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...
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 \( \text{BRCA1/2} \) were screened for small-range mutations in 176 patients [10]. In the other study 120 \( \text{BRCA1/2} \) 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 \( \text{BRCA1/2} \) 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.

#### \( \text{BRCA1/2} \) 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 \( \text{BRCA1} \) (Genbank accession number U14680) and \( \text{BRCA2} \) (Genbank accession number U43746) genes including exon-intron boundaries were screened for small-range \( \text{BRCA1/2} \) 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 \( \text{BRCA1/2} \) 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 \( \text{BRCA2} \) 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 \( \text{BRCA1/2} \) mutation frequencies according to family structure

| Risk group | Phenotype of families | No. of families | No. of families with mutations (%) in BRCA1 | No. of families with mutations (%) in BRCA2 |
|------------|----------------------|----------------|---------------------------------------------|---------------------------------------------|
|            |                      |                | 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)* |
| 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) |

*Including 57 previously reported families [11, 18]

LGRs: large genomic rearrangements

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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.nlm.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 <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 | Reported in databases (No. of entries) |
|--------|------|-----------------|------------------|--------------|----------------------------------------|
| BRCA1-small-range mutations |
| 432    | 2    | 185             | 185InsA          | c.66dup      | p.(Glu23Argfs*18) FS BIC (32) |
| 723    | 2    | 185             | 185delAG         | c.68_69del   | p.(Glu23Valfs*17) FS BIC (2036) |
| 372    | Intron 4 | IVS4-2 | -                | c.135-2A>G   | Splice site SP BIC (1) |
| 254a   | 7    | 454             | 454delA          | c.335del     | p.(Asn112Ifs*7) FS ClinVar (2) |
| 449    | 7    | 509             | Y130X            | c.390C>G     | p.(Tyr130*) NS LOVD (3) |
| 296, 317, 340a, 511, 521, 626a, 742 |
| 470a   | 11   | 903             | Q262X            | c.784C>T     | p.(Gln262*) NS ClinVar (3) |
| 711    | 11   | 1014            | 1014delGT        | c.895_896del | p.(Val299Argfs*4) FS BIC (2) |
| 669a   | 11   | 1127            | 1127delA         | c.1008del    | p.(Glu337Lysfs*4) FS No |
| 748    | 11   | 1307            | 1307delT         | c.1188del    | p.(Asp396Glufs*14) FS LOVD (1) |
| 241a   | 11   | 1309            | 1309delA         | c.1190del    | p.(Asp397Alafs*13) FS ClinVar (3) |
| 722    | 11   | 1518            | 1518_1572dupS5   | c.1399_1453dup | (Ava485Glufs*13) FS No |
| 336a   | 11   | 1590            | Q491X            | c.1471C>T    | p.(Gln491*) NS BIC (4) |
| N12    | 11   | 1898            | 1898delTATGGAA   | c.1779_1785del | p.(Met594Serfs*3) FS LOVD (2) |
| N28, 328a, 557a |
| 574a   | 11   | 2080            | 2080InsA         | c.1961dup    | p.(Tyr655Valfs*18) FS BIC (13) |
| 488a   | 11   | 2268            | E717X            | c.2149G>T    | p.(Glu717*) NS ClinVar (2) |
| 236a, 283a, 489a, 493a, 593 |
| 363    | 11   | 2388            | 2388delG         | c.2266del    | p.(Val575Phefs*8) FS BIC (10) |
| 362, 469 |
| 421a, 442, 510a, 542, 619a |
| N34    | 11   | 2657            | 2657delAAT-insG  | c.2538_2540delinsG | (Met847Glyfs*4) FS LOVD (2) |
| 415a, 660a |
