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# Spectrum and prevalence of *BRCA1/2* germline mutations in Pakistani breast cancer patients: results from a large comprehensive study

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## Abstract

**Background:** Pathogenic germline mutations in *BRCA1* and *BRCA2* (*BRCA1/2*) account for the majority of hereditary breast and/or ovarian cancers worldwide. To refine the spectrum of *BRCA1/2* mutations and to accurately estimate the prevalence of mutation in the Pakistani population, we studied 539 breast cancer patients selected for family history and age of diagnosis.

**Methods:** Comprehensive screening for *BRCA1/2* germline mutations was performed using state-of-the-art technologies.

**Results:** A total of 133 deleterious mutations were identified in 539 families (24.7%), comprising 110 in *BRCA1* and 23 in *BRCA2*. The prevalence of *BRCA1/2* small-range mutations and large genomic rearrangements was 55.4% (36/65) for families with breast and ovarian cancer, 27.4% (67/244) for families with two or more cases of breast cancer, 18.5% (5/27) for families with male breast cancer, and 12.3% (25/203) for families with a single case of early-onset breast cancer. Nine mutations were specific to the Pakistani population. Eighteen mutations in *BRCA1* and three in *BRCA2* were recurrent and accounted for 68.2% (75/110) and 34.8% (8/23) of all identified mutations in *BRCA1* and *BRCA2*, respectively. Most of these mutations were exclusive to a specific ethnic group and may result from founder effects.

**Conclusions:** Our findings show that *BRCA1/2* mutations account for one in four cases of hereditary breast/ovarian cancer, one in five cases of male breast cancer, and one in eight cases of early-onset breast cancer in Pakistan. Our study suggests genetic testing of an extended panel of 21 recurrent *BRCA1/2* mutations for appropriately selected patients and their families in Pakistan.

**Keywords:** *BRCA1/2*, germline mutations, breast cancer, Pakistan

## Background

Individuals harboring *BRCA1/2* germline mutations have high lifetime risks of breast and ovarian cancer. The identification of individuals harboring *BRCA1/2* mutations is crucial to assess their cancer risk, consider preventive measures and tailor cancer management strategies.

Several studies have investigated the prevalence of *BRCA1/2* small-range mutations and/or large genomic rearrangements (LGRs) with frequencies varying from 17.6% to 29.8% in white populations from Europe and Australia [1–5] and 9.4% to 21.7% in non-whites from Asia [6–8]. The prevalence and distribution of *BRCA1/2* mutations vary across populations, mainly due to population-specific recurrent or founder mutations. Accurate identification of the population-specific mutation spectrum is therefore the first step towards incorporating appropriate genetic *BRCA1/2* testing into clinical practice in a particular population. This information is not fully elucidated in Pakistan, a country with one of the highest rates of breast cancer in Asia.

To date, no large comprehensive studies evaluating the *BRCA1/2* mutations have been reported in the Pakistani

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population and mutations in males have not been identified so far. Small-range mutations were previously reported in 341 unselected breast and 120 ovarian cancer patients, in which the analysis was restricted to a few exons only [9]. We conducted two studies in early-onset and familial breast/ovarian cancer patients from Pakistan. In the initial study the complete coding regions and exon-intron boundaries of *BRCA1/2* were screened for small-range mutations in 176 patients [10]. In the other study 120 *BRCA1/2* small-range mutations negative patients were screened for LGRs [11]. Other Asian studies also had small sample sizes [12, 13], reported small-range mutations only [14, 15], and/or restricted LGR analyses to a small number of study participants [6, 16, 17].

Here, we refined the spectrum of *BRCA1/2* mutations and more precisely estimated the mutation frequencies including small-range mutations and LGRs in 539 early-onset and familial breast cancer patients from Pakistan.

## Methods

### Enrollment of families

Five hundred and ninety-three breast cancer only or breast and ovarian cancer families were enrolled through index breast and/or ovarian cancer patients who presented at the Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC) in Lahore, Pakistan, from September 2004 to August 2015. The recruited families were classified into five risk groups based on family history of breast/ovarian cancer or age at diagnosis (Table 1) as described previously [19]. After enrollment, 54 families were excluded (Fig. 1), leaving 539 families in the study.

Clinical and histopathological data and comprehensive information on personal and family history of cancer(s), and ethnicity were obtained from all study participants. The Institutional Review Board of the SKMCH&RC approved the study. All study participants signed an informed written consent before providing a blood sample.

### *BRCA1/2* mutation screening

Genomic DNA was extracted from 9 to 18 ml of whole blood samples, as described previously [20]. The entire coding regions of the *BRCA1* (Genbank accession number U14680) and *BRCA2* (Genbank accession number U43746) genes including exon-intron boundaries were screened in 139 patient DNA samples for small-range mutations using denaturing high-performance liquid chromatography (DHPLC) analysis as previously described [21, 22]. Each sample revealing variants was sequenced as described elsewhere [18]. Four-hundred female patients selected based on triple-negative vs. non triple-negative breast cancer phenotype had been screened for small-range *BRCA1/2* mutations and 33 mutations were described [18]. All small-range mutation-negative patients had been screened for LGRs using multiplex ligation-dependent probe amplification and three LGRs were described [11]. For the current study, families were selected on the basis of family history of breast/ovarian cancer, male breast cancer or age at diagnosis.

### Mutation classification

All *BRCA1/2* alterations identified in the current study were classified into pathogenic mutations, variants of unknown significance, or polymorphisms. Pathogenic mutations were defined as (i) small-range mutations which affect one or a few nucleotides including frameshift, nonsense, or splice-site mutations and generate a premature termination codon, except *BRCA2* exon 27 variants generating a premature termination codon after codon 3010 [23] and (ii) LGRs that span one or more exons. Mutations were designated using the Human

**Table 1** *BRCA1/2* mutation frequencies according to family structure

| Risk group | Phenotype of families | No. of families | No. of families with mutations (%) in | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | *BRCA1* | | | *BRCA2* | | | *BRCA1/2* |
| | | | Small-range | LGRs | All | Small-range | LGRs | All | |
| | All families | 539 | 101 (18.7) | 9 (1.7) | 110 (20.4) | 23 (4.3) | 0 (0) | 23 (4.3) | 133 (24.7)[a] |
| | Female breast cancer families | 447 | 67 (15.0) | 7 (1.6) | 74 (16.6) | 18 (4.0) | 0 (0) | 18 (4.0) | 92 (20.6) |
| A1 | 1 case ≤ 30 years | 203 | 20 (9.8) | 2 (1.0) | 22 (10.8) | 3 (1.5) | 0 (0) | 3 (1.5) | 25 (12.3) |
| A2 | 2 cases, ≥1 diagnosed ≤50 years | 131 | 20 (15.3) | 4 (3.0) | 24 (18.3) | 6 (4.6) | 0 (0) | 6 (4.6) | 30 (22.9) |
| A3 | ≥3 cases, ≥1 diagnosed ≤50 years | 113 | 27 (23.9) | 1 (0.9) | 28 (24.8) | 9 (8.0) | 0 (0) | 9 (8.0) | 37 (32.7) |
| A4 | Male breast cancer families | | | | | | | | |
| | ≥1 case of male breast cancer | 27 | 1 (3.7) | 0 (0) | 1 (3.7) | 4 (14.8) | 0 (0) | 4 (14.8) | 5 (18.5) |
| B | Breast-ovarian cancer families | | | | | | | | |
| | ≥1 breast cancer and ≥1 ovarian cancer | 65 | 33 (50.8) | 2 (3.0) | 35 (53.8) | 1 (1.5) | 0 (0) | 1 (1.5) | 36 (55.4) |

