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ORIGINAL RESEARCH

Germline *BRCA1* and *BRCA2* deleterious mutations and variants of unknown clinical significance associated with breast/ovarian cancer: a report from North India

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Background: The spectrum of BRCA mutations that predispose to development of breast/ovarian cancer in Indian population remains unexplored. We report incidence and various types of pathogenic, likely pathogenic and variants of unknown significance (VUS) mutations in *BRCA1* and *BRCA2* genes observed at a tertiary cancer center in North India.

Materials and methods: A total of 206 unrelated breast and/or ovarian cancer patients, who met the National Comprehensive Cancer Network (NCCN) guidelines for genetic testing, were screened for germline *BRCA1*/*BRCA 2* mutations on high-throughput sequencing platform; large genomic rearrangements were assessed by multiple ligation probe assay. Mutations were mined in mutational databases, PubMed, and discerned into classes. Furthermore, the clinicopathological correlation of BRCA mutation status with prognostic markers in breast cancer and tumor histology in ovarian cancer was performed.

Results: In total, 45/206 and 17/206 cases showed positivity for *BRCA1* and *BRCA2* mutations, respectively, whereas 1/206 was positive for a mutation in both the genes. Altogether, 33 distinct *BRCA1* mutations were observed, among which 27 were deleterious (12 frameshifts, 8 nonsense, 1 missense, 3 splice-site variants, 2 big deletions and 1 large duplication) and 6 were VUS. Five novel *BRCA1* mutations (c.541G>T, c.1681delT, c.2295delG, c.4915C>T and exon 23 deletion) were identified. Seven mutations (c.2214_2215insT, c.2295delG, c.3607C>T, c.4158_4162delCTCTC, c.4571C>A, splicesite_3 (C>T) and exon 21–23 duplication) occurred more than once, whereas 16 distinct *BRCA2* mutations were noted – 9 were lethal (6 frameshifts, 2 nonsense and 1 big deletion) and 7 VUS. One unique pathogenic *BRCA2* mutation (c.932_933insT) was recognized. Two mutations (c.9976A>T and c.10089A>G) recurred twice. No significant difference in hormone receptor status was observed among *BRCA1* carriers, *BRCA2* carriers and noncarriers.

Conclusion: We have documented various pathogenic and VUS mutations in *BRCA1* and *BRCA2* genes observed in the cohort. Six novel mutations were identified. The knowledge shared would assist genetic testing in enabling more focused site-specific screening for mutations in biological relatives.

Keywords: genetic screening, high-throughput sequencing, multiplex ligation-dependent probe amplification assay, novel mutations, recurrent mutations

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Introduction

The breast cancer susceptibility genes *BRCA1* (MIM# 113705) and *BRCA2* (MIM# 600185) produce tumor suppressor proteins that participate in the repair of

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double-stranded DNA breaks by the "homologous recombination" (HR) DNA repair pathway that restores the DNA to its original self. The absence of BRCA proteins or dysfunctional BRCA proteins makes the HR pathway ineffective and the double-stranded breaks are thus repaired by "nonhomologous end joining", which is mutagenic and hence carcinogenic. It is for this reason that individuals carrying deleterious germline mutations in *BRCA1*/*BRCA2* genes have an elevated lifetime risk of developing breast cancer (by 60%–85%) and ovarian cancer (by 26%–54% for *BRCA1*; 10%–23% for *BRCA2*), when compared to 12.5% and 1% lifetime risks of breast and ovarian cancers, respectively, in women with intact BRCA genes.[1–5] In mutation carrier men, the risk of developing breast cancer are 1% and 6% for mutations in *BRCA1* and *BRCA2* genes, respectively.[6] These mutations are inherited with high penetrance in *BRCA1* (>50%) and intermediate penetrance in *BRCA2* (20%–50%) and account for 5%–10% of breast cancer and 15% of ovarian cancer.[7,8] More than 20,000 unique variants have been identified, together for both *BRCA1* and *BRCA2* genes, which are spread throughout the coding, splice site and intervening sequences of these large genes.[9] Mutational landscape of *BRCA1*/2 is a greenfield area, where novel mutations are still being identified regularly due to wider availability of sequencing facilities, the large size of these genes and ethnic and racial polymorphism. These novel mutations are analyzed in functional assays and through predictive algorithms (in silico) for their pathogenic role. Although most of these mutations are classifiable either as pathogenic (including likely pathogenic) or as benign (including likely benign), about 10% of these genetic alterations are unclassifiable now and are gathered under "variants of unknown significance" (VUS).[10]

The frequency and types of mutations in *BRCA1* and *BRCA2* genes seem to be differentially represented among the different population. The knowledge of the pathogenic and likely pathogenic mutations is useful to predict prognosis, make therapeutic decisions and apply risk reduction strategies in patients and carry out cost-effective screening in the first-degree relatives.[11] Although the VUS are currently uninformative, they have a 30% likelihood of being pathogenic when functional assays, clinical data, pedigree and predictive data are fully analyzed.[12] It is therefore necessary to catalog these too carefully.

The incidence and type of pathogenic mutations and VUS in *BRCA1*/*BRCA2* genes in North Indian population that predispose to the development of breast and ovarian cancers are largely unknown, except in one publication,[13] and are only now being slowly discovered. Therefore, this study is an effort to report specific damaging *BRCA1*/2 mutations in the North Indian population and discuss the clinicopathological features in mutation carriers.

More specifically, the aim of this study is to report the incidence and spectrum of pathogenic, likely pathogenic and VUS mutations in *BRCA1* and *BRCA2* genes that were observed in breast and ovarian cancer patients tested at a tertiary cancer center in North India on high-throughput sequencing platform followed by multiplex ligation probe assay for long genomic rearrangements (Big Indel) in cases who were found to be negative for pathogenic mutation on direct sequencing. Special attention has been paid to frequently recurring and some novel mutations; those were identified during the course of the study. Clinicopathological correlation with prognostic biomarker status in breast cancer and histology in ovarian cancer has also been performed.

