

## Founder *BRCA1* mutations in Nepalese population

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**Background:** Founder mutation is a heritable genetic alteration observed with high frequency in a geographically and culturally isolated population where one or more ancestors becomes the forebearer of the altered gene. The current study reports two founder mutations in the *BRCA1* gene in the Nepalese people. **Methods:** Germline *BRCA* testing in all surface epithelial ovarian cancers and the selected case of breast, prostate, and pancreatic cancers has been the standard practice from 2016 to 2021. One thousand one hundred thirty-three probands were screened for germline *BRCA* variants by next generation sequencing. The variants were classified as per the American Society of Medical Genetics and Genomics recommendations. Pathogenic (class V) and likely pathogenic (class IV) were considered clinically relevant and utilized for cascade screening. **Results:** Nepalese population made up a subcohort of 5.12% (58/1,133) of probands tested for germline *BRCA1/2* variants. Twenty-seven of these 58 tested harbored pathogenic genetic alterations in *BRCA1/2* genes, with 23 being *BRCA1* mutant. Sixteen of 23 *BRCA1* mutant cases shared one common pathogenic mutation c.2214\_2215insT (p.Lys739Ter) (NM\_007294.4). Additionally, a second highly recurrent mutation in *BRCA1* gene c.5068A>T (p.Lys1690Ter) (NM\_007294.4) was noted in six patients from this population. **Conclusions:** The overwhelming abundance of the above two variants in a geographically confined population confers these two genetic alterations a status of founder mutations amongst the people of Nepal. A more extensive population-based study to reaffirm these findings will help establish a dual site-specific germline testing similar to the “Multisite-3-asay” in Ashkenazi Jews as the primary screening tool, especially in a resource-constrained environment.

**Key Words:** *BRCA1*; *BRCA2*; Founder mutation; Nepal

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The founder effect amplifies a particular genotypic alteration in a geographically or culturally confined population caused by several generations of interbreeding. Deleterious germline alterations of the *BRCA1* (OMIM \*113705) and *BRCA2* (OMIM \*600185) gene result in breast and ovarian cancer susceptibility. The lifetime risk of breast cancer is 55%–72% and 45%–69% in mutation carriers of *BRCA1* and *BRCA2* deleterious germline alterations, respectively [1-4]. Likewise, the risk of ovarian cancer is 39%–44% and 11%–17% in the *BRCA1* and *BRCA2* germline variants [1-4].

Germline alterations of *BRCA2* variants and, to a lesser extent, *BRCA1* variants in males predispose them to the breast [5] and prostate carcinoma [6-8]. The inherited pathogenic *BRCA1/BRCA2* variants also increase the risk of pancreatic cancers, albeit to a lower extent [9]. The prevalence of *BRCA1* or *BRCA2* mutations varies considerably between ethnic groups and geo-

graphical areas [10,11]. Founder *BRCA* mutations that are population-specific mutation(s) have been described in Iceland, Netherlands, Sweden, Norway, Germany, France, Spain, countries of central and eastern Europe, and Ashkenazi Jews [10,11]. The prevalence of *BRCA* mutation carriers in the general population is estimated at 1/800 to 1/1,000; however, the rate of occurrence of *BRCA* mutation increases several-fold in the population carrying founder mutation, for instance, the rate of a founder mutation in Ashkenazi Jews goes up to 1 in 40 [12,13]. Nepal is a Himalayan country with limited groupings of Tibeto-Burman and Indo-Aryan stock [14]. The geographically confined existence and restricted interaction with the outside world due to rugged terrain and the land-locked nature of the country provided an appropriate milieu for a deleterious mutation to breed, dominate and produce the founder effect. The current study reports the concentration of two particular *BRCA1*-specific vari-

ants in the Nepalese population tested for *BRCA1* and *BRCA2* deleterious mutations.