*LGRs* large genomic rearrangements
[a]Including 57 previously reported families [11, 18]

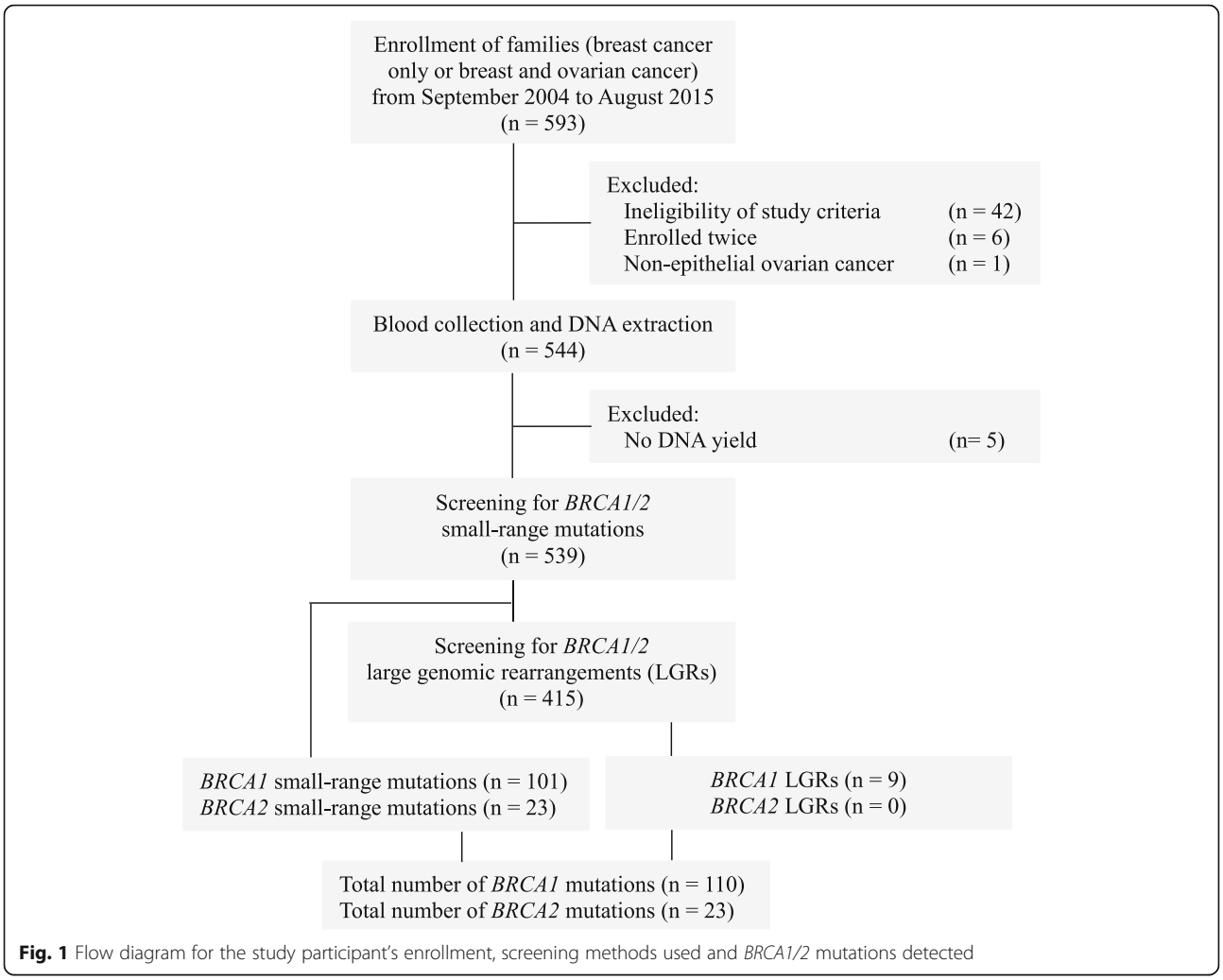


**Fig. 1** Flow diagram for the study participant's enrollment, screening methods used and *BRCA1/2* mutations detected

Genome Variation Society (HGVS) and the Breast Cancer Information Core (BIC) committee nomenclature.

All identified mutations were searched in various mutation databases including BIC (https://research.nhgri.nih.gov/bic/), ClinVar (http://www.ncbi.nim.nih.gov/clinvar/), LOVD (http://databases.lovd.nl/shared/genes/BRCA2), ARUP (http://arup.utah.edu/database/BRCA/), and BRCA Exchange (http://brcaexchange.org/). Mutations not reported in these databases were considered as novel and specific to Pakistani population.

### Statistical analyses

Distribution of clinical and histopathological characteristics between *BRCA1/2* carriers and non-carriers were estimated using Fisher's exact test for categorical variables and the Wilcoxon rank-sum test for quantitative variables. All statistical tests were two-sided. Results were considered significant at a $p$ value of $\leq 0.05$. All statistical analyses were done using StatXact 4 for Windows (Cytel, Cambridge, USA) and R, version 2.1.

## Results

A total of 539 index patients from unrelated families were enrolled and stratified into five risk groups (Table 1). The mean age of disease onset was 35.4 years (range 18-78) for female breast cancer (n=502), 45.4 years (range 23-66) for ovarian cancer (n=30) and 54.5 years (range 27-76) for male breast cancer (n=27) patients.

### Spectrum of *BRCA1/2* mutations

Evaluation of pooled data from 539 patients yielded 71 distinct pathogenic mutations in 133 families (24.7%) (Table 1). Fifty-three *BRCA1* mutations were detected in 110 families (20.4%) and 18 *BRCA2* mutations in 23 families (4.3%). Five mutations in *BRCA1* (9.4%) and four mutations in *BRCA2* (22.2%) were novel (Table 2). The phenotypes of all families carrying *BRCA1/2* mutations are presented in Table 3.