Materials and methods

Research setting and subjects

This study was conducted in a tertiary cancer care center in North India.

A total of 206 unrelated patients with breast and/or ovarian cancer, who met the National Comprehensive Cancer Network (NCCN) recommendations,[14] were comprehensively tested for germline mutations in a time frame of 3 years (2015–2018). There were 126 breast cancer subjects, 74 ovarian cancer subjects and 6 subjects diagnosed with both breast and ovarian cancers. Among the breast cancer subjects, there were three male breast cancer cases.

This study was approved by the institutional review board (Rajiv Gandhi Cancer Institute and Research Center); vide the ethical approval letter number RGCIRC/IRB/229/2018, dated September 14, 2018, for the presentation of the study. All the patients had provided written informed consent for participation in the research study and for genetic testing. This study was conducted in accordance with the Declaration of Helsinki.

Isolation of DNA from blood, next-generation sequencing (NGS) and data analysis

Genomic DNA was isolated from 0.2 mL of peripheral blood of the patients using the commercially available DNA isolation kit (Qiagen DNeasy Blood and Tissue kit; Qiagen NV, Hilden, Germany), following the manufacturer's instructions. Isolated DNA was quantified by Qubit 3.0 Fluorometric quantitation (Thermo Fisher Scientific, Waltham, MA, USA).

The library for NGS was prepared manually with 10 ng of the isolated DNA using Oncomine BRCA assay – A328400 (Thermo Fisher Scientific), containing 265 primer pairs for 100% exonic coverage with large intronic flanking regions of *BRCA1* and *BRCA2* genes. "Template" was prepared using the HiQ OT-200 template kit by using 100 pmol of library. The template was further enriched by employing Ion One Touch ES instrument with the help of Streptavidin MyOne beads. The barcoded and enriched template was loaded on the Ion Torrent 316/318 V2 sequencing chip for deep sequencing on personal genome machine (PGM). Data generated from the runs were assessed for quality metrics on Torrent Suite Viewer (Ion Torrent Suite 5.6; Thermo Fisher Scientific). Successively, variant calling was performed on Oncomine Ion Reporter (Ion Reporter 5.6), and the final report was generated using Oncomine Knowledge Reporter.

Multiplex ligation-dependent probe amplification (MLPA) assay

Cases tested negative for *BRCA1*/*BRCA2* mutations were further investigated for possible large genomic rearrangements by MLPA assay. SALSA MLPA P002:BRCA1 and P090:BRCA2 kits (MRC Holland, Amsterdam, the Netherlands) were used as per the manufacturer's instructions. Briefly, 100 ng (5 µL) of blood leukocyte DNA was denatured (98°C, 5 minutes), cooled, hybridized with *BRCA1*/*BRCA2* probe mixture (95°C, 1 minute) and incubated overnight (60°C, 16 hours). This was followed by the addition of buffers and ligase provided with the kit (54°C, 15 minutes). Thereafter, the ligase enzyme was heat inactivated (98°C for 5 minutes). After the ligated sample attains room temperature, to 10 µL of the sample, 6 carboxyfluorescein (FAM) labeled primers, deoxyribonucleotide mixture and Taq DNA polymerase were added, and the reaction volume was made up in sterile water and amplified by PCR (35 cycles [95°C, 30 seconds; 60°C, 30 seconds and 72°C, 60 seconds]). The fragments were subjected to capillary electrophoresis on SeqStudio Genetic Analyzer (Thermo Fisher Scientific), and the data generated were analyzed using the Coffalyser.Net software (Amsterdam, the Netherlands).

Classification and identification of *BRCA1* and *BRCA2* variants including novel variants

The Ion Reporter Software used for variant call listed all the five classes of mutations and polymorphisms. The reported mutations were further checked in linked dbSNP[15] and ClinVar databases.[16] Additionally, other databases such as BRCA Exchange,[9] LOVD[17] and Breast Cancer Information Core (BIC)[18] were mined along with PubMed publications to reaffirm the assigned class of the mutation, the level of evidence and discern novel mutations. VarSome,[19] the integrated search engine, was used to access multiple databases, prediction tools and publications at a single site. In the absence of universal functional assay availability, in silico predictions by assessing phylogenetic conservation and the likelihood of severe physiochemical alterations in the protein were utilized as prediction tools, including Variant Effect Predictor (Ensembl),[20] SIFT, PolyPhen, TraP, Mutation Analyzer and other prediction tools available at VarSome.[19]All genetic annotations and nomenclature were done on GRCh37/hg19 build. GenBank BRCA1: NM_007300 and BRCA2: NM_000059 were used as the reference sequences.

Furthermore, the cDNA position/sequence of the alteration was inputted in MutationTaster[21] against Ensembl transcript ID ENST00000471181 for BRCA1 and ENST00000544455 for BRCA2 to obtain the sequence snippets of complementary DNA strand along with bioinformatics prediction for the altered sequence variant.

The variants were classified according to the American Society of Medical Genetics and Genomics (ACMG) recommendations for standards of interpretation and reporting of sequence variations. The variants were organized into five classes as follows: 1) pathogenic/Class 5, 2) likely pathogenic/Class 4, 3) variant of uncertain significance/Class 3 and 4) likely benign/Class 2 and 5) benign/Class 1.[22] Without departing from the scope of this study, we have considered the pathogenic, likely pathogenic and VUS mutations.

