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Status of *BRCA1/2* in Breast Cancer Patients; where Pakistan stands?

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Abstract
Pakistan has diverse genealogical foundation with rushes of movement from neighboringareas.TherisingtrendofbreastcancerisonsuchafastpaceinPakistan, that it is now important to check the contributing causes. Investigation about *BRCA1/2* is necessary to augment the cancer genetics. Especially the national level statistical data about hereditary mutations in different ethnicities in Pakistan is the need of an hour. Knowledge about the information regarding prevalence, underscores the formulation of a hereditary and non-hereditary *BRCA1/2* mutational database in Pakistan. This review article is delved to summarize the past studies and investigations about *BRCA1/2* in the country. Function, structure, different variants in ethnic groups and involvement of the *BRCA1/2* genes is assessed. Initiatives taken by the government of Pakistan for the surveillance of breast cancer in Pakistan are debated. Questions like what are the most common *BRCA1/2* mutation in Pakistani population is also answered by summing up the available data and studies.
Keywords: BRCA1/2, Breast cancer, Pakistan, genealogical, hereditary

Introduction

With 2.4 million new cases each year, breast cancer is the commonest and one of theoldestmalignanciesaroundtheglobe.Every1in4womenaccountedforbreastcancer in 2018 (Bray *et al*., 2018). Whereas, developing Asiatic countries like Pakistan is bearing twofold weight of the disease as half of the Pakistan's population is based upon women (Menhas & Umer, 2015).Karachi,Pakistanalonerepresents69.1per1millionproportionofbreastcancerin 2006 out of which the greater part of the cases introduced were in stages of III and IV (Arshad *et al.*,2019).The reasons for breast cancer can't be clarified by an only etiologic agent. Because of its dubious etiology, breast diseases have caught the attention of many physicians throughout all ages. However, with scientific and logical inquiries researchers aretryingtodrillthetruth. Butinspiteofhundredsofyears,breast cancerstaysoneofthe most feared human diseases. The breast cancer genesis could be because of damage cells over many years. Induction of proliferation of cells and mutations in DNA are the two broad terms for moleculartraumawhichcausescancer.IfItalkaboutmutations inDNAwithinthecritical genes, functions like cell growth regulation, differentiation, death and chromosomal replications are affected. The second broad term which causes breast cancer, promotes or induces the cell proliferation by two mechanisms. One is the induction of proliferation directly through mitogenic agents and the other is the proliferation in the surviving cells in the damage tissue. Whereas the latter is due to toxic agents. However, the induction of proliferation is notjustbecauseofmutationincellgrowthgenes.Buttheenvironment(internalorexternal factors)isalsoresponsibleto providetheplatformtomutatedcells.Inthiswaythemutated cells growth exceeds their normal neighbors and may acquire additional mutations (Parsons,2007).

1.1.Breast Cancer Genetics

Cancer is a complex disease with number of classified mutations in tumor suppressor and oncogenes which are gatekeeper, caretaker or landscaper, oncogenes which are drivers (necessary for tumor formation and survival) other mutations that are partial or complete loss of gene function, gain of any novel function, gain of opposite of normal function and lossofheterozygosityetc.Onefeaturethattumorsuppressorsandoncogeneshavecommon in both are key components in controlling the signal transduction pathways and hence regulate cell cycle by controlling the entry into S phase. Furthermore, besides mutations, alteration of exact copy number and loss of telomeric DNA can be the two

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other causes of cancer (Bland *et al*.,2017). Most of the breast cancers are sporadic and studies suggest that 12-13% of breast cancer cases have heritable genetic element (Cohen & Ellwein, 1990; Loeb, 1991) and out of which only 5-10% are autosomal dominant predisposition genes which means that if someone has just one copy of mutated gene then its risk of developing breast cancer is sufficiently high. The pattern of inheritance depends upon the genes which are involved. In case of *BRCA1* and *BRCA2* the pattern of inheritance is autosomal dominant (Parsons, 2007). Geneswithhighpenetrancelike*BRCA1/2,TP53,PTEN,STK11,*and*CDH1*etcarelinked with a relative risk higher than 5, instead of low-penetrant genes that have a relative risk of about 1.5 (Economopoulou*et al.,*2015).