Twenty-one (21/71; 29.6%) mutations including 18 in *BRCA1* and three in *BRCA2* occurred more than once (Fig. 2a, b). These mutations were identified in 83

**Table 2** Deleterious *BRCA1/2* germline mutations in Pakistani breast/ovarian cancer families

| Family | Exon | BIC designation | | | HGVS designation | | Mutation type[b] | Reported in databases (No. of entries)[c] |
|---|---|---|---|---|---|---|---|---|
| | | Nucleotide | Codon | Designation | Nucleotide change[a] | Effect on protein | | |
| *BRCA1*-small-range mutations | | | | | | | | |
| 432 | 2 | 185 | 23 | 185insA | c.66dup | p.(Glu23Argfs*18) | FS | BIC (32)[d] |
| 723 | 2 | 185 | 23 | 185delAG | c.68_69del | p.(Glu23Valfs*17) | FS | BIC (2036)[d] |
| 372 | Intron 4 | IVS4-2 | - | IVS4-2A>G | c.135-2A>G | Splice site | SP | BIC (1) |
| 254[e] | 7 | 454 | 112 | 454delA | c.335del | p.(Asn112Ilefs*7) | FS | ClinVar (2) |
| 449 | 7 | 509 | 130 | Y130X | c.390C>G | p.(Tyr130*) | NS | LOVD (3) |
| 296, 317, 340[e], 511, 521, 626[e], 747 | 11 | 804 | 229 | 804delT | c.685del | p.(Ser229Leufs*5) | FS | BIC (2) |
| 470[e] | 11 | 903 | 262 | Q262X | c.784C>T | p.(Gln262*) | NS | ClinVar (3) |
| 711 | 11 | 1014 | 299 | 1014delGT | c.895_896del | p.(Val299Argfs*4) | FS | BIC (2) |
| 669[e] | 11 | 1127 | 336 | 1127delA | c.1008del | p.(Glu337Lysfs*4) | FS | No[d] |
| 748 | 11 | 1307 | 396 | 1307delT | c.1188del | p.(Asp396Glufs*14) | FS | LOVD (1) |
| 241[e] | 11 | 1309 | 397 | 1309delA | c.1190del | p.(Asp397Alafs*13) | FS | ClinVar (3) |
| 722 | 11 | 1518 | 485 | 1518_1572dup55 | c.1399_1453dup | p.(Ala485Glufs*13) | FS | No |
| 336[e] | 11 | 1590 | 491 | Q491X | c.1471C>T | p.(Gln491*) | NS | BIC (4)[d] |
| N12 | 11 | 1898 | 593 | 1898delTATGGAA | c.1779_1785del | p.(Met594Serfs*3) | FS | LOVD (2) |
| N28, 328[e], 557[e] | 11 | 1912 | 598 | L598X | c.1793T>G | p.(Leu598*) | NS | BIC (1)[d] |
| 574[e] | 11 | 2080 | 655 | 2080insA | c.1961dup | p.(Tyr655Valfs*18) | FS | BIC (13)[d] |
| 488[e] | 11 | 2268 | 717 | E717X | c.2149G>T | p.(Glu717*) | NS | ClinVar (2) |
| 236[e], 283[e], 489[e], 493[e], 593 | 11 | 2388 | 757 | 2388delG | c.2269del | p.(Val757Phefs*8) | FS | BIC (10)[d] |
| 363 | 11 | 2433 | 772 | 2433delGT | c.2314_2315del | p.(Val772Thrfs*4) | FS | LOVD (2) |
| 550[h] | 11 | 2457 | 780 | Q780X | c.2338C>T | p.(Gln780*) | NS | BIC (36) |
| 362, 469 | 11 | 2459 | 780 | 2459delGGAA | c.2340_2343del | p.(Glu781Valfs*10) | FS | LOVD (2) |
| 421[e], 442, 510[e], 542, 619[e] | 11 | 2524 | 802 | 2524delTG | c.2405_2406del | p.(Val802Glufs*7) | FS | BIC (5)[d] |
| N34 | 11 | 2657 | 846 | 2657delAAT-insG | c.2538_2540delinsG | p.(Met847Glyfs*4) | FS | LOVD (2) |
| 415[e], 660[e] | 11 | 2722 | 868 | S868X | c.2603C>G | p.(Ser868*) | NS | BIC (11)[d] |
| 411[e] | 11 | 3090 | 991 | K991X | c.2971A>T | p.(Lys991*) | NS | ClinVar (2) |
| 247[e] | 11 | 3100 | 994 | 3100delGT | c.2981_2982del | p.(Cys994*) | FS | ClinVar (5) |
| 299 | 11 | 3248 | 1043 | 3248delTATTAATGAA | c.3129_3138del | p.(Asn1043Lysfs*2) | FS | ClinVar (3) |
| N13[e], 399[e] | 11 | 3458 | 1113 | 3458delTGA | c.3339_3341del | p.(Tyr1113*) | FS | ClinVar (2) |
| 610 | 11 | 3531 | 1138 | G1138X | c.3412G>T | p.(Gly1138*) | NS | ClinVar (3) |
| N25 | 11 | 3596 | 1159 | 3596delAAAG | c.3477_3480del | p.(Ile1159Metfs*50) | FS | BIC (3) |
| 279[e], 445[e] | 11 | 3717 | 1200 | Q1200X | c.3598C>T | p.(Gln1200*) | NS | BIC (21)[d] |
| 382 | 11 | 3726 | 1203 | R1203X | c.3607C>T | p.(Arg1203*) | NS | BIC (36) |
| 646 | 11 | 3819 | 1234 | 3819delGTAAA | c.3700_3704del | p.(Val1234Glnfs*8) | FS | BIC (61) |
| 210[e], 211[e], 313[e], 316, 332[e], 463, 520, 624, 652[e], 653 | 11 | 3889 | 1257 | 3889delAG | c.3770_3771del | p.(Glu1257Glyfs*9) | FS | BIC (23)[d] |
| N4[e], 687, 724, 743 | 11 | 4184 | 1355 | 4184delTCAA | c.4065_4068del | p.(Asn1355Lysfs*10) | FS | BIC (144)[d] |
| 318[e] | 12 | 4302 | 1395 | Q1395X | c.4183C>T | p.(Gln1395*) | NS | BIC (28)[d] |
| 408[e] | 13 | 4446 | 1443 | R1443X | c.4327C>T | p.(Arg1443*) | NS | BIC (128) |

