering protein synthesis and function (34% BRCA1 and 38% BRCA2 mutations), and others are missense types that alter amino acids but do not truncate the protein.[8,9] Hence, the mutation of BRCA1/2 results in the gene's function silencing or over-activation.

According to the GLOBOCAN project set up by the International Agency for Research on Cancer (IARC),[10] 145,000 new cases were diagnosed and 70,000 deaths occurred in India in 2012. Considering the size of the population, the incidence of BC in India appears to be lower, when compared to developed countries such as the USA (233,000 cases) and the European union (362,000 cases). But the ratio of death in India was much higher (48.3%) in comparison with the USA (18.9%) and the European union (25.4%).[11,12] Thus, the frequency of BC patients seems to be lower than in the developing countries, but mortality is higher in our country attributable to lifestyle changes, delay in introduction of cancer screening centers and limited medical awareness including treatment.[12–14]

The relatively high death rate from BC in the Indian health care system thus demonstrates a need for a cost-effective method of early detection, screening and treatment.[12] However, early detection of hereditary BC is essential to reduce this type of cancer in a developing country like India. Hence, this study was undertaken to detect the germline mutations using molecular diagnostic methods and their correlation with pathogenic events for its better treatment in addition to other factors, such as age, in our cohort. Emphasis is also paid to the involvement of missense mutations occurring in BRCA1 and BRCA2 genes of BC patients in the Indian population.

Materials and methods

Patient selection

A total of 79 referral, diagnosed, pre- and post-menopausal BC cases from India were selected. EDTA blood samples and a request for biological specimens were collected from the respondents after completion of consent forms and counseling. The patients provided test reports which were used for our current research. This work was approved by Gujarat University (Ahmedabad, India) Human Ethical Committee (GUHEC/001/2015) for investigation.

DNA extraction

Genomic DNA was extracted from 3–4 mL of peripheral EDTA blood using automated DNA extraction method (PerkinElmer Inc., Waltham, MA, USA). The concentration of DNA was determined using the Qubit 2.0 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

Targeted next-generation sequencing (NGS) (amplification)

For targeted NGS analysis, the Ion AmpliSeq™ BRCA1 and BRCA2 Panel (Thermo Fisher Scientific) containing 167 primer pairs in three pools (Pool 1 is 56 pairs, 2 is 56 pairs and 3 is 55 pairs) was used. Multiplex PCR was performed using 10–30 ng genomic DNA with a premixed primer pool and Ion AmpliSeq™ HiFi master mix; Ion AmpliSeq™ Library Kit 2.0; Cat. no. 4480441) for 2 min at 99°C, followed by 19 cycles of denaturation at 99°C for 15 s and annealing and elongation at 60°C for 4 min, ending with a holding period at 10°C. The PCR amplicons were treated with 2 µL FuPa reagent to partially digest primer sequences and phosphorylate the amplicons at 50°C for 10 min, followed by 55°C for 10 min, then 60°C for 20 min. The amplicons were ligated to adapters with the diluted barcodes of the Ion Xpress™ Barcode Adapters kit (Thermo Fisher Scientific) for 30 min at 22°C, then 72°C for 10 min. Adaptor-ligated amplicon libraries were purified using Agencourt AMPure XP (Cat no. A63881) reagents (Beckman Coulter, CA, USA). The library concentration was determined using an Ion Library Quantitation Kit (Cat no. 4468802) (Thermo Fisher Scientific), then each library was diluted to 4–8 pmol/L and the same amount of libraries was pooled for one sequence reaction. Next, emulsion PCR was carried out using the Ion OneTouch™2.0 System and Ion PGM™ Hi-Q™ OT2 (Cat no. A27743) reagents, or Ion 540™ OT2 (Cat no. A27752) reagents (Thermo Fisher Scientific), according to the manufacturer's instructions. Template-positive Ion Sphere™ particles were then enriched with Dynabeads MyOne™ Streptavidin C1 Beads (Cat no. 65001) (Thermo Fisher Scientific) using an Ion OneTouch™ ES system (Thermo Fisher Scientific). Purified Ion Sphere particles were loaded on any of chip types 314, 316, or 318 (Compatible with Ion PGM) or 520, 530, or 540 (Compatible with Ion S5 System) Chip. Massively parallel sequencing was carried out on a Personal Genome Machine (PGM) sequencer (Ion Torrent™) using the Ion PGM™ Hi-Q™ Sequencing Kit or Ion S5 sequencer (Thermo Fisher Scientific) using Ion S5 Sequencing kit according to the manufacturer's instructions. Sequencing was performed using 500 flow runs that generated ~200 bp reads.