## MATERIALS AND METHODS

### Research setting and subjects

One thousand one hundred thirty-three patients diagnosed with breast/ovarian/prostate/pancreatic cancer and who fulfilled the *BRCA* testing criteria recommended by National Comprehensive Cancer Network (NCCN) were included in the study [15]. Each eligible subject was explicitly explained and counseled about the pros and cons of undergoing *BRCA* testing by the institutional genetic counselor, and informed written consent was obtained for testing and using the information for research. The included participants were screened for the mutations in the *BRCA* genes by next generation sequencing (NGS), and the large genomic rearrangements (Big Indels) were examined through Multiplex Ligation-dependent Probe Amplification (MLPA).

### Isolation of DNA from blood, NGS, and data analysis

Genomic DNA was isolated from 2 mL of peripheral blood of the index case 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). NGS library was prepared manually with 10 ng of the isolated DNA using Oncomine *BRCA* assay – A328400 (Thermo Fisher Scientific) as detailed elsewhere [16]. Data generated from the runs was assessed for quality metrics on Torrent Suite Viewer (Ion Torrent Suite 5.10, Thermo Fisher Scientific) for parameters like the number of mapped reads, average base coverage depth, uniformity of coverage, coverage at 1×, 20×, and 100×, strand bias, end to end amplicon reads. Thresholds were employed as enunciated in The National Cancer Institute-Molecular Analysis for Therapy Choice (NCI-MATCH) trial [17]. Mapped reads of > 1,00,000 with 90% uniformity, average base coverage depth ≥ 100, and

> 90% coverage at 100x were considered optimal for reporting though the base coverage and mapped reads were usually far more. The variants were classified according to the American Society of Medical Genetics and Genomics recommendations for standards of interpretation and reporting of sequence variations [18].

Cases tested negative for *BRCA1/BRCA2* mutations were further investigated for possible large genomic rearrangements (Big Indels) by MLPA assay described previously elsewhere [16].

### Statistical analysis

Descriptive statistics were used to summarize the data. The data were analyzed statistically using SPSS ver. 23.0 (IBM Corp., Armonk, NY, USA). The statistical analysis comprised of calculating means and proportions. Appropriate tests of significance were applied, and a p-value of < .05 was considered significant.

## RESULTS

One thousand one hundred thirty-three comprehensive *BRCA1* and *BRCA2* testing for different ethnic populations, comprising the Indian, Nepalese, Bangladeshi, Afghani, Kenyan, Iraqi, and the Myanmar, were performed at the Molecular Diagnostic laboratory of a tertiary cancer care hospital in India. Of the 1,133 patients tested, 58 were Nepalese descent (5.1%) with a mean age of 47.3 years. Twenty-seven of the 58 Nepalese patients harbored pathogenic *BRCA1/BRCA2* genetic alteration, 16 breast, and 11 ovarian carcinoma cases. The mean age was 46.5 years in the Nepalese against 46.8 years in the rest of the cohort (95% confidence interval, -4.288 to 5.088; p = .867). Strikingly 23 of these 27 affected probands carried *BRCA1* mutation and, far more significantly, just two recurrent nonsense mutations, as shown in Table 1 below. Only one proband out of 23 with *BRCA1* inactivating alteration had a frameshift mutation outside these two genetic alterations. The two recurrent *BRCA1* alterations (c.2214\_2215insT and c.5068A>T) were not found in any other ethnic population in the study (Fig. 1).

A *BRCA* mutation is seen in 27 Nepalese patients (27/58), ac-

**Table 1.** *BRCA1/BRCA2* variants encountered in 27 Nepalese patients in our study

Gene	Variant	NM transcript	No. of patients with the variant
<i>BRCA1</i>	c.2214_2215insT (p.Lys739Ter)	NM_007294.4	16
<i>BRCA1</i>	c.5131A>T(p.Lys1690Ter)	NM_007294.4	6
<i>BRCA1</i>	c.2157_2158insA (p.Glu720ArgfsTer6)	NM_007294.4	1
<i>BRCA2</i>	c.1303_1304insA(p.Arg435fs)	NM_000059.4	1
<i>BRCA2</i>	c.2808_2811 del ACAA (p.Ala938fs)	NM_000059.4	1
<i>BRCA2</i>	c.6270_6271delTA(p.His2090GlnfsTer9)	NM_000059.4	1
<i>BRCA2</i>	c.7030delA(p.Ile2344TyrfsTer23)	NM_000059.4	1