1.1.2. Genome Godfather *BRCA1*

The location of *BRCA1* on chromosome is 17 q-21 with the gene ID 672 and ENSG00000012048 in Ensemble. The gene is also known as PSCP, BRCC1, RNF53, and IRIS. The presence of *BRCA1* is on the negative strand. Hall *et al*., in 1990 was first one to map the gene and found the location of gene on chromosome number 17 by Linkage method. Later on in 1994, Miki et al., revealed the structure further by using cloning method.

Therevelationof*BRCA1*hasincrediblyrevolutionizedtheunderstandingofbreastcancer. There are 24 exons in *BRCA1* and 1863 amino acid protein (Miki *et al*., 1994) Alu repeats make up 40% of the gene while there are some other repeats in low frequency. (Zhang *et al.,* 2010). The size of *BRCA1* is 117kbp with the biggest human exon (11th exon) covering alone 3426 base pair (Raponi *et al*., 2014). Through alternative splicing the gene has 3 isoforms; 1) An isoform having all exons called as full isoform, 2) Another including all exons except exon 11 known as r11, and 3) the isoform incorporating just 117 bases from 11th exon along with the rest of exons, this 1399 amino acid isoformiscalledIRIS(In-frameof*BRCA1*intron11splice)orr11q.(Xu*et al.,*1999). The full isoform comprises of preserved functional domains like N-terminus RING domain,2nuclearlocalizationregionsandBRCTdomainontheCterminus (Bollati*et al.,* 2017) Breast cancer susceptibility gene *BRCA1* is among the most important tumor suppressor genes which is classified as the "genome guardians", hence the proteins encoded by *BRCA1* have many significant binding areas on DNA and involves regulating and guiding for DNA repairing pathways and apoptosis. *BRCA1* also harbors about 15 othergenes,like*BRCA2,CtIP,ATM*and*P53*whichareheldby*BRCA1*forsuchguidelines, and in this manner, it is supposed to be the key controller for the upkeep of genomic stability and integrity (Cable *et al.,*2003). Thenuclearphosphoproteinencodeby*BRCA1*combineswithotherproteinsandmaintains the genome stability (Claus *et al*., 1991). It forms the multiunit complex called *BRCA1*-associated genome surveillance complex with other tumor suppressors, signal transducers, and DNA damagesensors. *BRCA1* is associated with HBOC and can increase a lifetime chance of developing breast cancer to 80% and that of ovarian cancer to 40%. The multidomain protein mostly have mutations in N-terminal RING domain, exons 11-13, and BRCT domain. Other domain is Serine cluster domain (SCD) which is located in exon 11-13 (Hollis *et al.,* 2017).

'RING'isstandforReallyInterestingNewGeneisresponsiblefortheinteractionsbetween the*BRCA1*andotherproteinsandisinvolvedintheE3ubiquitinligaseactivity.TheBRCT domainof*BRCA1*bindstoparticularphosphoproteinsequenceswhichareidentifybyboth *BRCA1* and ATR/ATM kinases. 66% of the *BRCA1* sequence is covered in between exon 11-13but despitethisverylittleisknownaboutitsatomiclevelstructureswhencompared to RING and BRCT domains. It encode two NLS (Nuclear localization sequence) and binding sites for many proteins including RB (Retinoblastoma protein) which progresses cell cycle, Rad50, Rad51, and transcription factor cMyc which repairs DNA. *BRCA1* encodes for such amino acids whose sequences interacts with *PALB2*. Many mutations in exon 11-13 region gives evident that this region is significant for thetumor suppressor job of*BRCA1*.