Sanger DNA sequencing

Possible high confidence variants were confirmed by Sanger sequencing. PCR primers were designed using the OligoCalc (http://biotools.nubic.northwestern.edu/OligoCalc.html) to determine the properties of designed primers and Primer Blast (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) was also used to check whether our region of interest was covered. Primers were synthesized at 100 nM scale and cartridge purified (Eurofins, Bengaluru, India). PCR amplification was carried out using Takara Taq™ Hot Start Version 2.0 PCR mix with each primer at 10 pM concentration under standard conditions. These PCR amplifications were all carried out in duplicate along with a known normal control cell line sample. Successful PCR products were purified prior to sequencing using ExoSAP-IT (Cat no. 78201) reagent (Thermo Fisher Scientific) according to the manufacturer's protocol. Purified PCR products were subjected to Sanger sequencing technique in both forward and reverse orientations with the same primer sequences used for PCR at 10 pM final concentration using BigDye® v3.1(Cat no. 4336917) according to the manufacturer's cycling conditions. BigDye® v3.1 sequencing reactions were then purified using EDTA (Cat no. 194822) and sodium acetate (Cat no. 567418) according to the manufacturer's protocol and analyzed on 3500 Genetic Analyzer (Thermo Fisher Scientific). Sanger sequencing data were analyzed using Codon Code Aligner v5.0.2, (CodonCode Corporation, Centerville, MA, USA) and Mutation Surveyor v5.0, (SoftGenetics, State College, PA, USA).

Data analysis

The sequence data were processed using standard Ion Torrent Suite™ Software running on the Torrent Server (Thermo Fisher Scientific). Raw signal data were analyzed using Torrent Suite™ version 4.4, 4.6 or 5.0. The pipeline included signaling processing, base calling, quality score assignment, adapter trimming, PCR duplicate removal, read alignment to human genome 19 reference (hg19), quality control of mapping quality, coverage analysis, and variant calling. Coverage analysis and variant calling used Torrent Variant Caller plugin software (Version 4.4, 4.6 or 5.0) in the Torrent Server. The variant caller parameter setting was germline PGM high stringency. Following data analysis, annotation of single-nucleotide variants, insertions, deletions, and splice site alterations were performed by the Ion Reporter™ Server System (Thermo Fisher Scientific), which identified nonsynonymous mutations. Splice site alteration were analyzed 2 bp upstream or downstream of exon–intron boundaries. Sequence data were visually confirmed with the Integrative Genomics Viewer (IGV) and any sequence, alignment, or variant call error artifacts were discarded. Nonsynonymous mutations were annotated using the Breast Cancer Information Core (BIC) database (https://research.nhgri.nih.gov/projects/bic/index.shtml) and ClinVar (http://www.ncbi.nlm.nih.gov/clinvar/).[14] Minor allele frequency was determined from the 1000 Genomes Project database.[15] The 5000 Exome project (http://evs.gs.washington.edu/EVS/) and The Human Genetic Variation Database (http://www.genome.med.kyoto-u.ac.jp/SnpDB) were used to identify the pathogenic conditions of the BRCA1/2 gene mutations.

Statistics

Percentage was calculated wherever necessary.

Results

After analyzing 79 referral cases of women ranging in age between 23 and 72 years, 35 BC-positive (44.30%) cases were detected with germline gene mutations. Among them, 11 cases were with BRCA1 gene mutations (31.4%) followed by 15 with BRCA2 gene mutations (42.9%), and both BRCA1 and BRCA2 mutations were found in nine cases (25.7%) (Figure 1).

Mutation types in BRCA1 and BRCA2

A total of 82 mutations, 39 with BRCA1 (39/82; 47.6%) and 43 with BRCA2 (43/82; 52.4%), were identified in 35 cancer patients. A total of 72 (72/82) missense mutations (87.8%) with amino acid replacement (BRCA1, 36/82, 43.9%; BRCA2, 36/82, 43.9%) were detected with two novel mutations (BRCA1; two splice sites). Among other types, five frameshift mutations (5/82:6.1%) were detected in five cases (exon 1, exon 14 in BRCA1; exon 6, exon 10, exon 11 in BRCA2), where three were novel types. Others were four nonsense mutations of BRCA2 (4/82:4.9%) and