**Table 2** Deleterious *BRCA1/2* germline mutations in Pakistani breast/ovarian cancer families *(Continued)*

| Family | Exon | BIC designation | | | HGVS designation | | Mutation type[b] | Reported in databases (No. of entries)[c] |
|---|---|---|---|---|---|---|---|---|
| | | Nucleotide | Codon | Designation | Nucleotide change[a] | Effect on protein | | |
| 523[e], 555, N18, 598[e], 612, 621 | Intron 14 | IVS14-1 | - | IVS14-1G>A | c.4485-1G>A | Splice site | SP | BIC (2)[d] |
| 220[e], 275[e], 512[e] | 15 | 4627 | 1503 | S1503X | c.4508C>A | p.(Ser1503*) | NS | BIC (1)[d] |
| 609[e] | 15 | 4784 | 1558 | 4784delG | c.4665del | p.(Arg1555Serfs*4) | FS | No |
| 611[e] | 16 | 4981 | 1621 | 4981delA | c.4862del | p.(Asp1621Valfs*12) | FS | No |
| 249[e], 658 | 17 | 5154 | 1679 | 5154delC | c.5035del | p.(Leu1679*) | FS | BIC (2) |
| 276[e], 679 | Intron 17 | IVS17+1 | - | IVS17+1G>A | c.5074+1G>A | Splice site | SP | BIC (3) |
| 685 | 20 | 5358 | 1747 | 5358delC | c.5239del | p.(Gln1747Lysfs*18) | FS | LOVD (2) |
| 734 | 20 | 5385 | 1756 | 5385dupC | c.5266dup | p.(Gln1756Profs*74) | FS | LOVD (376) |
| 706 | Intron 20 | IVS20-1 | - | IVS20-1G>C | c.5278-1G>C | Splice site | SP | LOVD (5)[d] |
| 678 | 21 | 5429 | 1771 | 5429dupG | c.5310dup | p.(Pro1771Alafs*59) | FS | LOVD (1) |
| 278, 338[e] | 22 | 5480 | 1787 | 5480delTG | c.5361_5362del | p.(Cys1787Trpfs*42) | FS | ClinVar (3) |
| 682 | 22 | 5496 | 1793 | K1793X | c.5377A>T | p.(Lys1793*) | NS | ClinVar (1) |
| 248[e] | Intron 23 | IVS23-2 | - | IVS23-2A>T | c.5468-2A>T | Splice site | SP | ClinVar (1) |
| 260, 264, 329[e], 377[e], 389, 439, 481, 501, 522 | 24 | 5622 | 1835 | R1835X | c.5503C>T | p.(Arg1835*) | NS | BIC (74)[d] |
| *BRCA1*-large genomic rearrangements[e] | | | | | | | | |
| 229, 291, 314, 379, 406, 498, 549 | 1-2 | - | - | del exon 1-2 | g.41271967_41308900del[f] | | LGR | (42)[g] |
| 261, 719 | 21-24 | - | - | del exon 21-24 | g.41172653_41205744del[f] | | LGR | No |
| *BRCA2*-small-range mutations | | | | | | | | |
| 497, 700 | 3 | 320 | 31 | W31X | c.92G>A | p.(Trp31*) | NS | ClinVar (4) |
| N26 | Intron 4 | IVS4-2 | - | IVS4-2A>G | c.426-2A>G | Splice site | SP | ClinVar (4) |
| 545 | 9 | 993 | 255 | 993delCACAA | c.765_769del | p.(Asn255Lysfs*19) | FS | No |
| 330 | 10 | 1528 | 434 | 1528delAAAA | c.1300_1303del | p.(Lys434Glufs*25) | FS | ClinVar (2) |
| 602 | 11 | 3048 | 941 | 3048delA | c.2820del | p.(Val941Cysfs*19) | FS | No |
| 206 | 11 | 3063 | 945 | 3063delA | c.2835del | p.(Asp946Ilefs*14) | FS | ClinVar (2) |
| 505 | 11 | 4088 | 1287 | 4088delA | c.3860del | p.(Asn1287Ilefs*6) | FS | BIC (2) |
| 222, 407[h], 525, 540[h] | 11 | 5450 | 1741 | 5450delGTAA | c.5222_5225del | p.(Ser1741Thrfs*35) | FS | BIC (1) |
| 627, 684 | 11 | 5910 | 1894 | Y1894X | c.5682C>A | p.(Tyr1894*) | NS | BIC (3) |
| 295[e] | 11 | 5950 | 1908 | 5950delCT | c.5722_5723del | p.(Leu1908Argfs*2) | FS | BIC (43)[d] |
| 447 | 11 | 6696 | 2156 | 6696delTC | c.6468_6469del | p.(Gln2157Ilefs*18) | FS | BIC (24)[d] |
| 548[h] | 11 | 7044 | 2274 | 7044delAAGAG | c.6816_6820del | p.(Gly2274Alafs*17) | FS | ClinVar (6) |
| 579 | 15 | 7803 | 2526 | 7803delA | c.7575del | p.(Ala2526Glnfs*2) | FS | LOVD (2) |
| 492 | Intron 17 | IVS17+2 | - | IVS17+2C>A | c.7976+2C>A | Splice site | SP | ClinVar (1) |
| 713 | 20 | 8773 | 2849 | 8773delAA | c.8545_8546del | p.(Lys2849Glyfs*19) | FS | No |
| 702 | 20 | 8779 | 2860 | 8779_8798dup20 | c.8551_8570dup | p.(Lys2860Asnfs*10) | FS | No |
| 207[h] | 21 | 8897 | 2890 | 8897insT | c.8669dup | p.(Thr2891Asnfs*16) | FS | ClinVar (1) |

**Table 2** Deleterious *BRCA1/2* germline mutations in Pakistani breast/ovarian cancer families *(Continued)*

| Family | Exon | BIC designation | | | HGVS designation | | Mutation type[b] | Reported in databases (No. of entries)[c] |
|---|---|---|---|---|---|---|---|---|
| | | Nucleotide | Codon | Designation | Nucleotide change[a] | Effect on protein | | |
| 538 | Intron 21 | IVS21+4 | - | IVS21+4A>G | c.8754+4A>G | Splice site | SP | BIC (7) |

[a]Numbering starts at the first A of the first coding ATG (located in exon 2) of NCBI GenBank accession number U14680 (*BRCA1*) and U43746 (*BRCA2*)
[b]*FS* frameshift mutation, *LGR* large genomic rearrangement, *MS* missense mutation, *NS* nonsense mutation, *SP* splice-site mutation
[c]*BIC* Breast Cancer Information Core database (https://research.nhgri.nih.gov/projects/bic/), *LOVD* Leiden Open Variation Database (http://databases.lovd.nl/shared/genes/BRCA2); ClinVar (https://www.ncbi.nlm.nih.gov/clinvar/), date last accessed June 26, 2018
[d]Previously reported in Pakistani breast/ovarian cancer cases [9, 10]
[e]Families and mutation description have been previously reported [11, 18]
[f]Genomic locale for chromosome 17, from the UCSC genome browser, Feb 2009 assembly
[g]Not available in databases; reported in various studies [1, 11]
[h]Families with male breast cancer

unrelated families and accounted for 62.4% (83/133) of all families with mutations. The most common *BRCA1* mutation was c.3770_3771del (ten Punjabi families), followed by c.5503C>T (nine Punjabi families), exon 1-2 deletion (seven Punjabi families), c.685del (five Punjabi and two Balochi families), c.4485-1G>A (six Punjabi families), c.2269del (one Punjabi and four Pathan families), c.2405_2406del (five Punjabi families), c.4065_4068del (three Punjabi and one Pathan families), c.1793T>G and c.4508C>A (three Punjabi families each), exon 21-24 deletion, c.2603C>G, c.3339_3341del, c.3598C>T, c.5035del, c.5074+1G>A, and c.5361_5362del (two Punjabi families each) and c.2340_2343del (one Pathan and one Mohajir families). The most common *BRCA2* mutation was c.5222_5225del (one Punjabi and three Mohajir families), followed by c.92G>A (two Punjabi families) and c.5682C>A (two Pathan families).

In addition to the deleterious mutations, 153 (28.4%) distinct *BRCA1/2* sequence variants were detected: 79 missense variants, 48 non-coding variants, 24 synonymous variants, one in-frame deletion, and one polymorphic nonsense variant in exon 27 of *BRCA2* (data not shown).