counting for 46.5% of tested patients. In comparison, *BRCA* mutation is observed in 227 non-Nepalese patients (227/1,075), accounting for 21.1%. The preceding emphasizes that *BRCA* mutation is significantly higher in the Nepalese population than in the non-Nepalese population (Fisher exact test, statistical value is <0.001). Also, *BRCA1* mutation was more common in Nepalese subcohort (23/58) than non-Nepalese subcohort (167/1,075) (Fisher exact test, statistical value is <0.001).

Of the total *BRCA* mutant cases, c.2214\_2215insT is the most frequently observed *BRCA1* variant in the Nepalese population (16/27), constituting 59.2% of the total *BRCA* mutant cases followed by c.5068A>T (6/27) (22.2%). Thirty three point three three percentage of these 27 *BRCA* mutant cases constituted triple-negative breast carcinoma with 29.6% luminal type breast cancer and 25.9% high-grade serous ovarian carcinoma, as summarized in Table 2. Only 11 (11/27) *BRCA* mutant cases had a family history of breast/ovarian cancer.

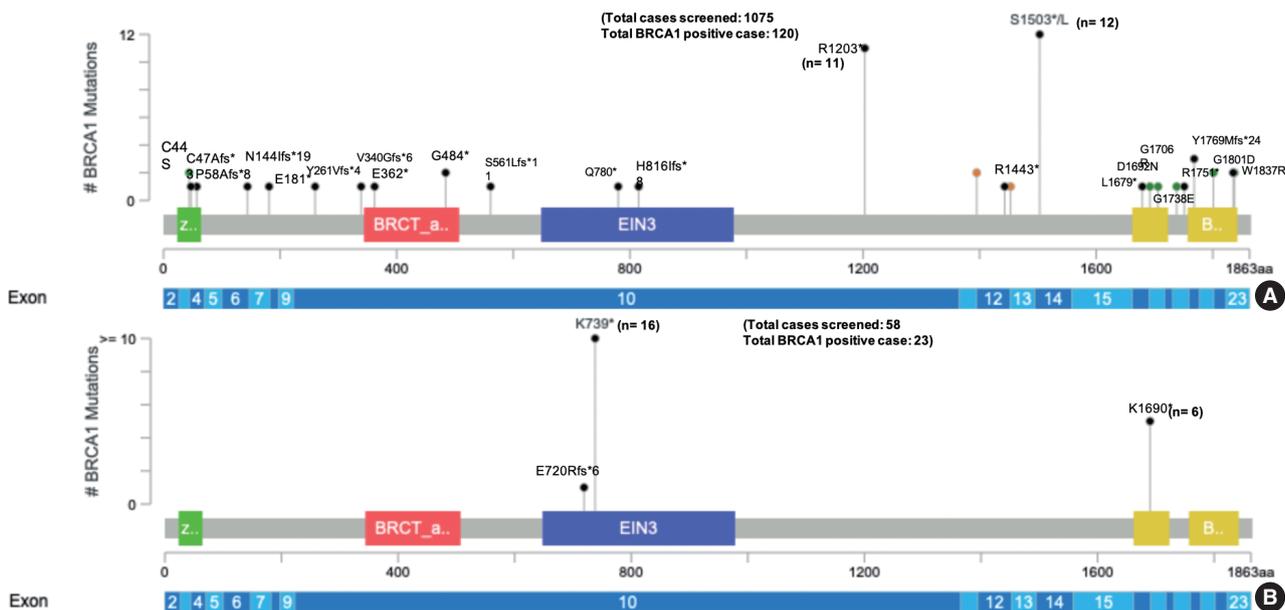
One of the probands was tested for somatic alteration in the *BRCA1* gene in ovarian carcinoma, and p.Lys1690Ter was detected in the *BRCA1* gene at a high variant allele frequency (VAF). Combined with our observation of this variant being highly repetitive in the Nepalese people and the high VAF, a peripheral blood testing was done by an orthogonal method (site-specific Sanger sequencing) and confirmed as germline.