| 411a   | 11   | 2722            | 2722             | c.2603C>G    | p.(Ser868*) NS BIC (11) |
| 318a   | 12   | 4302            | 4302             | c.4183C>T    | p.(Gln1395*) NS BIC (28) |
| 408a   | 13   | 4446            | 4446             | 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 | Reported in databases (No. of entries) |
|--------|------|-----------------|------------------|---------------|----------------------------------------|
| 523<sup>e</sup>, 555, N18, 598<sup>e</sup>, 612, 621 | Intron 14 | IVS14-1 | - | IVS14-1G>A | c.4485-1G>A | Splice site | SP | BIC (2)<sup>d</sup> |
| 220<sup>e</sup>, 275<sup>e</sup>, 512<sup>e</sup> | 15 | 4627 | 1503 | 51503X | c.4508C>A | p.(Ser1503*) | NS | BIC (1)<sup>d</sup> |
| 609<sup>e</sup> | 15 | 4784 | 1558 | 4784delG | c.4665del | p.(Arg1555Serfs*4) | FS | No |
| 611<sup>e</sup> | 16 | 4981 | 1621 | 4981delA | c.4862del | p.(Asp1621Valfs*12) | FS | No |
| 249<sup>e</sup>, 658 | 17 | 5154 | 1679 | 5154delC | c.5035del | p.(Leu1679*) | FS | BIC (2) |
| 276<sup>e</sup>, 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 | 5385delC | 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)<sup>d</sup> |
| 678 | 21 | 5429 | 1771 | 5429dupG | c.5310dup | p.(Pro1771Alafs*59) | FS | LOVD (1) |
| 278, 338<sup>e</sup> | 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<sup>e</sup> | Intron 23 | IVS23-2 | - | IVS23-2A>T | c.5468-2A>T | Splice site | SP | ClinVar (1) |
| 260, 264, 329<sup>e</sup>, 377<sup>e</sup>, 389, 439, 481, 501, 522 | 23 | 5622 | 1835 | R1835X | c.5503C>T | p.(Arg1835*) | NS | BIC (74)<sup>d</sup> |
| BRCA1-large genomic rearrangements<sup>j</sup> | 1-2 | - | - | del exon 1-2 | g.41271967_41308900del | LGR | (42)<sup>d</sup> |
| 229, 291, 314, 379, 406, 498, 549 | 24 | 5480 | 1787 | 5480delTG | c.5361_5362del | p.(Cys1787Trpfs*42) | FS | ClinVar (3) |
| 261, 719 | 21-24 | - | - | del exon 21-24 | g.41172653_41205744del | LGR | No |
| BRCA2-small-range mutations | | | | | | |
| 497, 700 | 3 | 320 | 31 | W61X | 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 | 993delCAACA | c.765_769del | p.(Asn255Lysfs*19) | FS | No |
| 330 | 10 | 1528 | 434 | 1528delAAA | c.1300_1303del | p.(Lys434Glufs*25) | FS | ClinVar (2) |
| 602 | 11 | 3048 | 941 | 3048delA | c.2820del | p.(Val941Cysfs*19) | 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<sup>e</sup>, 525, 540<sup>h</sup> | 11 | 5450 | 1741 | 5450delGTAA | c.5222_5225del | p.(Ser1741Thrfs*35) | FS | BIC (1) |
| 627, 684 | 11 | 5910 | 1894 | 1894X | c.5682C>A | p.(Tyr1894*) | NS | BIC (3) |
| 295<sup>e</sup> | 11 | 5950 | 1908 | 5950delCT | c.5722_5723del | p.(Leu1908Argfs*2) | FS | BIC (43)<sup>d</sup> |
| 447 | 11 | 6696 | 2156 | 6696delTC | c.6468_6469del | p.(Gln2157Ilefs*18) | BIC (24)<sup>d</sup> |
| 548<sup>h</sup> | 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.(Lys2849Glufs*19) | FS | No |
| 702 | 20 | 8779 | 2860 | 8779_8798dup20 | c.8551_8570dup | p.(Lys2860Asns*10) | FS | No |
| 207<sup>h</sup> | 21 | 8897 | 2890 | 8897insT | c.8660dup | p.(Thr2891Asns*16) | FS | ClinVar (1) |
unrelated families and accounted for 62.4% (83/133) of all families with mutations. The most common \( \text{BRCA1} \) 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 (three Punjabi families), c.4269del (one Punjabi and four Pathan families), c.2405_2406del (five Punjabi families), c.4065_4068del (three Punjabi and one Pathan families), c.1793T>G (two Pathan families), c.4508C>A (three Pathan families), c.4613_4614del (one Pathan family), c.4508C>A (two Pathan families), and c.5074+1G>A (two Punjabi families).

The most common \( \text{BRCA2} \) mutation was c.5682C>A (two Pathan families). In addition to the deleterious mutations, 153 (28.4%) 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 \( \text{BRCA2} \) (data not shown).