Clinical correlation

Medical records of the subjects were curated from the "Computerized Patient Record System" maintained at the Institute. The status of breast prognostic markers, estrogen receptor (ER), progesterone receptor (PR) and HER2/neu in breast cancer subjects and tumor histopathology of the ovarian cancer subjects were retrieved from the laboratory information management system.

Statistical analysis

Descriptive statistics were used to summarize the data. Categorical variables were expressed as frequencies and corresponding percentages. Pearson's chi-squared test of association/Fisher's exact test was used to compare categorical data, setting the limits of statistical significance as 0.05. Statistical analysis was conducted using SPSS version 23.0 software package (IBM Corporation, Armonk, NY, USA).

Results

A total of 206 breast and/or ovarian cancer subjects were screened for mutations. The baseline characteristics of the study group are presented in Table 1. Among the cases screened, 30.1% (62/206) of the cases were positive for *BRCA1*/BRCA*2* gene mutations, whereas one breast cancer subject was positive for a mutation in both the genes. Overall, *BRCA1*-positive cases exceeded *BRCA2*-positive cases (45/206, 21.8% vs 17/206, 8.3%). Also, among the mutation-positive cases, it was observed that BRCA mutations were about twice more common in the ovarian cancer group (42.9%, 31/74) than in the breast cancer group (22.3%, 28/126) (Table 1). Furthermore, mutation frequency was relatively higher in the dual malignancy (breast and ovarian cancers) subjects (4/6, 66.7%). These *BRCA1* and *BRCA2* mutations identified in the cohort were further carefully examined and profiled into appropriate classes.

The spectrum of *BRCA1* and *BRCA2* germline mutations

BRCA1 mutations

Various *BRCA1* germline mutations were detected in the breast and/ovarian cancer subjects. These mutations along with their location on the chromosome, predicted variant effect, class and frequency of occurrence are enlisted in Table 2. Altogether, 33 distinct *BRCA1* mutations were identified. These mutations spanned from exon 2 to exon 24 of the *BRCA1* gene and were predominantly distributed around exon 10 (Table 2 and Figure 1A). One intronic indel (on IVS7) was also observed. Altogether, based on the predicted variant effect of these mutations, 12 frameshift mutations, 5 missense mutations, 8 nonsense mutations, 3 splice-site variants, 1 big duplication (doubling of exons 21–23), 2 big deletions that caused skipping of exon 23 and skipping of exon 24 and 1 synonymous substitution were observed in the exonic regions of *BRCA1* gene in the subjects (Table 2). Most of the *BRCA1* mutations identified were pathogenic (Class 5, 79%), one mutation was likely pathogenic (Class 4, 3%), whereas six mutations were grouped in the VUS category (Class 3, 18%) (Table 2 and Figure 1B).

BRCA2 mutations

Next, we analyzed *BRCA2* sequence variants that were detected in the subjects (Table 3). Half of the mutations detected were concentrated around exon 11 (Figure 1C). In total, 16 different mutations were detected in *BRCA2* gene, including 6 frameshifts, 7 missense, 2 nonsense mutations and a big deletion of exon 12. Furthermore, 7/16 (44%) of these mutations were VUS, among which 6/7 were missense mutations (Figure 1D and Table 3).

A comparison of Class 4/Class 5 *BRCA1* and *BRCA2* gene mutations based on the predicted variant effect, observed in the study, is shown in Figure 1E. As evident from Figure 1E, frameshift and protein decaying nonsense mutations were found to be relatively common in the cohort.

In silico analysis of novel mutations

These variants were compared in various databases and existing literature. Although most of the mutations had been recognized previously, five novel mutations (c.541G>T, c.1681delT, c.2295delG, c.4915C>T and large deletion of exon 23) in *BRCA1* gene and one novel *BRCA2* variant (c.932_933insT)

Table 1 Baseline characteristics of the study group (n=206)

	Breast cancer, n (%)	Ovarian cancer, n (%)	Breast and ovarian cancers, n (%)	Total, n (%)
	(n=126)	(n=74)	(n=6)	(n=206)
Age (years)				
≤50	94 (74.6)	26 (35.1)	–	120 (58.3)
>50	32 (24.4)	48 (64.9)	6 (100)	86 (41.7)
Median age (range)	41.5 (25–74)	55 (32–74)	56.5 (53–70)	47 (25–74)
Gender				
Male	3 (2.4)	–	–	3 (1.5)
Female	123 (97.6)	74 (100)	6 (100)	203 (98.5)
Mutation status				
BRCA1 positive	20 (15.9)	21 (28.4)	4 (66.7)	45 (21.8)
BRCA2 positive	7 (5.6)	10 (13.5)	0 (0)	17 (8.3)
Both *BRCA1* and *BRCA2* positive	1 (0.8)	0 (0)	0 (0)	1 (0.5)
No mutation detected	98 (77.8)	43 (58.1)	2 (33.3)	143 (69.4)
Positive family history of cancer	40 (31.7)	20 (27)	1 (16.7)	61 (29.6)