## DISCUSSION

*BRCA1* and *BRCA2* mutations show variable prevalence in different ethnic populations. The prevalence of *BRCA1/2* mutation in the general population is estimated to be around 1/800 to 1/1,000 or at the rate of 0.125 to 0.1%. In contrast, the prevalence increases several-fold in the geographically confined ethnic groups with founder mutation as in Ashkenazi Jews, where *BRCA* mutation is observed at a frequency of 2.5% [12,13]. The present study highlighted a similarly high incidence of *BRCA* mutation in the Nepalese patient cohort (46.5%), with the *BRCA1* gene altered in 39.6% and the *BRCA2* in 7%.

The discovery of a founder mutation in Ashkenazi Jews population paved the way to the “Multisite 3 assay” testing for *BRCA1* 185delAG, *BRCA1* 5382insC, and *BRCA2* 6174delT. The present study identifies two founder mutations in the *BRCA1* gene (c.2214\_2215insT and c.5068A>T) in the Nepalese population. Significantly, none from the non-Nepalese cohort of the 1,075 probands tested positive for these two mutations. This exclusiveness and high frequency of just two variants in an ethnic group support the founder nature of these variants.

These two alterations account for 85.2% of all cases detected with germline *BRCA* mutation in the Nepalese subcohort, which is far higher than the three mutations that make up 73.2% of all germline *BRCA* mutations amongst Ashkenazi Jews [21].



**Fig. 1.** The commonly encountered *BRCA1* mutations in the non-Nepalese population (A) and the Nepalese population (B) in the present study [19,20]. Note the dominance of just two genetic alterations in the Nepalese people and contrast to the widespread distribution of pathogenic mutation in the full-length *BRCA1* gene in the non-Nepalese population.

**Table 2.** Clinical and morphological profile of *BRCA* mutant and *BRCA* wild-type cases in Nepalese and Non-Nepalese subcohorts

	<i>BRCA</i> mutant Nepalese cohort (n=27)	<i>BRCA</i> wild Nepalese cohort (n=31)	<i>BRCA</i> mutant non-Nepalese subcohort (n=227)	<i>BRCA</i> wild non-Nepalese cohort (n=848)
Age (yr)				
Mean <sup>a</sup>	46.5	51.6	46.8	51.6
Median (range)	44.5 (32–76)	52 (27–72)	44.5 (29–66)	52 (29–86)
Ovarian carcinoma with the morphology of HGSC	7 (25.9)	10 (32.5)	90 (39.6)	160 (18.8)
TNBC cases	9 (33.3)	11 (35.5)	96 (42.3)	300 (35.4)
Luminal type breast carcinoma cases	8 (29.6)	6 (19.3)	23 (10.1)	207 (24.4)
HER-2 enriched breast carcinoma	1 (3.7)	1 (3.2)	2 (0.1)	26 (3.1)
Cases with ovarian HGSC and TNBC	1 (3.7)	0	10 (4.4)	2 (0.02)
Cases with endometrioid ovarian carcinoma and TNBC	0	0	0	2 (0.02)
Cases with mixed ovarian carcinoma	0	0	2 (0.1)	0
Cases with ovarian low-grade serous carcinoma	0	0	0	2 (0.02)
Cases with ovarian endometrioid carcinoma	0	2 (6.4)	0	5 (0.6)
Prostate carcinoma cases	0	1 (3.2)	2 (0.1)	62 (7.3)
Pancreatic carcinoma cases	0	0	2 (0.1)	82 (9.7)
Cases with positive family history	11 (40.71)	4 (12.9)	54 (23.8)	43 (5.1)

Values are presented as number (%) unless otherwise indicated.