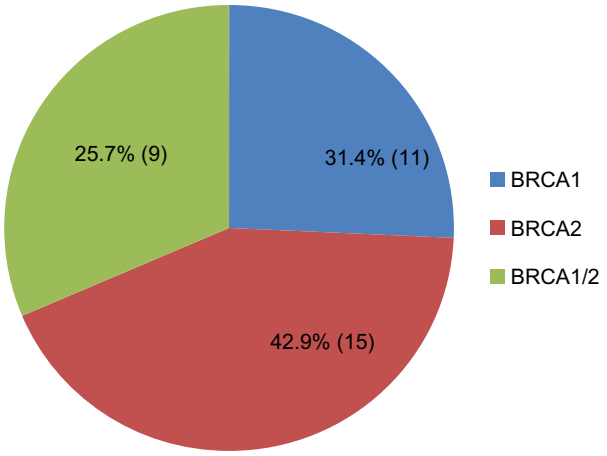

Figure 1 Patients (n=35) with BRCA1 and BRCA2 mutations.

one in-frame deletion of BRCA1 (1/82:1.2%). A total of five novel mutations were present with three frameshift and two missense (splice site) mutations in our study (Table 1; Figure 2). Moreover, familial (20) and sporadic (15) cases were not significantly different (Table 1).

Frequency of SNPs in BRCA1 and BRCA2 and pathologic variants

The single-nucleotide polymorphism (SNP) frequency was higher in exons 10, 11 and 16 of BRCA1 and BRCA2 followed by exons 9, 2 and 3. In BRCA1, exon 9 had two splice site SNPs which were pathogenic and one SNP likely benign (total of three). Exon 10 had seven SNPs benign, two each with likely pathogenic and variant of unknown significance (VUS) (total of four). Exon 11 contributed 12 SNPs, each with two amino acid changes showing likely benign and two with VUS having two amino acid changes. The exon 16 possessed six benign and two with VUS. In BRCA2, exon 10 showed nine SNPs likely benign/benign/likely pathogenic, four SNPs likely benign/likely pathogenic and one with VUS, respectively. Exon 11 exhibited seven SNPs likely benign/benign/likely pathogenic and three likely benign/benign. It also had three VUS with two amino acid changes followed by one likely benign. Exon 16 showed six SNPs likely benign, whereas exon 3 and exon 2 had one each of VUS and benign SNPs (two), respectively (Table 2). The SNPs of each exon percent of two germline genes, i.e., BRCA1 (36) and BRCA2 (36), are shown in Figures 3 and 4.

Age at diagnosis and mutation frequency

The average age was 47.3 ± 3.2 ranging from 23 to 72 years. The age group 41–60 years (18 patients) had a high frequency