### *BRCA1/2* mutation frequencies

The frequencies of *BRCA1/2* mutations by risk group are provided in Table 1. For *BRCA1,* the highest mutation frequency was noted in families with breast and ovarian cancer (53.8%), followed by families with at least three breast cancer cases (24.8%), families with two breast cancer cases (18.3%), or families with one early-onset breast cancer case (≤30 years) (10.8%). For *BRCA2,* the highest frequency was observed in families with male breast cancer (14.8%).

### Patient and tumors characteristics by *BRCA1/2* status

*BRCA1* carriers (n=110) were more often identified among female patients (99.1% *vs.* 94.6%, $p$=0.039) and belonged to the Punjabi ethnic group (81.8% *vs.* 68.7%, $p$=0.030) compared to non-carriers (n=406). In contrast, *BRCA2* carriers (n=23) were more common among male patients (17.4% *vs.* 5.4%, $p$=0.043) and more often belonged to Pathan ethnic group (34.8% *vs.* 15.5%, $p$=0.009).

Female breast cancer patients with mutations in *BRCA1* (n=106) or *BRCA2* (n=19) had a similar mean age of diagnosis (34.0 years (range 21–72) and 37.7 years (range 23-56), respectively, $p$=0.073, Wilcoxon rank-sum test), which did not differ to that of non-carriers (n=377) (35.7 years (range 18-78). In contrast, male breast cancer patients harboring *BRCA2* mutations (n=4) had an older mean age of diagnosis than non-carriers (n=22) (66.5 years (range 54-76) and 52.5 years (range 27-69) years, respectively, $p$=0.039, Wilcoxon rank-sum test).

*BRCA1*-associated breast tumors more often were invasive ductal carcinomas (99.0% *vs.* 91.4%, $p$=0.004), triple-negative (60.8% *vs.* 22.6%, $p$=<0.0001), and of higher tumor grade (grade 3: 94.9% *vs.* 63.2%, $p$=<0.0001) compared to tumors of non-carriers. *BRCA2*-associated breast tumors more often were PR positive compared to tumors of non-carriers (81.8% *vs.* 57.2%, $p$=0.025) (data not shown).

## Discussion

To our knowledge, this is the largest Pakistani study that comprehensively investigated the spectrum of *BRCA1/2* small-range mutations and LGRs and prevalence of mutations in 539 high-risk families. Mutations were identified in 24.7% (133/539) of families. Eighteen *BRCA1* and three *BRCA2* mutations were recurrent and accounted for 68.2% and 34.8% of all mutations in *BRCA1* and *BRCA2*, respectively. Nine mutations were specific to the Pakistani population, whereas other mutations had been reported elsewhere.

The most common type of identified mutations were frameshift mutations (60.6%) followed by nonsense mutations (25.4%). These data are consistent with our previous report [10] and a recent worldwide study [25]. In Pakistani patients, *BRCA1* mutations were about 5-

**Table 3** Characteristics of the 133 families with deleterious *BRCA1/2* mutations

| Family | No. of cancers | | Age at onset (years) | | Other cancer(s)[c] (age at onset in years) | Ethnicity |
|---|---|---|---|---|---|---|
| | Female BC (Bilateral) | OC (OC+BC) | BC | OC | | |
| Families carrying *BRCA1*-small-range mutations | | | | | | |
| 236[a] | 1 | - | 22[b] | - | - | Pathan |
| 316 | 1 | - | 25[b] | - | - | Punjabi |
| 264 | 1 | - | 26[b] | - | - | Punjabi |
| 706 | 1 | - | 26[b] | - | Uterus (67) | Punjabi |
| N12 | 1 | - | 26[b] | - | - | Punjabi |
| 624 | 1 | - | 27[b] | - | - | Punjabi |
| N25 | 1 | - | 28[b] | - | - | Punjabi |
| 276[a] | 1 | - | 28[b] | - | - | Punjabi |
| 610 | 1 | - | 28[b] | - | - | Punjabi |
| 678 | 1 | - | 28[b] | - | - | Punjabi |
| 411[a] | 1 | - | 29[b] | - | Stomach (70) | Punjabi |
| 724 | 1 | - | 29[b] | - | Renal (48), lung (65), throat (65), unknown | Punjabi |
| N28 | 1 | - | 30[b] | - | - | Punjabi |
| 279[a] | 1(1) | - | 27/36[b] | - | - | Punjabi |
| 278 | 2 | - | 25[b],32 | - | - | Kashmiri |
| 332[a] | 2 | - | 26[b],51 | - | Leukemia (45) | Punjabi |
| 682 | 2 | - | 28[b],40 | - | Uterus (<62,65), throat (<72) | Punjabi |
| N18 | 2 | - | 29[b],<50 | - | - | Punjabi |
| 421[a] | 2 | - | 30[b],33 | - | - | Punjabi |
| 432 | 2 | - | 30[b],53 | - | Skin (12), oral (54) | Punjabi |
| 520 | 2 | - | 30[b],47 | - | Uterus (32) | Punjabi |
| 449 | 2 | - | 32[b],55 | - | - | Punjabi |
| 557[a] | 2 | - | 32[b],45 | - | Unknown (<55), renal (70) | Punjabi |
| 747 | 2 | - | 33[b],38 | - | - | Unknown |
| 722 | 2 | - | 20,34[b] | - | Unknown (<18,<40) | Punjabi |
| 687 | 2 | - | 37[b],45 | - | - | Punjabi |
| 470[a] | 2 | - | 40[b],40 | - | Stomach (46), colon (59), lung | Punjabi |
| 510[a] | 2 | - | 40[b],55 | - | - | Punjabi |
| N13[a] | 2 | - | 40[b],>50 | - | - | Punjabi |
| 593 | 2 | - | 43,44[b] | - | Leukemia (22) | Pathan |
| 299 | 2(1) | - | 24/27[b],55 | - | - | Punjabi |
| 660[a] | 2(1) | - | 25/26[b],70 | - | Bladder | Punjabi |
| 260 | 2(1) | - | 25/26[b],28 | - | - | Punjabi |
| 511 | 2(1) | - | 30/33[b],<32 | - | Brain (75) | Punjabi |
| N34 | 3 | - | 24[b],<30,31 | - | - | Punjabi |
| 669[a] | 3 | - | 25[b],<40,<50 | - | Brain (<78), oral (<80) | Punjabi |
| 685 | 3 | - | 26[b],26,? | - | Blood (2x) | Mohajir |
| 723 | 3 | - | 28[b],40,? | - | - | Pathan |
| 612 | 3 | - | 29[b],<30,40 | - | Throat (45), uterus (48) | Punjabi |
| 313[a] | 3 | - | 30[b], 48,? | - | - | Punjabi |