HGSC, high-grade serous carcinoma; TNBC, triple-negative breast carcinoma; HER-2, human epidermal growth factor receptor 2.

<sup>a</sup>p = .867; 95% confidence interval, -4.288 to 5.088 (Nepalese vs. non-Nepalese *BRCA* mutant population).

Such high occurrence of just two mutations opens up the possibility that, like in Ashkenazi Jews, the first tier of testing can be reduced to dual site-specific testing for *BRCA1* c.2214\_2215insT and *BRCA1* c.5068A>T for the Nepalese people. Failing to identify one of the two nonsense mutations shall prompt further testing by a more extensive NGS-based assay.

One of the index cases of ovarian carcinoma screened for somatic *BRCA* testing had c. 5068A>T mutation in *BRCA1* gene at a high VAF and confirmed as germline mutation on orthogonal testing by site-specific Sanger sequencing on peripheral blood. Further, even when detected in tumor tissue, these two mutations shall get full consideration of germline origin, and germline testing should follow.

A positive family history of breast/ovarian carcinoma was found in 40.71% of *BRCA* mutant cases in the Nepalese subcohort, clearly far more common than non-Nepalese subcohort (23.8%). The observation of a significantly high *BRCA* mutant population sans a family history of cancer predisposition thus mandates testing all patients for *BRCA* irrespective of the family history as emphasized by the NCCN guidelines [15].

c.2214\_2215insT is located at chr17:41245334; exon 10 of 24 results in a frameshift that generates a new stop codon in place of Lysine, either causing the formation of a truncated *BRCA1* protein or lack of it due to Nonsense-mediated mRNA decay [22,23]. Likewise, c.5068A>T is located at chr17:41219631; exon 17 of 23; position 57 of 78 (on assembly GRCh37) [22] also forms a premature stop codon in place of Lysine with similar ef-

fects as for the other founder mutation. These mutations were reported by the first author in the LOVD Database [24]. The variants above are not reported in the population databases (ExAC) [25]. The Clinvar has annotated these variants to be pathogenic [26].

The present hospital-based study on cancer patients acting as probands reveals founder mutations in the Nepalese people. A more extensive population-based study confirming their high-frequency and lack of other randomly distributed deleterious mutations in *BRCA* genes shall permit the development of a dual site-specific *BRCA1* testing as the first-line screen for probands and kindred. Such a strategy shall prevent the need for NGS, drastically reduce the cost of *BRCA* testing, allow deeper reach, especially in a resource-constrained setting. This strategy shall also provide a fillip to a preventive approach towards cancer.

*BRCA1* c.2214\_2215insT and *BRCA1* c. 5068A>T are the two germline alterations in the Nepalese population with founder effect and incidence of 85.2%. Further confirmation of our findings by a more extensive population-based study shall allow developing a limited dual site-specific germline test as a first-line screening tool, especially in a resource-restricted environment with limited accessibility to NGS. Additionally, Founder mutations can help conclude or rule out an anthropological hypothesis and has immense research potential in that field.

**Ethics Statement**

This study was approved by the institutional review board (Rajiv Gandhi

Cancer Institute and Research Center), vide the ethical approval letter number RGCIRC/IRB-BHR/41/2020. The study was conducted per the Declaration of Helsinki. Informed written consent was obtained for testing and using the information for research.

### Availability of Data and Material

The datasets generated or analyzed during the study are available from the corresponding author on reasonable request.

### Code Availability

Not applicable.

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### Author Contributions

Conceptualization: AM. Data curation: GG, HD, SA, SN. Formal analysis: AM, GG, HD, SN. Investigation: AM, HD, GG, SN, SA, SD. Methodology: AM. Project administration: AM. Resources: SD. Supervision: AM. Visualization: HD, SN. Writing—original draft: GG, HD. Writing—review & editing: AM.

### Conflicts of Interest

The authors declare that they have no potential conflicts of interest.

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