Table 1 Patients with BRCA1 and BRCA2 mutations

Pt. No.	Location	Gene	Age (years)	c.dna	Amino acid change	Mutation type	F/S	Mutation effect	Novel/ Reference
1	Exon 27	BRCA2	56	c.9976A>T	p.Lys3326Ter	SNP	Familial	Nonsense	rs11571833:a, b
2	Exon 2	BRCA1	51	c.66_67delAG	p.Glu23Val	Deletion	NA	Frameshift	-, a
3	Exon 10	BRCA1	53	c.3119G>A	p.Ser1040Asn	SNP	Sporadic	Missense	rs4986852:a, b
4	Exon 11	BRCA1	49	c.2412G>C	p.Gln804His	SNP	Familial	Missense	rs55746541:a, b
5	Exon 6	BRCA2	29	c.484_484delG	p.Pro163fs	Deletion	Familial	Frameshift	Novel
6	Exon 11	BRCA2	31	c.2971A>G	p.Asn991Asp	SNP	Familial	Missense	rs1799944:a, b
				c.4779A>C	p.Glu1593Asp				rs80358703:a, b
	Exon 16	BRCA1		c.5019G>A	p.Met1673Ile				rs1799967:b
	Exon 10	BRCA2		c.865A>C	p.Asn289His				rs766173:a, b
7	Exon16	BRCA1	23	c.5019G>A	p.Met1673Ile	SNP	Sporadic	Missense	rs1799967:b
	Exon 10	BRCA2		c.865A>C	p.Asn289His				rs766173:a, b
8	Exon 10	BRCA1	39	c.3113A>G	p.Glu1038Gly	SNP	Sporadic	Missense	rs16941:a, b
9	Exon 09	BRCA1	45	c.2077G>A	p.Asp693Asn	SNP	Familial	Missense	rs4986850:a, b
10	Exon 11	BRCA1	55	c.2612C>T	p.Pro871Leu	SNP	Familial	Missense	rs799917:a, b
	Exon 10			c.3113A>G	p.Glu1038Gly				rs16941:a, b
	Exon 11			c.3548A>G	p.Lys1183Arg				rs16942:a, b
	Exon 16			c.4837A>G	p.Ser1613Gly				-, a
	Exon 11	BRCA2		c.5744C>T	p.Thr1915Met				-, a
	Exon 14			c.7397T>C	p.Val2466Ala				-, a
11	Exon 10	BRCA2	63	c.1114 A>C	p.Asn372His	SNP	Familial	Missense	rs144848:a, b
	Exon 11			c.2971A>G	p.Asn991Asp				rs1799944:a, b
	Exon 10			c.865A>C	p.Asn289His				rs766173:a, b
12	Exon 18	BRCA2	52	c.8117A>G	p.Asn2706Ser	SNP	Familial	Missense	rs80359055:a, b
13	Exon 11	BRCA1	40	c.2521C>T	p.Arg841Try	SNP	Sporadic	Missense	rs1800709:a, b
	Exon 10	BRCA1		c.3119G>A	p.Ser1040Asn				rs4986852:a, b
14	Exon 11	BRCA2	49	c.2971A>G	p.Asn991Asp	SNP	Sporadic	Missense	rs1799944:a, b
	Exon 10	BRCA2		c.865A>C	p.Asn289His				rs766173:a, b
15	Exon 10	BRCA2	39	c.943T>A	p.Cys315Ser	SNP	Familial	Missense	rs79483201:a, b
16	Exon 10	BRCA2	59	c.1114A>C	p.Asn372His	SNP	Familial	Missense	rs144848:a, b
	Exon 11	BRCA1		c.2612C>T	p.Pro871Leu				rs799917:a, b
	Exon 10			c.3113A>G	p.Glu1038Gly				rs16941:a, b
	Exon 11			c.3548A>G	p.Lys1183Arg				rs16942:a, b
	Exon 16			c.4837A>G	p.Ser1613Gly				-, a
	Exon 14	BRCA2		c.7397T>C	p.Val2466Ala				-, a

(*Continued*)

Table 1 (*Continued*)