Table 2 *BRCA1* germline mutations in the breast and/or ovarian cancer group

Locus	Exon/ intron	Variation	Amino acid change	Variant effect	Class	Frequency	Previously reported
Breast cancer							
chr17:41276044	2	c.68_69delAG	p.Glu23fs	Frameshift	5	1	Yes
chr17:41251798	**7**	**c.541G>T**	**p.Glu181Ter**	**Nonsense**	**4**	**1**	**Novel**[a]
chr17:41245333	10	c.2214_2215insT	p.Lys739Ter	Nonsense	5	2	Yes
chr17:41245278	10	c.2269delG	p.Val757fs	Frameshift	5	1	Yes
chr17:41245252	**10**	**c.2295delG**	**p.Ser766fs**	**Frameshift**	**5**	**1**	**Novel**
chr17:41245185	10	c.2362delG	p.Val788fs	Frameshift	5	1	Yes
chr17:41244217	10	c.3328-3330delAAG	p.Lys1110del	Missense	3	1	Yes
chr17:41243037	**11**	c.4108delT	p.Ser1370fs	Frameshift	5	**1**	Yes
chr17:41242983	**11**	c.4158_4162delCTCTC	p.Ser1387fs	Frameshift	5	**2**	Yes
chr17:41234450	12	c.4328G>A	p.Arg1443Gln	Missense	3	1	Yes
chr17:41226515	15	c.4571C>A	p.Ser1524Ter	Nonsense	5	2	Yes
chr17:41219624	17	Splicesite_3 (C>T)	.	Splicing	5	1	Yes
chr17:41203135	21	c.5278-1 G>T	.	Splicing	5	1	Yes
chr17:41199538	21–23	Duplication (exons 21–23)	.	Big duplication	5	2	Yes[b]
chr17:41199538	**23**	**Deletion (exon 23)**	**Truncated protein at codon 1770**	**Big deletion**	**5**	**1**	**Novel**
chr17:41201142	23	c.5465G>A	p.Gly1822Asp	Missense	3	1	Yes[c]
chr17:41197601	24	Deletion (exon 24)	.	Big deletion	5	1	Yes[d]
Ovarian cancer							
chr17:41256148	6	c.431delA	p.Asn144fs	Frameshift	5	1	Yes
chr17:41246098	10	c.1450G>T	p.Gly484Ter	Nonsense	5	1	Yes
chr17:41245866	**10**	**c.1681delT**	**p.Ser561fs**	**Frameshift**	**5**	**1**	**Novel**
chr17:41245346	10	c.2188_2195delGAAAAAGA insAAAAAGG	p.Glu730fs	Frameshift	5	1	Yes
chr17:41245333	10	c.2214_2215insT	p.Lys739Ter	Nonsense	5	3	Yes
chr17:41245306	10	c.2241delC	p.Asp749fs	Frameshift	5	1	Yes
chr17:41245252	**10**	**c.2295delG**	**p.Ser766fs**	**Frameshift**	**5**	**1**	**Novel**
chr17:41244159	10	c.3389C>G	p.Ser1130Ter	Nonsense	5	1	Yes
chr17:41243941	10	c.3607C>T	p.Arg1203Ter	Nonsense	5	2	Yes
chr17:41242961	11	c.4185G>A	p.Gln1395=	Synonymous	3	1	Yes
chr17:41226405	15	c.4681G>T	p.Glu1561Ter	Nonsense	5	1	Yes
chr17:41223097	**16**	c.4897C>T	p.Gln1633Ter	Nonsense	5	**1**	Yes
chr17:41223079	**16**	**c.4915**C>T	**p.His1639Tyr**	**Missense**	**3**	**1**	**Novel**
chr17:41219624	17	Splicesite_3 (C>T)		Splicing	5	2	Yes
chr17:41215948	18	c.5158C>T	p.Arg1720Trp	Missense	5	1	Yes[e]
chr17:41203107	21	c.5367delC	p.Tyr1790fs	Frameshift	5	1	Yes
chr17:41256089	IVS7	delAAAAAAAAAGAAAAG>A		Deletion	3	1	Yes
Breast and ovarian cancers							
chr17:41243941	10	c.3607C>T	p.Arg1203Ter	Nonsense	5	1	Yes
chr17:41243505	10	c.4041_4042delAG	p.Gly1348fs	Frameshift	5	1	Yes
chr17:41226515	15	c.4571C>A	p.Ser1524Ter	Nonsense	5	1	Yes
chr17:41226347	15	Splicesite_3 (G>T)		Splicing	5	1	Yes

Notes: Bold indicates novel mutation. Previous reports denoted Yes are cited in the databases mentioned in the "Materials and methods" section. [a]Reported previously by Suryavanshi et al.[23] [b]Reported by Hogervorst et al.[24] [c]Reported by Machackova et al.[25] [d]Reported by Armaou et al,[26] Engert et al.[27] and Sedghi et al.[28] [e]Reported by Zorrieh Zahra et al.[29]

were identified, for which previous reports were unavailable. We further sought to look into these mutations in detail. Mutation plots showing the placement of the mutation and predicted amino acid variation caused by the mutation on BRCA1/2 protein are depicted in Figure 2A and B.

The first novel mutation c.541G>T (E181X) is a transversion mutation, a single-nucleotide variation which results in the conversion of glutamic acid triplet codon (GAA) at position 541 to a stop codon (TAA). The second mutation c.1681delT is a frameshift mutation wherein deletion of a single base pair