**BRCA1/2 mutation frequencies**

The frequencies of \( \text{BRCA1/2} \) mutations by risk group are provided in Table 1. For \( \text{BRCA1} \), 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 \( \text{BRCA2} \), the highest frequency was observed in families with male breast cancer (14.8%).

**Patient and tumors characteristics by \( \text{BRCA1/2} \) status**

\( \text{BRCA1} \) 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, \( \text{BRCA2} \) 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 \( \text{BRCA1} \) (n=106) or \( \text{BRCA2} \) (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 \( \text{BRCA2} \) 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).

\( \text{BRCA1} \)-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. \( \text{BRCA2} \)-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 \( \text{BRCA1/2} \) 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 \( \text{BRCA1} \) and three \( \text{BRCA2} \) mutations were recurrent and accounted for 68.2% and 34.8% of all mutations in \( \text{BRCA1} \) and \( \text{BRCA2} \), 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, \( \text{BRCA1} \) mutations were about 5-
| Family No. | No. of cancers | Age at onset (years) | Other cancer(s)\(^c\) (age at onset in years) | Ethnicity |
|-----------|---------------|----------------------|-----------------------------------------------|-----------|
| Females 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 |
| 482 | 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 |
| 669\(^a\) | 3 | 25\(^b\)/<40, <50 | Brain (<78), oral (<80) | Punjabi |
| 685 | 3 | 26,26\(^b\) | Blood (2x) | Mohajir |
| 723 | 3 | 28\(^b\)/40 | - | Pathan |
| 612 | 3 | 29, <30,40 | Throat (45), uterus (48) | Punjabi |
| 313\(^a\) | 3 | 30\(^b\)/48,7 | - | Punjabi |
Table 3 Characteristics of the 133 families with deleterious BRCA1/2 mutations (Continued)