Pt. No.	Location	Gene	Age (years)	c.dna	Amino acid change	Mutation type	F/S	Mutation effect	Novel/ Reference
17	Splice site 3	BRCA1	74	c.5137+1G>A	-		Familial	Missense	Novel
18	Exon 11	BRCA2	25	c.6231G>C	p.Lys2077Asn	SNP	Sporadic	Missense	rs541826447:b
	Exon 27			c.9976A>T	p.Lys3326Ter			Non sense	rs11571833:a, b
19	Exon 10	BRCA2	68	c.865A>C	p.Asn289His		Sporadic	Missense	rs766173:a, b
	Exon 25			c.9380G>A	p.Trp3127Ter			Nonsense	rs80359211:a, b
20	Exon 10	BRCA2	67	c.1114A>C	p.Asn372His	SNP	Familial	Missense	rs144848:a, b
	Exon 11	BRCA1		c.2612C>T	p.Pro871Leu				rs799917:a, b
	Exon 10			c.3113A>G	p.Glu1038Gly				rs16941:a, b
	Exon 11			c.3548A>G	p.Lys1183Arg				rs16942:a, b
	Exon 16			c.4837A>G	p.Ser1613Gly				-, a
	Exon 14	BRCA2		c.7397T>C	p.Val2466Ala				-, a
21	Exon 11	BRCA1	49	c.2612C>T	p.Pro871Leu	SNP	Familial	Missense	rs799917:a, b
	Exon 11	BRCA2		c.2971A>G	p.Asn991Asp				rs1799944:a, b
	Exon 10	BRCA1		c.3113A>G	p.Glu1038Gly				rs16941:a, b
	Exon 11			c.3548A>G	p.Lys1183Arg				rs16942:a, b
	Exon 16			c.4837A>G	p.Ser1613Gly				-, a
	Exon 14	BRCA2		c.7397T>C	p.Val2466Ala				-, a
	Exon 10			c.865A>C	p.Asn289His				rs766173:a, b
22	Exon 10	BRCA1	30	c.3328_3330delAAG	p.Lys1110del	Deletion	Familial	In-frame del.	rs80358335:a, b
23	Exon 14	BRCA1	37	c.4442_4442delG	p.Ser1481fs	Deletion	Familial	Frameshift	NOVEL
24	Exon 11	BRCA1	72	c.2612C>T	p.Pro871Leu	SNP	Familial	Missense	rs799917:a, b
	Exon 11	BRCA2		c.2971A>G	p.Asn991Asp				rs1799944:a, b
	Exon 10	BRCA1		c.3113A>G	p.Glu1038Gly				rs16941:a, b
	Exon 11			c.3548A>G	p.Lys1183Arg				rs16942:a, b
	Exon 16			c.4837A>G	p.Ser1613Gly				-, a
	Exon 14	BRCA2		c.7397T>C	p.Val2466Ala				-, a
	Exon 10			c.865A>C	p.Asn289His				rs766173:a, b
25	Splice site 3	BRCA1	44	c.5137+1G>A	-		Sporadic	Missense	Novel
26	Exon 11	BRCA1	52	c.2612C>T	p.Pro871Leu	SNP	Sporadic	Missense	rs799917:a, b
	Exon 11	BRCA2		c.2971A>G	p.Asn991Asp				rs1799944:a, b
	Exon 10	BRCA1		c.3113A>G	p.Glu1038Gly				rs16941:a, b
	Exon 11			c.3548A>G	p.Lys1183Arg				rs16942:a, b
	Exon 16			c.4837A>G	p.Ser1613Gly				-, a
	Exon 14	BRCA2		c.7397T>C	p.Val2466Ala				-, a
	Exon 10			c.865A>C	p.Asn289His				rs766173:a, b
27	Exon 03	BRCA2	48	c.125 A>G	p.Try42Cys	SNP	Familial	Missense	rs4987046:a, b
	Exon 11			c.3167_3170delAAAA	p.Gln1056	Deletion		Frameshift	rs80359372:a, b
28	Exon 25	BRCA2	44	c.9380G>A	p.Trp3127Ter		Familial	Nonsense	rs80359211:a, b
29	Exon 11	BRCA2	57	c.2971A>G	p.Asn991Asp	SNP	Sporadic	Missense	rs1799944:a, b
				c.5744C>T	p.Thr1915Met				-, a
	Exon 10	BRCA2		c.865A>C	p.Asn289His				rs766173:a, b
30	Exon 10	BRCA2	50	c.2169_2170insA	p.Val726fs	Insertion	NA	Frameshift	Novel
31	Exon 11	BRCA2	53	c.5744C>T	p.Thr1915Met	SNP	Familial	Missense	rs4987117:a, b
32	Exon 10	BRCA2	39	c.1114A>C	p.Asn372His	SNP	Familial	Missense	rs144848:a, b
		BRCA1		c.2077G>A	p.Asp693Asn				rs4986850:a, b
33	Exon 11	BRCA2	34	c.4258G>T	p.Asp1420Tyr	SNP	Sporadic	Missense	rs28897727:a, b
34	Exon 11	BRCA2	55	c.4779A>C	p.Glu1593Asp	SNP	Sporadic	Missense	rs80358703:a, b
35	Exon 10	BRCA1	67	c.2077G>A	p.Asp693Asn	SNP	Sporadic	Missense	rs4986850:a, b

Notes: a, BIC; b, dbSNP; -, unknown; Familial n=20; Sporadic n=13; NA n =2.

Abbreviations: F, familial; S, sporadic; BIC, Breast Cancer Information Core; dbSNP, single-nucleotide polymorphism database; NA, not available; Pt, patient.