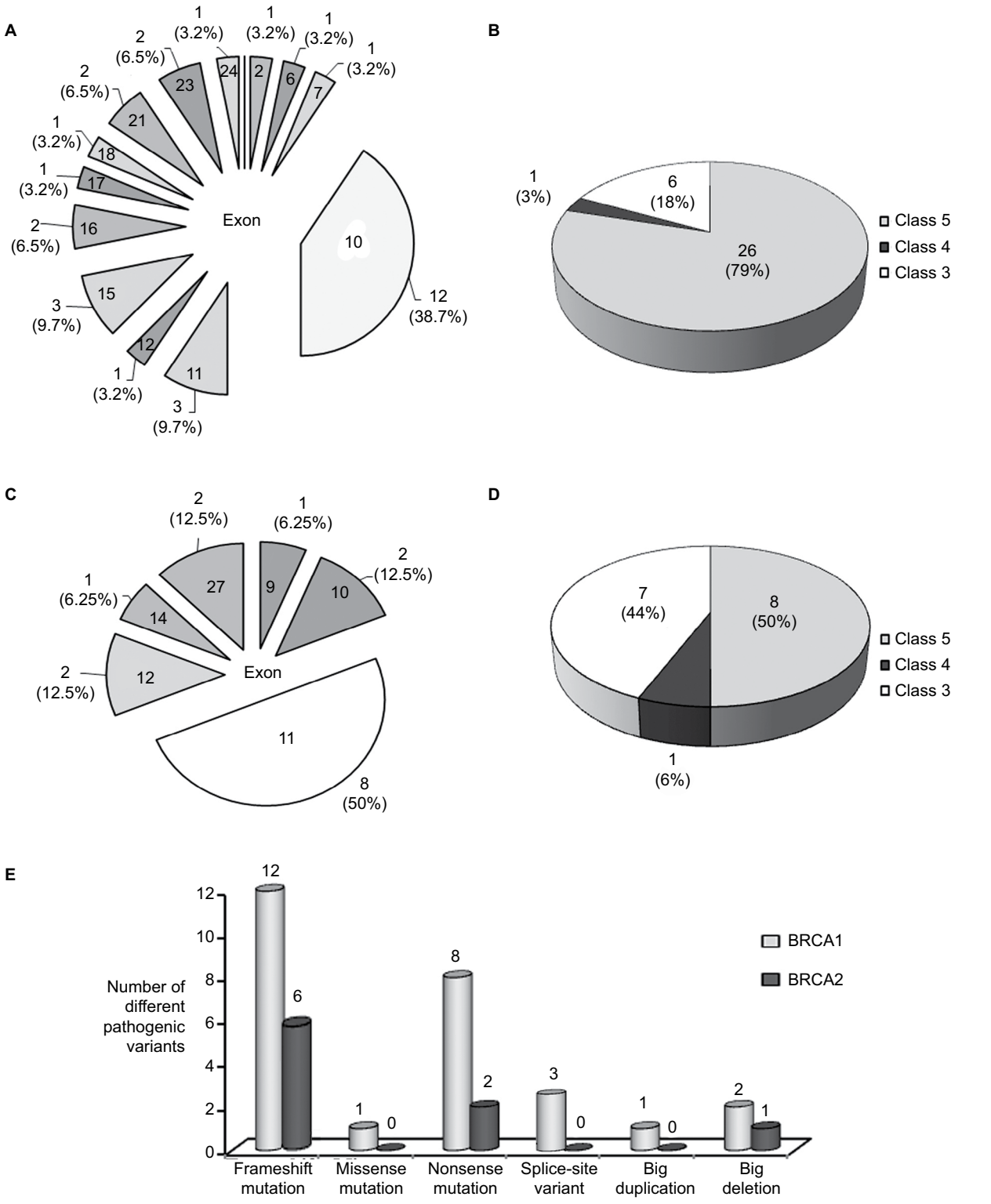

Figure 1 Mutational spectrum of BRCA genes in the breast and/or ovarian cancer group.

Notes: Pie chart depicting (**A**) exonic distribution of the distinct *BRCA1* mutations ([a]n=31). (**B**) Number of different types of Class 5, Class 4 and Class 3 mutations observed in *BRCA1* gene (n=33). (**C**) Exonic distribution of distinct *BRCA2* mutations (n=16). (**D**) Number of different types of Class 5, Class 4 and Class 3 mutations observed in *BRCA2* gene (n=16). (**E**) Bar graph comparing the number of different Class 4/5 mutations observed in *BRCA1*/*BRCA2* genes (n=36). [a]Duplication (exons 21–23) and one intronic mutation have been excluded from the pie diagram.

Table 3 *BRCA2* germline mutations in the breast/ovarian cancer patients

Locus	Exon	Variation	Amino acid change	Variant effect	Class	Frequency	Previously reported
Breast cancer							
chr13:32906547	**10**	**c.932_933insT**	**p.Ser313fs**	**Frameshift**	**5**	**1**	**Novel**
chr13:32906565	10	c.950_951insA	p.Asn319fs	Frameshift	5	1	Yes[a]
chr13:32911297	11	c.2808_2811delACAA	p.Ala938fs	Frameshift	5	1	Yes
chr13:32913587	11	c.5095G>A	p.Asp1699Asn	Missense	3	1	Yes
chr13:32914617	11	c.6125A>C	p.Gln2042Pro	Missense	3	1	Yes
chr13:32918739	12	c.6886A>C	p.Ile2296leu	Missense	3	1	Yes
chr13:32929291	14	c.7301A>C	p.Lys2434Thr	Missense	3	1	Yes
chr13:32972626	27	c.9976A>T	p.Lys3326Ter	Nonsense	5	1	Yes
Ovarian cancer							
chr13:32905126	9	c.752C>T	p.Thr251Ile	Missense	3	1	Yes
chr13:32911620	11	c.3128C>G	p.Ala1043Gly	Missense	3	1	Yes
chr13:32913032	11	c.4540_4541insA	p.Ile1516fs	Frameshift	5	1	Yes
chr13:32913032	11	c.4544_4545insA	p.Ile1516fs	Frameshift	5	1	Yes
chr13:32913475	11	c.4983T>G	p.Try1661Ter	Nonsense	4	1	Yes
chr13:32913558	11	c.5070_5073delAAAA	p.Lys1690fs	Frameshift	5	1	Yes
chr13:32918653	12	Deletion (exon 12)	.	Big deletion	5	1	Yes[b]
chr13:32972626	27	c.9976A>T	p.Lys3326Ter	Nonsense	5	1	Yes
chr13:32972739	27	c.10089A>G	p.Ile3363Met	Missense	3	2	Yes