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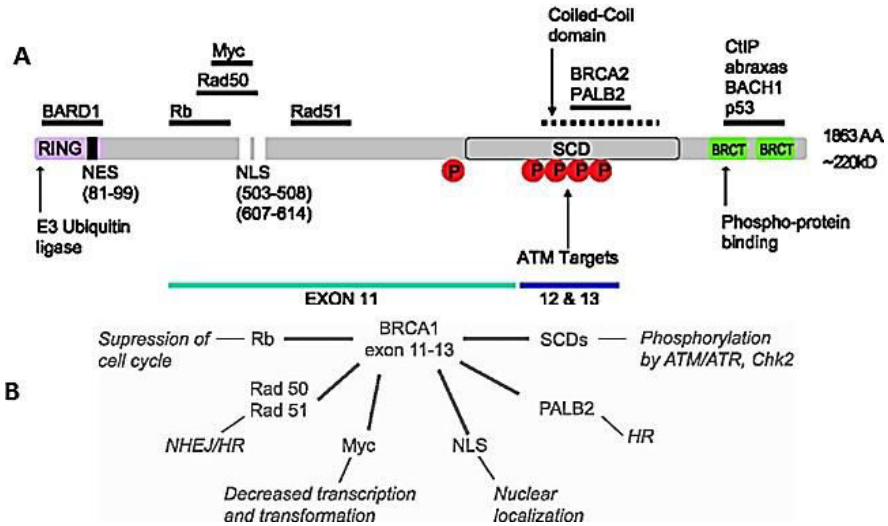

Figure 2: A)RING,BRCT andSCDdomainsof*BRCA1*areshown. NLSandNESaredepicted.Blacklines are indicating the domains where protein binds. Sites where phosphorylation occurs are observed as red circles B) Exon 11 which is the largest human exon has many binding sites for proteins such as Retinoblastoma and RAD50 and 51 protein, PALB2, and c-Myc. SCD and NLS can be seen. Adapted from: (Clark *et al.,* 2012) and (Hollis *et al*.,2017).

1.1.3. BRCA2

Like*BRCA1,BRCA2*(13q12-13)genestablesthegenomeand hasanimportantroleinthe Homologous DNA repairing. In 1995, *BRCA2* was identified by Wooster (Wooster *et al.,*1995). *BRCA2* is also a tumor suppressor gene on positive strand and have 27 exons and many isoforms which codes for 3412 amino acids (Collins *et al*.,1995). *BRCA2* is important as it is involved in the stability of chromosomes, RAD51 controlling during the DNA repairing process, cytokinesis, centromere duplication, telomeres replication and regulation of products of transcription. Mutations in *BRCA2* also stalled and collapsed the replication fork (Rosenthal, 2012). Male carriers of *BRCA2* have lifetime risk of Prostrate (20%), Breast (6%) and pancreatic cancer (3% risk). While females carriers have about 26%-84% chances of developing breast cancer and 20% risk of ovarian cancer (Thorlacius *et al*., 1998). *BRCA2* contains 27 exons and mutations can happen all through the large gene. Most of these mutations are frameshifts, yet there are also various missense mutations. *BRCA2*- related breast cancers show estrogen and progesterone receptors and have features similar to that of sporadic cancers, unlike *BRCA1*.