| Family No. | No. of cancers | Age at onset (years) | Other cancer(s) (age at onset in years) | Ethnicity |
|------------|----------------|----------------------|----------------------------------------|-----------|
|            |                |                      | Prostate (29)                          | Punjabi   |
| 336‡       | 3              | -                    | -                                      | Pathan    |
| 493‡       | 3              | -                    | -                                      | Punjabi   |
| 382        | 3              | -                    | -                                      | Punjabi   |
| 489‡       | 3              | -                    | Bone (60), leukemia (60)               | Pathan    |
| 743        | 3              | -                    | -                                      | Punjabi   |
| 658        | 3              | -                    | -                                      | Punjabi   |
| 377‡       | 3              | -                    | Thyroid (59), intestine (70), bladder (75), liver | Punjabi   |
| 550‡       | 3              | -                    | Lung, unknown                          | Punjabi   |
| 372        | 3(1)           | -                    | Squamous cell carcinoma scalp (22)    | Pathan    |
| 626‡       | 3(1)           | -                    | -                                      | Balochi   |
| 389        | 3(1)           | -                    | Brain (36), uterus (70)               | Punjabi   |
| 247‡       | 4              | -                    | Uterus (31, 55)                       | Siriaki   |
| 652‡       | 4              | -                    | -                                      | Punjabi   |
| 362        | 4              | -                    | Liver (>40), abdomen                  | Punjabi   |
| 399‡       | 4              | -                    | Abdomen (45), lung (45), prostate (53) | Punjabi   |
| 338‡       | 4              | -                    | Stomach (73)                          | Punjabi   |
| 408‡       | 4(1)           | -                    | Abdomen (54), esophagus (74)          | Punjabi   |
| 521        | 4(1)           | -                    | Stomach (60, 65), lung, unknown       | Punjabi   |
| 653        | 5              | -                    | Colon (42), throat (66)               | Punjabi   |
| 734        | 5              | -                    | -                                      | Punjabi   |
| 296        | 7(1)           | -                    | -                                      | Punjabi   |
| 439        | 8              | -                    | Uterus (40), prostate, unknown        | Punjabi   |
| 249‡       | 8(1)           | -                    | -                                      | Punjabi   |
| 619‡       | 1              | 1                    | -                                      | Punjabi   |
| 646        | 1              | 1(1)                 | 36*                                    | Punjabi   |
| 748        | 1              | 1(1)                 | 52*                                    | Punjabi   |
| 542        | 1              | 2(1)                 | 46*                                    | Punjabi   |
| 210‡       | 1              | 4                    | 35                                      | Punjabi   |
| 241‡       | 2              | 1                    | 29*                                    | Punjabi   |
| 598‡       | 2              | 1                    | 30*                                    | Punjabi   |
| 463        | 2              | 1                    | 35*                                    | Punjabi   |
| 481        | 2              | 1                    | 25*                                    | Punjabi   |
| 621        | 2              | 1                    | 50*                                    | Punjabi   |
| 211‡       | 2              | 1(1)                 | 50*                                    | Punjabi   |
| 415‡       | 2              | 1(1)                 | 35*                                    | Punjabi   |
| 679        | 2              | 1(1)                 | 28*                                    | Punjabi   |
| N4‡        | 2              | 1 (1)                | 41*                                    | 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) | Ethnicity |
|--------|----------------|----------------------|-----------------|-----------|
|        | Female BC (Bilateral) | OC (OC+BC) | BC | OC | (age at onset in years) |                          |                       |
|        | 2 | 2(1) | 40b,55 | 42b,45 | - | Pavajhi |
| 317 | 2 | 3(1) | 41,46 | 47b,52,55 | Leukemia (10), vocal cord (45) | Pavajhi |
| 318 | 2 | 4(2) | 40,46b | 40,42b,44,58 | Bladder (50, 50) | Pavajhi |
| 442 | 2(1) | 2 | 34,50/50b | 28,52 | Leukemia (15) | Pavajhi |
| 283 | 2(1) | 3 | 34/38,56 | 54,55,65 | - | Pavajhi |
| 254 | 3 | 1 | 27,32b,43 | 41 | Brain | Pavajhi |
| 711 | 3 | 1 | 40b,77 | ? | Gall bladder | Sindhi |
| 445 | 3 | 1(1) | 44b,>60,73 | 74 | Gall bladder | Pavajhi |
| 363 | 3 | 2 | 32,35,70 | 47b,7 | Lung (65), oral (70), liver | Kashmari |
| 329 | 3 | 3 | 34b,39,7 | 39,50,7 | - | Pavajhi |
| 609 | 4(1) | 1 | 29b,31,48/55,56 | 30 | - | Mohajir |
| 328 | 4 | 1 | 29,30b,31,39 | 55 | - | Pavajhi |
| 501 | 4 | 1 | 34b,35,50,7 | ? | Brain (42) | Pavajhi |
| 522 | 4 | 2(1) | 30b,40,45,45 | 35,60 | Uterus (41) | Pavajhi |
| 611 | 4 | 2(1) | 31b,36,37,42 | 50,55 | Blood (30) | Pavajhi |
| 523 | 3(1) | 4 | 39,46,50,53b | 30,45,60 | - | Pavajhi |
| 275 | 4(1) | 1(1) | 34,40,42,50 | 40 | - | Pavajhi |
| 469 | 5 | 1(1) | 29b,30,35,55 | 32b | - | Mohajir |
| 340 | 6 | 1 | 34b,42,50,50,50 | 54 | - | Balochi |
| 574 | 6 | 2 | 32,32,35,45b,48,50 | 48,55 | - | Pavajhi |
| 512 | 6(1) | 1 | <25,30,40,46,55b,50,50 | <50,50 | Uterus (<50) | Kashmari |
| 248 | 7 | 2(2) | 23b,25,34,40,46,46,60 | 50,60 | - | Pavajhi |
| 220 | 8 | 1(1) | 25,27b,30,53,58,63,77 | <30 | - | Pavajhi |

Families carrying BRCA1-LGRsa

|        | No. of cancers | Age at onset (years) | Other cancer(s) | Ethnicity |
|--------|----------------|----------------------|-----------------|-----------|
|        | Female BC (Bilateral) | OC (OC+BC) | BC | OC | (age at onset in years) |                          |                       |
| 229 | 1 | - | 28b | - | - | Pavajhi |
| 379 | 2 | - | 29,31b | - | Liver (38) | Pavajhi |
| 261 | 2 | - | 33,34b | - | - | Pavajhi |
| 406 | 2 | - | 39b,40 | - | Abdomen (65) | Pavajhi |
| 498 | 2 | - | 40,41b | - | - | Siriaki |
| 549 | 2 | - | 38,72b | - | Unknown | Pavajhi |
| 314 | 6 | - | 32b,42,56,70,7 | - | Uterus (54), pharynx (59), brain (63), abdomen | Pavajhi |
| 291 | 3 | 1 | 39,42,48 | 48b | Stomach, brain | Pavajhi |
| 719 | 3(1) | 1 | >40,42b | ? | - | Pavajhi |