**Table 3** Characteristics of the 133 families with deleterious *BRCA1/2* mutations *(Continued)*

| Family | No. of cancers | | Age at onset (years) | | Other cancer(s)[c] (age at onset in years) | Ethnicity |
|---|---|---|---|---|---|---|
| | Female BC (Bilateral) | OC (OC+BC) | BC | OC | | |
| 336[a] | 3 | - | 23,30[b],38 | - | Prostate (29) | Punjabi |
| 493[a] | 3 | - | 35[b],55,>55 | - | - | Pathan |
| 382 | 3 | - | 36[b],50,? | - | - | Punjabi |
| 489[a] | 3 | - | 25,42[b],45 | - | - | Punjabi |
| 743 | 3 | - | 40,44[b],62 | - | Bone (60), leukemia (60) | Pathan |
| 658 | 3 | - | 26,44[b],50 | - | - | Punjabi |
| 377[a] | 3 | - | 31,50[b],85 | - | Thyroid (59), intestine (70), bladder (75), liver | Punjabi |
| 550[d] | 3 | - | 50[b],55,>50 | - | Lung, unknown | Punjabi |
| 372 | 3(1) | - | 21/21[b],29,45 | - | Squamous cell carcinoma scalp (22)[b] | Pathan |
| 626[a] | 3(1) | - | 35[b],36/37, 42 | - | - | Balochi |
| 389 | 3(1) | - | 22/32,42,48[b] | - | Brain (36), uterus (70) | Punjabi |
| 247[a] | 4 | - | 27,28[b],40,42 | - | Uterus (31, 55) | Siriaki |
| 652[a] | 4 | - | 31[b],33,42,50 | - | - | Punjabi |
| 362 | 4 | - | 31,32,35[b],45 | - | Liver (>40), abdomen | Pathan |
| 399[a] | 4 | - | 43, 44[b],50,? | - | Abdomen (45), lung (45), prostate (53) | Punjabi |
| 338[a] | 4 | - | 30,40,44,48[b] | - | Stomach (73) | Punjabi |
| 408[a] | 4(1) | - | 24[b],31/31,33,50 | - | Abdomen (54), esophagus (74) | Punjabi |
| 521 | 4(1) | - | 25/38[b],27,33,70 | - | Stomach (60, 65), lung, unknown | Punjabi |
| 653 | 5 | - | 24,32,35[b],37,50 | - | Colon (42), throat (66) | Punjabi |
| 734 | 5 | - | 37,38[b],55,?,? | - | - | Punjabi |
| 296 | 7(1) | - | 21[b],<30,34,43,44/44,51,52 | - | - | Punjabi |
| 439 | 8 | - | 30,<35,40[b],40,40,40,>50,>65 | - | Uterus (40), prostate, unknown | Punjabi |
| 249[a] | 8(1) | - | 30/31[b],37,45,?,?,?,?,? | - | - | Punjabi |
| 619[a] | 1 | 1 | 30[b] | >50 | - | Punjabi |
| 646 | 1 | 1(1) | 34[b] | 36[b] | - | Punjabi |
| 748 | 1 | 1(1) | 47[b] | 52[b] | Skin (45), liver (50) | Punjabi |
| 542 | 1 | 2(1) | 40[b] | 46[b],70 | Leukemia (74) | Punjabi |
| 210[a] | 1 | 4 | 45[b] | ?,?,?,? | Brain (32), abdomen, lung, leukemia | Punjabi |
| 241[a] | 2 | 1 | 29[b],64 | 35 | Lymphoma | Punjabi |
| 598[a] | 2 | 1 | 30[b],56 | >50 | Stomach (>50, >50), tongue (>50) | Punjabi |
| 463 | 2 | 1 | 35[b],58 | 48 | - | Punjabi |
| 481 | 2 | 1 | <25,47[b] | 36 | Lung (55), uterus | Punjabi |
| 621 | 2 | 1 | 50,>60 | 50[b] | Prostate (65) | Punjabi |
| 211[a] | 2 | 1(1) | 26[b],50 | 50 | - | Punjabi |
| 415[a] | 2 | 1(1) | 34,35[b] | 35[b] | Leukemia (45), unknown | Punjabi |
| 679 | 2 | 1(1) | 28[b],<33 | 49[b] | - | Punjabi |
| N4[a] | 2 | 1 (1) | 41[b],45 | 45 | Uterus (38) | Punjabi |

**Table 3** Characteristics of the 133 families with deleterious *BRCA1/2* mutations *(Continued)*

| Family | No. of cancers | | Age at onset (years) | | Other cancer(s)[c] (age at onset in years) | Ethnicity |
|---|---|---|---|---|---|---|
| | Female BC (Bilateral) | OC (OC+BC) | BC | OC | | |
| 488[a] | 2 | 2(1) | 40[b],55 | 42[b],45 | - | Punjabi |
| 555 | 2 | 2(1) | 29[b],36 | 36[b],>50 | Leukemia (10), vocal cord (45) | Punjabi |
| 317 | 2 | 3(1) | 41,46 | 47[b],52,55 | Fallopian tube (47)[b] | Punjabi |
| 318[a] | 2 | 4(2) | 40,46[b] | 40,42[b],44,58 | Bladder (50, 50) | Pathan |
| 442 | 2(1) | 2 | 34,50/50[b] | 28,52 | Leukemia (15) | Punjabi |
| 283[a] | 2(1) | 3 | 34/38[b],56 | 54,55,65 | - | Pathan |
| 254[a] | 3 | 1 | 27,32[b],43 | 41 | Brain | Punjabi |
| 711 | 3 | 1 | 40[b],?,? | ? | Gall bladder | Sindhi |
| 445[a] | 3 | 1(1) | 44[b],>60,73 | 74 | Gall bladder | Punjabi |
| 363 | 3 | 2 | 32,35,70 | 47[b], ? | Lung (65), oral (70), liver | Kashmiri |
| 329[a] | 3 | 3 | 34[b],39,? | 39,<50,? | - | Punjabi |
| 609[a] | 4(1) | 1 | 29[b],31,48/55,65 | 30 | - | Mohajir |
| 328[a] | 4 | 1 | 29,30[b],31,39 | 55 | - | Punjabi |
| 501 | 4 | 1 | 34[b], 35, >50,? | ? | Brain (42) | Punjabi |
| 522 | 4 | 2(1) | 30[b],<40,45,45 | 35,60 | Uterus (41) | Punjabi |
| 611[a] | 4 | 2(1) | 31[b],36,37,42 | 50,55 | Blood (30) | Punjabi |
| 523[a] | 3(1) | 4 | 39/46,40,53[b] | 30,45,51,60 | - | Punjabi |
| 275[a] | 4(1) | 1(1) | 34/40[b],40,42,50 | 40 | - | Punjabi |
| 469 | 5 | 1(1) | 29[b],29,<35,>35,>55 | 32[b] | - | Mohajir |
| 340[a] | 6 | 1 | 34[b],42,<50,<50,<50,<50 | 54 | - | Balochi |
| 574[a] | 6 | 2 | 32,32,35,45[b],48,>50 | 48,55 | - | Mohajir |
| 512[a] | 6(1) | 1 | <25,<30,<40,46/55[b],<50,>50 | <50 | Uterus (<50) | Kashmiri |
| 248[a] | 7 | 2(2) | 23[b],<25,34,<40,<46,<60,? | 23[b],<60 | - | Punjabi |
| 220[a] | 8 | 1(1) | 25,27[b],<30,53,58,63,77 | <30 | - | Punjabi |
| Families carrying *BRCA1*-LGRs[a] | | | | | | |
| 229 | 1 | - | 28[b] | - | - | Punjabi |
| 379 | 2 | - | 29,31[b] | - | Liver (38) | Punjabi |
| 261 | 2 | - | 33,34[b] | - | - | Punjabi |
| 406 | 2 | - | 39[b],40 | - | Abdomen (65) | Punjabi |
| 498 | 2 | - | 40,41[b] | - | - | Siriaki |
| 549 | 2 | - | 38,72[b] | - | Unknown | Punjabi |
| 314 | 6 | - | 32[b],42,56,70,?,? | - | Uterus (54), pharynx (59), brain (63), abdomen | Punjabi |
| 291 | 3 | 1 | 39,42,48 | 48[b] | Stomach, brain | Punjabi |
| 719 | 3(1) | 1 | >40,42[b],? | ? | - | Punjabi |
| Families carrying *BRCA2*-small-range mutations | | | | | | |
| 330 | 1 | - | 29[b] | - | Lung (48, 58, 66), tongue (55) | Punjabi |
| 206 | 1 | - | 30[b] | - | - | Pathan |
| 540[d] | 1 | - | 67[b] | - | - | Mohajir |
| 207[d] | 1 | - | 76[b] | - | Intestine (60) | Punjabi |
| 295[a] | 1(1) | - | 23/23[b] | - | Leukemia (49), esophagus (50) | Punjabi |