Notes: Bold indicates novel mutation. Previous reports denoted Yes are cited in the databases mentioned in the "Materials and methods" section. [a]Reported previously by Suryavanshi et al.[23] [b]Reported by Rauh-Adelmann et al.[30]

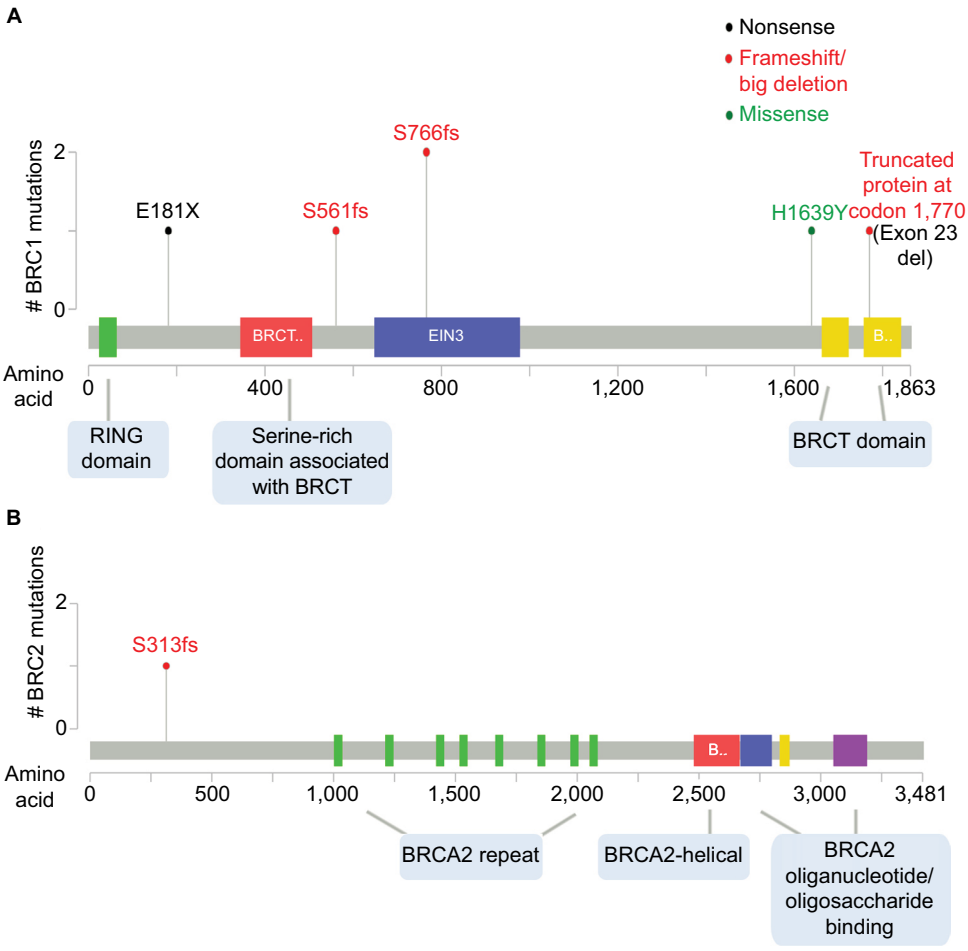

Figure 2 Novel *BRCA1*/*BRCA2* mutations identified in the cohort.
Notes: Lollipop plots showing the distribution of germline mutations in (**A**) *BRCA1* and (**B**) *BRCA2* genes. Predicted amino acid change has been represented for each mutation. The plots were generated using the online tool MutationMapper – cBioPortal for Cancer Genomics[31,32] (GenBank Reference BRCA1: NM_007300 and GenBank Reference BRCA2: NM_000059).

(T) changes the reading frame from serine (TCT) at the 561st position to leucine (CTA), thereby shuffling the frame by a single base forward, generating stop codon (TAG) after 11 codons. The third novel mutation c.2295delG is a frameshift mutation, wherein the deletion of base G shifts the frame of glutamic acid (GAG) to again glutamic acid (GAA). But this alteration further changes the next codon serine (AGT) at the 766th position to valine (GTA) and further slips the coding sequence by a single base ahead leading to the generation of a stop codon (TAG) at the 26th codon downstream of the frameshift position. The fourth novel variant identified in this study is c.4915C>T, which causes a missense mutation (H1639Y) where C>T transition replaces histidine (CAT) with tyrosine (TAT). This mutation was predicted to be VUS. Finally, the last variant is a large deletion causing skipping of exon 23, predicted to be protein-truncating at codon 1770.

In the single novel *BRCA2* mutation, c.932_933insT, the insertion of a single base T modifies the frame to generate two subsequent phenylalanines (TTT and TTC) and further formats an early stop codon (TAA) at serine (TCT) at the 313th amino acid position.

Specific mutation in BRCA1/BRCA2 genes occurred frequently

Seven different genetic alterations in *BRCA1* (c.2214_2215insT, c.3607C>T, c.4571C>A, c.4158_4162delCTCTC, c.2295delG, large duplication spanning exons 21–23 and splicesite_3 (C>T) variations) and two different mutations in *BRCA2* (c.9976A>T and c.10089A>G) were observed in the study group, which repeated more than once in the cohort (Table 4).

All the repeating *BRCA1* mutations were deleterious (Class 5). Among these recurrent mutations, c.2295delG was the only novel mutation observed in two subjects (Table 4).

Clinicopathological characteristics of the study group

To determine whether there exists any relationship between *BRCA1*/*BRCA2* mutation status and prognostic markers (ER, PR and Her2) in breast cancer or tumor histology in ovarian cancer in our study cohort, we extracted medical information from laboratory reports of the patients and analyzed the data. As shown in Table 5, the considerable fraction of *BRCA1* carriers (15/24, 62.5%) were triple-negative breast cancer (TNBC) cases, whereas *BRCA2* carriers were more or less equally distributed with respect to various breast cancer subtypes. However, no significant correlation was obtained between the prognostic marker status/subtype among the carriers of *BRCA1* mutation, *BRCA2* mutations and the noncarriers (Table 5).