Families carrying BRCA2-small-range mutations

|        | No. of cancers | Age at onset (years) | Other cancer(s) | Ethnicity |
|--------|----------------|----------------------|-----------------|-----------|
|        | Female BC (Bilateral) | OC (OC+BC) | BC | OC | (age at onset in years) |                          |                       |
| 330 | 1 | - | 29b | - | Lung (48, 58, 66), tongue (55) | Pavajhi |
| 206 | 1 | - | 30b | - | - | Pathan |
| 540d | 1 | - | 67b | - | - | Mohajir |
| 207d | 1 | - | 76b | - | Intestine (60) | Pavajhi |
| 295 | 1(1) | - | 23/23b | - | Leukemia (49), esophagus (50) | Pavajhi |
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.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. Fourteen 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.

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

| Family No. | Female BC (Bilateral) | OC (OC+BC) | Age at onset (years) | Other cancer(s) (age at onset in years) | Ethnicity |
|------------|-----------------------|------------|----------------------|-----------------------------------------|-----------|
|            |                       |            |                      |                                      |           |
| N26        | 2                     | -          | 26;35                | -                                      | Pathan    |
| 602        | 2                     | -          | 31;43                | -                                      | Punjabi   |
| 492        | 2                     | -          | 38;39                | -                                      | Mohajir   |
| 505        | 2                     | -          | 43;46                | -                                      | Pathan    |
| 713        | 2                     | -          | 35;56                | -                                      | Kashmiri  |
| 700        | 2(1)                  | -          | 35/43;46             | Throat (72)                            | Punjabi   |
| 627        | 2(1)                  | -          | 42;51/51             | -                                      | Pathan    |
| 545        | 3                     | -          | 35;36;47             | Brain (50), uterus (50), bone (54)     | Punjabi   |
| 497        | 3                     | -          | 51;55;50             | Brain                                  | Sriuki    |
| 548d       | 3                     | -          | 45;50,69             | -                                      | Pathan    |
| 702        | 4                     | -          | 26,30;33,70          | -                                      | Punjabi   |
| 579        | 4                     | -          | 35,49;50,51          | Oral (35), gall bladder (42)           | Kashmiri  |
| 407d       | 4                     | -          | 31,45(male),45,54     | Esophagus (39,59), leukemia (64)       | Mohajir   |
| 538        | 5                     | -          | 34;38,45,50,58       | Retinoblastoma (3), pancreas (73, liver (83) | Pathan |
| 684        | 5                     | -          | 40;45,48,50,57       | Throat (<48, <82), stomach (53), intestine (60) | Pathan |
| 447        | 3(1)                  | 2          | 31;50,55/65,50>50    | Abdomen (>50), colon (62), brain (65) | Punjabi   |
| 525        | 4(1)                  | 1          | 32,33/35;50,60       | <45                                    | Mohajir   |
| 222        | 7                     | 2(1)       | 35,42;43;50,54;60,7   | 47;53b                                | Kashmiri  |

BC breast cancer, OC ovarian cancer, Unknown cancer phenotype is not known

*Mutations previously described [11, 18]; *Proband; *Age at cancer diagnosis is mentioned along with cancer phenotype. For relatives with unknown age at cancer onset, only cancer phenotype is mentioned; *Families with male breast cancer
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 [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 cancer, 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

Acknowledgements
We are grateful to all study subjects for their participation in this study. We thank the clinicians (Neelam Siddiqui, Mazhar Ali Shah, Narjis Muzaffar, Usman Ahmad, Umm e Kaltoom, Amir Ali Syed, Huma Majeed, Zulqarnain Chaudhry, Muhammad Asad Parvaiz, and Amina Khan) for their help in recruitment of study participants. We thank Jörg Hoheisel for critical reading of the manuscript.

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.

Funding
The study was supported by the Shaukat Khanum Memorial Cancer Hospital and Research Centre (grant number ONC-BRCA-002) and the German Cancer Research Center.

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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Received: 23 May 2019 Accepted: 2 September 2019
Published online: 11 September 2019

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