**Table 3** Characteristics of the 133 families with deleterious *BRCA1/2* mutations *(Continued)*

| Family | No. of cancers | | Age at onset (years) | | Other cancer(s)[c] (age at onset in years) | Ethnicity |
|---|---|---|---|---|---|---|
| | Female BC (Bilateral) | OC (OC+BC) | BC | OC | | |
| N26 | 2 | - | 26[b],35 | - | - | Pathan |
| 602 | 2 | - | 31[b],43 | - | - | Punjabi |
| 492 | 2 | - | 38[b],39 | - | - | Mohajir |
| 505 | 2 | - | 43,46[b] | - | - | Pathan |
| 713 | 2 | - | 35,56[b] | - | - | Kashmiri |
| 700 | 2(1) | - | 35/43[b],46 | - | Throat (72) | Punjabi |
| 627 | 2(1) | - | 42[b],51/51 | - | - | Pathan |
| 545 | 3 | - | 35,36[b],47 | - | Brain (50), uterus (50), bone (54) | Punjabi |
| 497 | 3 | - | 51[b],55,50 | - | Brain | Siriaki |
| 548[d] | 3 | - | 45,50,69[b] | - | - | Pathan |
| 702 | 4 | - | 26,30[b],≤33,70 | - | Throat (72) | Punjabi |
| 579 | 4 | - | 35,49,<50,51[b] | - | Oral (35), gall bladder (42) | Kashmiri |
| 407[d] | 4 | - | 31,45(male),45,54[b] | - | Esophagus (39,59), leukemia (64) | Mohajir |
| 538 | 5 | - | 34[b],38,45,50,58 | - | Retinoblastoma (3), pancreas (75), liver (83) | Pathan |
| 684 | 5 | - | 40[b],45,48,50,<57 | - | Throat (<48, <82), stomach (53), intestine (60) | Pathan |
| 447 | 3(1) | 2 | 31[b],<50,55/65 | 50,>50 | Abdomen (>50), colon (62), brain (65) | Punjabi |
| 525 | 4(1) | 1 | 32,33/35[b],<50,60 | <45 | Bladder (>60) | Mohajir |
| 222 | 7 | 2(1) | 35,42,43,50,54[b],60,? | 47,53[b] | Lung (60), prostate (70), lung | Kashmiri |

*BC* breast cancer, *OC* ovarian cancer, *Unknown* cancer phenotype is not known
[a]Mutations previously described [11, 18]; [b]Proband; [c]Age at cancer diagnosis is mentioned along with cancer phenotype. For relatives with unknown age at cancer onset, only cancer phenotype is mentioned; [d]Families with male breast cancer

times more frequent than *BRCA2* mutations. A similar distribution was observed in two Asian studies from South India [26] and Saudi Arabia [27] and most studies among white populations [3–5, 28]. This is likely due to the predominance of recurrent *BRCA1* mutations in these populations. Contradictory results were reported in other Asian studies from China, Hong Kong, Korea, and Indonesia, where *BRCA2* mutations were observed at an equal or a higher frequency than *BRCA1* mutations [6, 12, 15–17].

Among the 133 mutations identified in our study, 18 *BRCA1* and three *BRCA2* mutations were recurrent, accounting for 68.2% and 34.8% of all mutations in *BRCA1* and *BRCA2*, respectively. The proportion of recurrent *BRCA1* mutations to the total number of identified *BRCA1* mutations is higher than our previous report [10], which is likely due to the larger size of the present study. Of the identified recurrent mutations, the majority was also reported as recurrent mutations in other populations [1, 4, 25], while few were exclusively identified in a specific ethnic group of Pakistan. Fourteen *BRCA1* mutations (c.3770_3771del, c.5503C>T, c.4485-1G>A, c.2405_2406del, c.1793T>G, c.4508C>A, c.2603C>G, c.3339_3341del, c.3598C>T, c.5035del, c.5074+1G>A, c.5361_5362del, exon 1-2 deletion, and exon 21-24 deletion) and one *BRCA2* mutation (c.92G>A) were identified only in the Punjabi ethnic group. One *BRCA2* mutation (c.5682C>A) was found only in the Pathan ethnic group. Five other recurrent mutations were identified in more than one ethnic group. Our findings imply that a panel of ethnic specific recurrent mutations may be useful for initial screening of high-risk patients from these ethnic groups. Founder effects were previously suggested for six of the 18 recurrent *BRCA1* mutations (c.3770_3771del, c.4065_4068del, c.4485-1G>A, c.4508C>A, c.5503C>T, exon 1-2 deletion) [9–11], while haplotype analyses of the remaining recurrent mutations have not been performed so far.