With respect to ovarian cancer subjects (including breast and ovarian cancer cases), almost all (34/35) *BRCA1*/*BRCA2* mutation-positive cases had high-grade serous carcinoma histology. Endometrioid carcinoma occurred rarely, observed in three cases belonging to the noncarrier group. Furthermore, tumor histology and BRCA status failed to achieve significant correlation (Table 5).

Discussion

This study is the first to report the spectrum of germline BRCA mutations in breast and ovarian cancer patients from a large cancer care hospital in North India. All coding regions

Table 4 Recurrent *BRCA1*/*BRCA2* gene mutations observed in the study cohort

Locus	Exon	Variation	Amino acid change	Variant effect	Class	Frequency (number of subjects)	Cancer type
BRCA1							
chr17:41245333	10	c.2214_2215insT	p.Lys739Ter	Nonsense	5	5	2-Br and 3-Ov
chr17:41243941	10	c.3607C>T	p.Arg1203Ter	Nonsense	5	3	2-Ov, 1-Br and Ov
chr17:41245252	**10**	**c.2295delG**	**p.Ser766fs**	**Frameshift**	**5**	**2**	**1-Br and 1-Ov**
chr17:41242983	11	c.4158_4162delCTCTC	p.Ser1387fs	Frameshift	5	2	2-Br
chr17:41226515	15	c.4571C>A	p.Ser1524Ter	Nonsense	5	3	2-Br, 1-Br and Ov
chr17:41219624	17	Splicesite_3 (C>T)		Splicing	5	3	1-Br, 2-Br and Ov
chr17:41199538	21–23	Duplication (exons 21–23)		Big duplication	5	2	2-Br
BRCA2							
chr13:32972626	27	c.9976A>T	p.Lys3326Ter	Nonsense	5	2	1-Br and 1-Ov
chr13:32972739	27	c.10089A>G	p.Ile3363Met	Missense	3	2	2-Ov

Note: Bold indicates novel mutation.
Abbreviations: Br, breast; Br and Ov, both breast and ovarian cancers; Ov, ovarian.

Table 5 Correlation between BRCA mutation status and prognostic markers/tumor histopathology

	BRCA1 carriers, n (%)	BRCA2 carriers, n (%)	Noncarriers, n (%)	n (%)	*P*-value
Cancer of breast/breast and ovary (n=131)[a]					
Prognostic markers	(n=24)	(n=7)	(n=100)	(n=131)	
ER					
Positive	7 (29.2)	2 (28.6)	43 (43.0)	52 (39.7)	0.43
Negative	17 (70.8)	5 (71.4)	57 (57.0)	79 (60.3)	
PR					
Positive	6 (25.0)	2 (28.6)	34 (34.0)	42 (32.1)	0.74
Negative	18 (75.0)	5 (71.4)	66 (66.0)	89 (67.9)	
HER2/neu					
Positive	2 (8.3)	3 (42.9)	11 (11.0)	16 (12.2)	0.07
Negative	22 (91.7)	4 (57.1)	89 (89.0)	115 (87.8)	
Subtypes					
HR+[b], HER2/neu+	0 (0.0)	0 (0.0)	5 (5.0)	5 (3.8)	0.08
HR+[b], HER2/neu–	7 (29.2)	2 (28.6)	38 (38.0)	47 (35.9)	
ER– PR– HER2– (TNBC)	15 (62.5)	2 (28.6)	52 (52.0)	69 (52.7)	
ER– PR– HER2+ (HER2 enriched)	2 (8.3)	3 (42.9)	5 (5.0)	10 (7.6)	
Cancer of ovary/breast and **ovary (**n=80)					
Tumor histopathology	(n=25)	(n=10)	(n=45)	(n=80)	
Serous carcinoma (HG)	25 (100)	9 (90.0)	42 (93.3)	76 (95.0)	0.29
Endometrioid carcinoma (HG)	0 (0.0)	0 (0.0)	1 (2.2)	1 (1.3)	
Endometrioid carcinoma (LG)	0 (0.0)	0 (0.0)	2 (4.4)	2 (2.5)	
Clear cell carcinoma	0 (0.0)	1 (10.0)	0 (0.0)	1 (1.3)	

Notes: [a]One breast cancer subject positive for a mutation in both *BRCA1* and *BRCA2* genes was excluded from this comparison. [b]HR+ include ER– and/or PR-positive subjects. Column percentage is shown in parentheses.
Abbreviations: ER, estrogen receptor; HG, high grade; LG, low grade; PR, progesterone receptor.

and splice sites with large flanking regions were sequenced, and large genomic rearrangements were identified using MLPA.

Forty-five and 17 patients had germline *BRCA1* and *BRCA2* mutations, respectively, belonging to Classes 3, 4 and 5. Among the breast cancer cases, 9 of 28 BRCA mutation carriers had a hormone receptor-positive tumor. Contrary to expectation, seven of these had *BRCA1* mutation and only two of the nine cases had the *BRCA2* mutation. The presence of *BRCA1* mutations in ER-positive tumors is known.[33–36] These investigators have reported *BRCA1* mutation carrier rates of 10%–17%, whereas in this study, the incidence of *BRCA1* mutations with ER-positive breast cancer is higher with a rate of 25%. This observation needs to be followed up on a larger data set as these cancers generally arise at a later age and have less aggressive biology compared to ER-negative *BRCA1*-mutated tumors[37] and may have important implications in the use of hormone modulation for chemoprevention. In the latter study by Lips et al,[37] the genomic profile of *BRCA1*-mutated ER-positive tumors was found to be similar to that of *BRCA2*-mutated ER-positive tumors. In addition, clinicopathological variables in *BRCA1*-mutated ER-positive cancer were similar to those of *BRCA2*-mutated ER-positive and sporadic ER-positive breast cancer compared to those of *BRCA1*-mutated ER-negative cancers. In this study, the ER-positive *BRCA1*-mutated cancer occurred in the age range of 32–74 years (median age being 46.5 years) and was majorly grade II (data not shown). Histologically, these revealed florid tubule formation and low mitotic activity similar to low-grade sporadic ER-positive breast cancers.