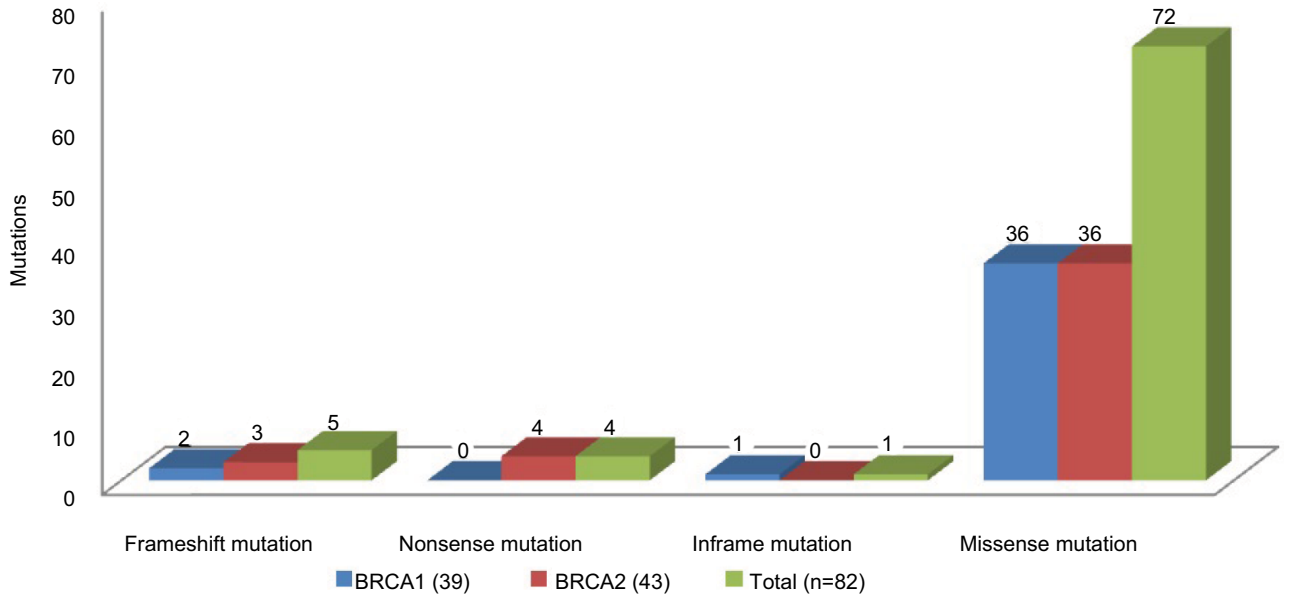

Figure 2 Mutation types in BRCA1 and BRCA2 mutations.

of mutations (45/82: 54.9%) followed by >61 years (six) with 20 (20/82: 24.4%) and <40 years (11) with 17 (17/82: 20.7%). However, the 23–60 years group had 62 mutations (62/82: 75.6%) (Figure 5).

Discussion

BC can be caused by germline mutations in BRCA1 and BRCA2, or by several other genes. In our cohort, we analyzed 79 referral cases using NGS and gene sequencing of Sanger with other reports of clinicians for phenotypic variations. Only 35 cases (44.30%) detected positive for BRCA1 or BRCA2. The mutations in BRCA2 (52.4%) were marginally higher than those in BRCA1 (47.6%) in our study. Similar results were observed in a North Indian population where BRCA2 had maintained equal distribution of BRCA1/2.[2] BRCA2 mutations contribute more than BRCA1 mutations in Philippine women in support of our data.[17] Jang et al[18] in Korean cases found no variations between BRCA1 and BRCA2 mutations. But in a South Indian population, BRCA1 mutations were more than BRCA2 germline mutations.[19]

Others included five frameshift mutations, where protein was truncated altering its structure and function. In these, three were novel types – BRCA2 (exon 6) of c.484-484delG, BRCA2 (exon 10) of c.2169_2170InsA and BRCA1 (exon 14) of c.4442_4442delG) – and the other two were referenced in BIC and the SNP database. All were pathogenic and familial in our study. Four nonsense mutations were found in our cancer patients, with two each of familial and sporadic in BRCA2 mutations, and these could lead to premature termination of protein due to change in specific amino acid proving lethal, and referenced in database. Furthermore, only one in-frame deletion in exon 10 of BRCA1(c.3328_3330delAAG) was detected in our cohort which was familial and deleterious. Finally, in our study, five novel mutations were detected, i.e., splice-site missense (two) and frameshift (three) types. Similar mutations are well reported globally in BC and ovarian cancer women except novel mutations in our report.[12,18,19]

High frequency of missense polymorphism (72) in women with BC is seen in our study, which was equal in both genes. Exons 10, 11 and 16 were more than exons 2, 3 and 9 of both genes, and all these may be responsible for BC in these patients depending upon amino acid substitution and its subsequent effect on protein structure and function.[2] Few missense polymorphic SNPs detected in Algerian populations were tested negative for both gene mutations, but could have a role as a susceptibility BC marker in BRCA1/2.[20] In both familial (20) and sporadic (15) cases, few expressed multiples of polymorphic sites as found by others.[20] In these mutations, two were found to be novel (two splice sites) in BRCA1 only, i.e., c.5137+1G>A in two cases in which G was replaced by A, and others were registered. In our cohort, as per database of ClinVar (National Center for Biotechnology Information [NCBI]), dbSNP and BIC revealed most of them were benign in BRCA1 except two splice site types (c.5137+1G>A) which were pathogenic (No. 8, 11 of Table. 1) and one with VUS (p.A841T). In BRCA2, five SNPs, p.Cys315Ser, p.Gly1593Asp(2), p.Lys2077Asn and p.Asn2106Ser were with VUS and others in both genes, and were benign only.