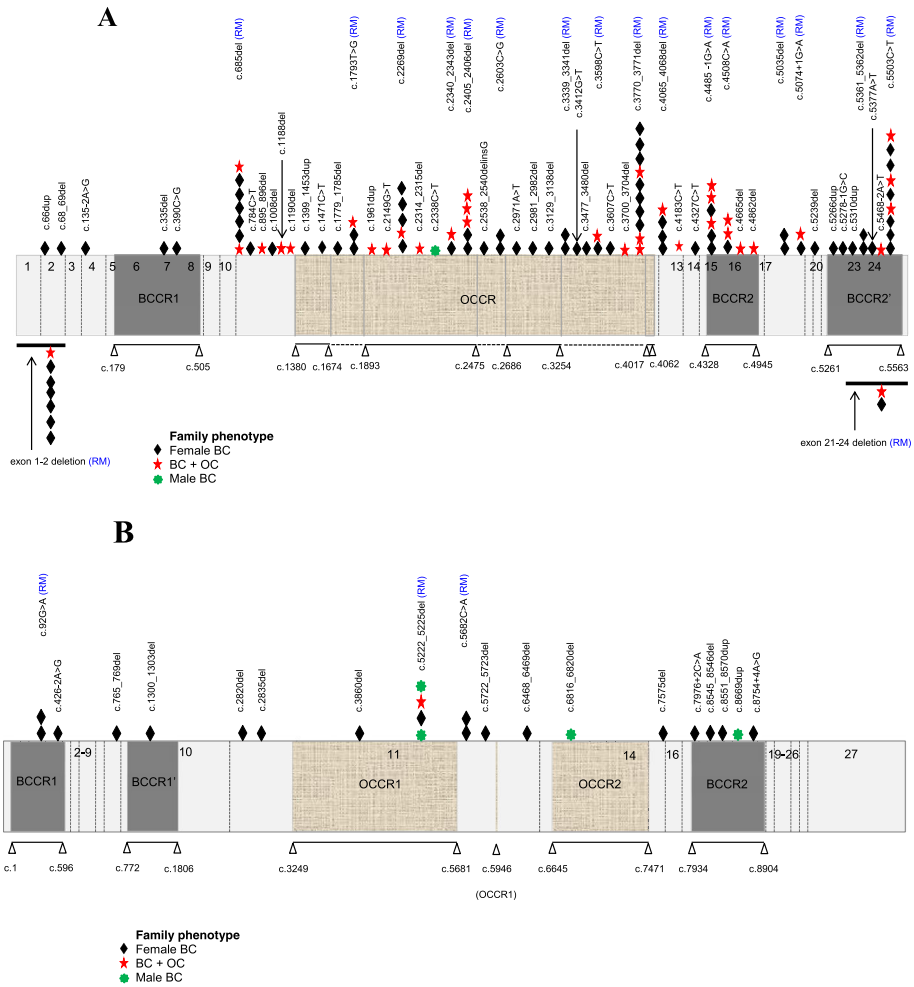


**Fig. 2** Distribution of deleterious germline mutations identified in Pakistani breast/ovarian cancer families across the *BRCA1* and *BRCA2* genes. Recurrent mutations are marked with (RM). The distribution of breast cancer (BC) and ovarian cancer (OC) in families according to the position of the mutations in *BRCA1* (**a**) and *BRCA2* (**b**) is shown. Regions inferred to be breast cancer cluster regions (BCCRs) and ovarian cancer cluster regions (OCCRs) according to Rebbeck and colleagues [24] are shown at the bottom

The high percentage of recurrent *BRCA1* mutations facilitates the development of a local, economical, and efficient ethnic-specific genetic testing strategy in Pakistan.

*BRCA1/2* mutations were identified in 24.7% of Pakistani breast cancer families. This frequency is higher than that from our initial report (17%) [10], probably due to the larger study size and comprehensive mutation analyses of both genes. This frequency is also higher than those from other Asian reports from Hong Kong, Malaysia, and Korea, ranging from 9.4% to 21.7% [6–8, 16, 17]. These findings further support the notion that the *BRCA1/2* mutation frequencies vary among different populations. Our data are similar to those reported in white populations, ranging from 17.6% to 29.8% [1, 2, 4]. We found the highest mutation frequency in breast and ovarian cancer families (55.4%), in agreement with previous studies from Pakistan [10], Korea [16], and studies in white populations [4, 28]. We observed a 2.52 fold (53.8% *vs.* 21.3%) increased occurrence of *BRCA1* mutations in breast and ovarian cancer families compared to breast cancer only families, in line with previous reports [1, 4, 6, 28]. Our findings support the notion that the presence of ovarian cancer in Pakistani breast cancer families increases the likelihood for the occurrence of *BRCA1* mutation.

In the present study on 27 families with male breast cancer, a *BRCA1/2* mutation frequency of approximately 19% was observed, with *BRCA2* mutations being about 4-times more common than *BRCA1* mutations. Our data are in line with previous studies [4, 14]. This observed frequency is higher than that reported in our initial much smaller study, in which no mutations were identified [10]. In agreement with the National Comprehensive Cancer Network (NCCN) guidelines, our data also

warrant *BRCA1/2* testing in families with male breast cancer (NCCN Guidelines Version 2.2019).

The main strength of this study is its large size of 539 high-risk families, the comprehensive screening of both genes for small-range mutations and LGRs using highly sensitive methods (allowing the identification of recurrent *BRCA1/2* mutations in the Pakistani population and the more accurate estimation of *BRCA1/2* mutation frequencies among high-risk families), and the confirmations of mutations in an independent patient's sample. However, our study also has some limitations. Participants were recruited at one tertiary care cancer center in Lahore, which may have introduced selection bias. Families belonging to Punjabi and Pathan ethnic groups are over-represented and, therefore, mutations in these groups may be over-represented. Nevertheless, Punjabi (44.7%) and Pathan (15.4%) are the most common ethnic groups reported in Pakistan (The World Factbook). Further, our data are based on self-reported ethnicity of study participants, which may lead to a misclassification of the ethnic origin of some of them.

## Conclusions

In summary, our study showed that *BRCA1/2* mutations account for 24.7% of high-risk breast cancer patients in Pakistan. Our results have important clinical implications, such as personalized treatment with platinum-based or PARP-inhibitor therapy for breast/ovarian cancer patients carrying a pathogenic *BRCA1/2* mutation and early detection, surgical prevention, and chemoprevention strategies for their unaffected *BRCA1/2* mutation positive relatives. Overall, *BRCA1/2* mutations account for one in four patients with a family history of breast cancer/breast and ovarian cancer, one in five patients with male breast cancer, and one in eight patients with early-onset breast cancer. Eighteen mutations in *BRCA1* and three in *BRCA2* were recurrent and accounted for 68.2% and 34.8% of all identified mutations in *BRCA1* and *BRCA2*, respectively. Our data suggest that *BRCA1* testing may be justified for families with multiple female breast cancers, breast and ovarian cancer or early-onset breast cancer and *BRCA2* testing for families with male breast cancer from Pakistan. Our findings will help in tailoring cost-effective genetic testing approach for the high-risk Pakistani population or for individuals of Pakistani origin residing in other countries.

### Abbreviations

BIC: Breast cancer information core; HGVS: Human genome variation society; LGRs: Large genomic rearrangements; LOVD: The Leiden open variation database; SKMCH&RC: Shaukat Khanum Memorial Cancer Hospital and Research Centre

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### Authors' contributions

MUR contributed to conception and design of the study, patient recruitment and data acquisition. In addition, he was involved in data analysis, interpretation and in drafting and revising the manuscript. NM performed the molecular analyses and contributed to data analysis and interpretation. He was also involved in writing the first draft of the manuscript. HN, FAK and SG performed the molecular analysis. MH and SF were involved in patient recruitment and clinical data acquisition. AA was involved in the recruitment of study subjects, clinical data collection and revising the manuscript. AL was involved in the pathological data acquisition and interpretation. UH contributed to conception and design of the study, data analysis and interpretation and led the writing of the manuscript. All authors read and approved the final manuscript.

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### Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

### Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines. It was approved by the ethics committee of Shaukat Khanum Memorial Cancer Hospital and Research Centre (SKMCH&RC), Lahore Pakistan. The ethics committee name is the "Institutional Review Board". The approval number is ONC-BRCA-002. Written informed consent was obtained from all study participants.

### Consent for publication

Not applicable

### Competing interests

The authors declare that they have no competing of interests.

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