Ten cases of breast cancer tested for germline BRCA mutations were Her2 overexpressing or amplified. Of these, 5 showed deleterious BRCA mutations with 2/24 in *BRCA1* and 3/7 in the *BRCA2* gene. This emphasizes the need to strictly abide by the guidelines for BRCA testing and not use HR or HER2 status to guide patient selection.

Sixty-two of 206 (30.1%) breast and/or ovarian cancer patients tested showed VUS or pathogenic mutations. A similar incidence of 35% BRCA mutation among breast and/or ovarian cancer patients has been reported in another Indian study.[38] To date, 510 distinct types of deleterious *BRCA1*/BRCA*2* mutations (268 *BRCA1* and 242 *BRCA2*) have been cataloged in Asian patients with breast cancer, most of which are frameshift or nonsense mutations.[39] From

the mutation listed in this compilation from Asian countries, only c.68_69delAG *BRCA1* mutation was found in one of our breast cancer patients. This founder mutation in Ashkenazi Jews has also been reported in Pakistan and Malaysia. All the other Class 5 and Class 4 mutations seen by us have not been reported either in the Asian population[39] or in the previous study from North Indian population.[13] Likewise, the worldwide study of BRCA mutations listing five commonest mutations from several neighboring Asian countries also does not match with the five commonest *BRCA1* mutations detected in this study.[40]

However, of the deleterious mutations identified in this study, c.2214_2215insT (p.Lys739Ter), nonsense *BRCA1* mutation was the most frequent, identified in 2/126 of the breast cancer patients and 3/74 of the ovarian cancer patients. This mutation has been reported at least nine times and has the approval of the expert committee.[40–42] This common mutation did not reach the proportion of a founder mutation and has not been reported from India in any other study, including the latest study by Singh et al[38] and other Indian studies.[5,43,44] The other four common *BRCA1* mutations seen in this study were c.3607C>T (p.Arg1203Ter); c.4158_4162delCTCTC (p.Ser1387fs); splice site_3C>T and c.2295delG (p.Ser766fs). We also identified three long genomic rearrangements in our cohort of patients, and all these were located toward the 3′-end of the *BRCA1* gene. These have been the duplication of exons 21–23 in two cases and deletion of exon 24 in one case. No ovarian or breast cancer cluster region was recognized. Exon 10 was the preferred location for *BRCA1* mutations and was mutated in 19/45 cases.

We have identified the following five novel mutations in *BRCA1*, which are not found in databases and in any publication so far: nonsense mutation (c.541G>T), two frameshift mutations (c.1681delT and c.2295delG), one missense mutation (c.4915C>T) and one big deletion (del exon 23).

With respect to the *BRCA2* gene, ambiguous VUS mutations were more common (7/16 of the mutation carriers). Indian population being a less tested and characterized group, a higher VUS frequency is anticipated and calls for future studies. One novel BRCA2 variant (c.932_933insT) was identified. Interestingly, this variant was observed in the subject carrying a dual mutation in both *BRCA1* and *BRCA2* genes. Two recurrent mutations were observed as follows: c.9976A>T (pathogenic nonsense mutation) and c.10089A>G (missense VUS), in two subjects each. Both the mutations have been reported previously. The c.9976A>T, a truncating allele mutation (creating stop codon Lys3326Ter), has been reevaluated by Thompson et al[45] in familial breast cancer cases. Based on a case–control study, it was identified as a low-to-moderate risk variant that was recommended to be included in the breast cancer risk evaluating panels.[45,46] This mutation has also been listed in the panel of top 20 BRCA2 mutation frequencies in the BIC database.[18]

Conclusion

BRCA mutations have a high lifetime risk of hereditary breast and ovarian cancers. Of all the hereditary germline mutations, BRCA mutations have a high penetrance and high incidence second only to Familial Adenomatous Polyposis and Lynch Syndrome, respectively. Identifying deleterious BRCA mutations can help both the patients and biological relatives. Creating this database is a long-drawn process and helps in identifying recurring, deleterious and VUS mutations in a population. Segregation studies and functional analysis over time can help classify VUS better. This study reports various deleterious and VUS mutations in *BRCA1* and *BRCA2* genes observed in North Indian population. Six novel mutations and nine recurrent mutations have been documented. The knowledge shared would help in deepening the process of screening and assist genetic services in enabling more focused site-specific screening for mutations in biological relatives.

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Author contributions

AM conceived the idea, supervised the experiments and bioinformatics analysis of mutations, critically evaluated the data and was involved in manuscript writing. SV performed data collection and data analysis, critically evaluated the data and organized and wrote the manuscript. SKS performed DNA extraction, DNA sequencing (NGS and MLPA) and data compilation. DK performed DNA extraction and DNA sequencing. MP performed DNA extraction and DNA sequencing. MS supervised the experiments, bioinformatics analysis of mutations and patient guiding. GG performed genetic counseling and data analysis. All authors contributed to data analysis, drafting and revising the article, gave final approval of the version to be published, 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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