Table 2 Types of polymorphic SNPs in BRCA1 and BRCA2

Serial No.	Exon No.	Amino acid change	Frequency	Effect
BRCA1				
1	Exon 10	p.Glu1038Gly	7	Benign
		p.Asp693Asn	2	Likely pathogenic
		p.Ser1040Asn	2	VUS
2	Exon 11	p.Pro871Leu	6	Likely benign
		p.Lys1183Arg	6	Likely benign
		p.Gln804His	1	VUS
		p.Arg841Typ	1	VUS
3	Exon 16	p.Ser1613Gly	6	Benign
		p.Met1673Ile	2	VUS
4	Exon 9	p.Asp693Asn	1	Likely benign
		C.5137+1G>A (Splice site*)	2	Pathogenic
BRCA2				
Serial No.	**Exon No.**	**Amino acid change**	**Frequency**	**Effect**
1	Exon 10	p.Asn289His	9	Likely benign/ Benign/Likely pathogenic
		p.Asn372His	4	Likely benign/ Likely pathogenic
		p.Cys315Ser	1	VUS
2	Exon 11	p.Asn991Asp	7	Likely benign/ Benign/Likely pathogenic
		p.Thr1915Met	3	Likely benign/ Benign
		p.Glu1593Asp	2	VUS
		p.Lys2077Asn	1	VUS
		p.Asp1420Tyr	1	Likely benign
3	Exon 16	p.Val2466Ala	6	Likely benign
4	Exon 2	p.Asn2706Ser	1	VUS
5	Exon 3	p.Tyr42Cys	1	Benign

Notes: *Missense of splice site types leading protein termination and novel. BRCA1 = 36 SNPs; BRCA2 = 36 SNPs. Effect is as per ClinVar, NCBI and BIC databases.
Abbreviations: VUS, variant of unknown significance; SNP, single-nucleotide polymorphism; NCBI, National Center for Biotechnology Information; BIC, Breast Cancer Information Core.

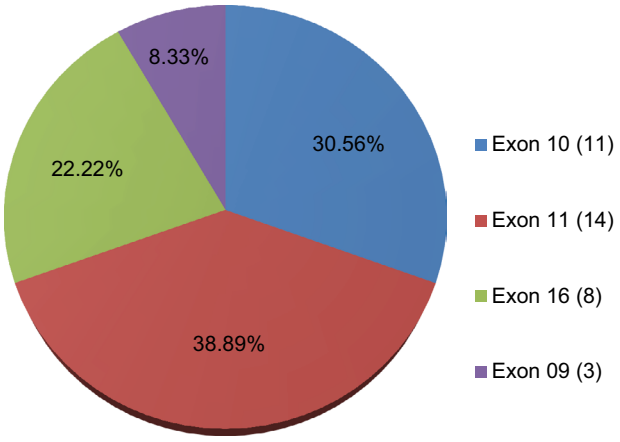

Figure 3 BRCA1 SNP mutations.
Abbreviation: SNP, single-nucleotide polymorphism.

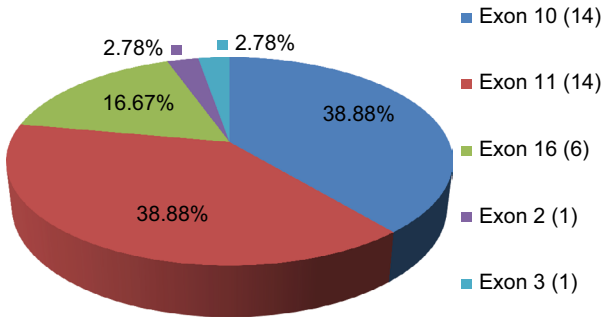

Figure 4 BRCA2 SNP mutations.
Abbreviation: SNP, single-nucleotide polymorphism.

However, these can be confirmed further by other predictive programs such as POLYPHEN 2, SIFT and PROVEN[20] as in silico analysis may not provide the pathogenicity of them. However, controversial reports exist about the role of SNPs in these BRCA1/2 genes for the induction of human breast and ovarian cancer (HBOC).[2,18,21,22,23] Dombernowsky et al[22] reported that missense mutations cannot explain the risk of HBOC in women with familial history. Contrarily, Dodova et al[21] in their study implicated the role of missense variants which may be deleterious. In an Iranian population, Neamatzadeh et al[23] detected these polymorphic SNPs at higher frequency in BRCA1 and BRCA2 genes compared to control subjects supporting our data. Valarmathi et al[7] in their study indicated that many of these variants are proposed to be deleterious in the Indian population. We further propose that polymorphic sites in BRCA1 and BRAC2 genes to cause cancer are dependent on the frequency and nature of amino acid replaced in the protein in regard to their lethality. These SNPs of BRCA1/2 could be related to the pathological condition of BC patients which differ from pathogenic, likely pathogenic, benign and likely benign, VUS or mixed types. It requires further elucidation by analyzing BRCA1/2 haplotypes in relation to gene mutations.[24]

In our study, young age groups (23–60 years) had more frequency of mutations 62/82 (75.6%). This indicated that age is also one of the factors to be noted including other lifestyles, ethnicity and geographic distribution for these differences in their frequency as reported earlier.[25,26,27] Mannan et al[12] found in their cohort, that the mutation rate was proportionally higher in patients aged <50 years. Wong et al[26] reported that early age is to be considered for higher rate of BRCA gene variants in HBOC patients in support of our observations. In the Indian population, the proportion of patients with early onset of mutations to BC is also much higher than in Caucasians. In North India,[2] lower BRCA mutations in early age were reported in comparison with other

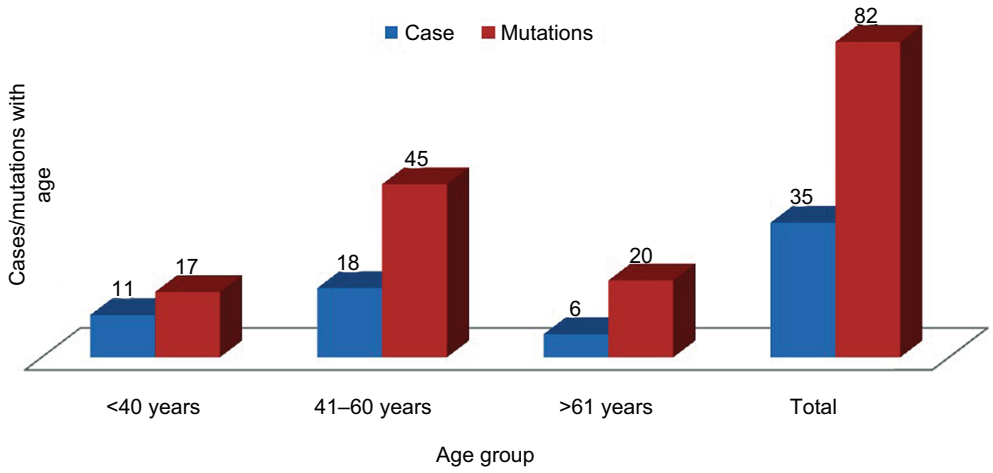

Figure 5 Cases and mutations in age groups of our study.

ethnic populations. Vaidyanathan et al[8] detected a high rate of germline mutations with early age, like that of Mannan et al[12] and our report. This discrepancy in these cohorts is related to the latest technologies including the gene panel used for monitoring gene mutations profiles in Indian patients.

In conclusion, BRCA2 gene mutations were marginally more than BRCA1 mutations. Young age groups had high frequency mutations which were also positively comparable. Polymorphic SNPs were higher than others, with five novel mutations, i.e., three frameshift and two spice site types, where synthesis of premature or truncating proteins occurs by amino acid replacement for their termination, respectively. Nonsense, in-frame and frameshift with splice site were pathogenic, and other polymorphic SNPs were benign, pathogenic and variant of unknown significance. The association of these mutations with haplotypes of BRCA1/2 needs to be evaluated in our future work in addition to age. This may help understand the role of SNPs in the causation of BC for better treatment and genetic testing highlighting sporadic and familial types in the Indian population.

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Author contributions

Drs Nidhi D Shah and Parth S Shah contributed to writing the results and discussion during manuscript preparation when they visited India. Hari Shankar P Ray and Yash Y Panchal were involved in the collection of blood from the patients after duly completed consent forms, blood analysis, DNA extraction, DNA sequencing, and data analysis of 79 patients. Dr Sandip C Shah, Dr Bhavini S Shah and Dr Mandava V Rao contributed to the preparation of reports after finalization of the results and the final preparation of the manuscript for submission to the journal. All authors contributed toward data analysis, drafting and critically revising the paper and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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