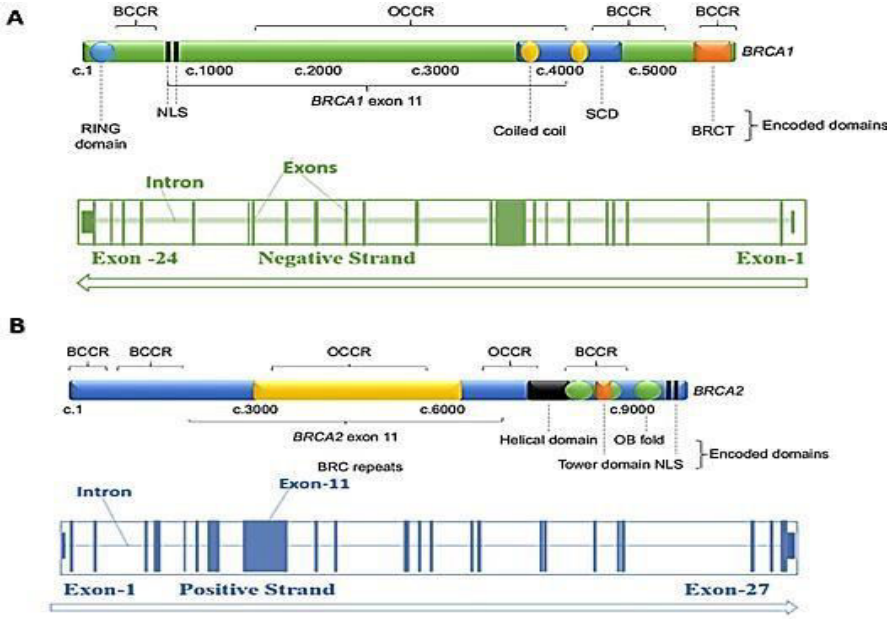

Figure3: A)*BRCA1*isgraphicallyrepresentedandexonregionsareshownasbars.Thenegativestrandcan be seen in figure 1A. B) *BRCA2* is shown with the positive strand. Adopted from Atlas of Genetics and Cytogenetics in Oncology and Hematology. Source of *BRCA1/2* map is (Hollis *et al.,*2017).

Pakistan ranks 6th according to its population on globe. Its location on the map is very pivotal. As more than half of

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the Pakistan's population is based upon women, the country bears twofold weight of the disease. Moreover, it's a multicultural society with many migrations in the country's past. Consanguineous marriages are also quite common which increases the risk of heritable cancers. A study in 2019 revealed that the number of breast cancer cases in the last 5 years were 119,710. (Majeed *et al*., 2020). While the ratio between female to male cases were 100:2.

In recent years, several studies have identified many factors which can cause high risk of breast cancer. Among them high penetrance of inheritable genetic mutations were also a contributing factor(Pasche, 2008) A person with *BRCA1* mutations has 80% lifetimerisk of breast cancer (Moatter *et al*., 2011). Even though 500 alterationsinthegenehasbeenidentifiedsofar,stillthemutationalspectrumisnotentirely characterized (Majeed *et al*., 2020). The reason behind this is that mutations are spread in many ethnicities in a diverse geographical range (Johannesdottir *et al*., 1996; Szabo & King, 1997). As we said that Pakistan also has a dynamic geography and multicultural population molecular analysis shows a diverse range in mutational statistics and factors responsible for breastcancer. If we compare the pathological features of Breast cancer patients having *BRCA1* mutations withthepatientswhodon't havethemutationsthanonecanseethat former breast cancercaseshave high tumor grade with more nodal involvement and less expression of estrogen and its partner in crime Progesterone. Moreover, breast cancer will be diagnosed in early age (36 years)withbilateralaffectedbreast tissuesascomparedto non*BRCA1*cancers (Majeed*et al.,*2020).Theserevelationshaveconfirmedthepreviousreporting'sfromPakistanandthe world. Another study confirmed that Pakistani women diagnosed with breast cancer in their 40s usually showed poor involvement of lymph node and high tumor grade (Muhammad Usman Rashid *et al*., 2016). Triple negative breast cancer with negative estrogen, progesterone and HER2/neu receptor status also gives the clue about the association of Breast cancer with mutations in the genes (Zhou *et al*., 2009). If a patient founds negative for *BRCA1* mutations then there is a possibility of variants in*BRCA2*.

In Pakistan, many breast cancer cases are presented in later stages. Women's late consultation is the major reason behind many deaths in the country. It can be due to lack of education, fewer screening programs, fear, financial, and culture obstacles (Mamoon *et al.,* 2009). This alarming situation needs screening and awarenessprogramsacrossthecountry,andcheapandeasyaccesstothemedicalfacilities is a need of thehour. Among many subtypes of breast cancer, the most common type is Infiltrating Ductal Carcinoma (IDC). Authors find that the incidence of IDC in population is 81% with type II grade tumor (Basra *et al.,* 2016). Following table shows different percentages of subtypes the country are listed in Table below.

Table 1: Common subtypes of breast cancer in Pakistan across the country with median age ranges and percentage.

Subtypes of Breast cancer	Area	Age Range	Age%
Infiltrating ductal carcinoma	Hyderabad, Jamshoro, Sindh	33-45	90%
	Karachi	48	81%
	Peshawar	40-59	82.60%
	Karachi	15-80	78%
	Rawalpindi, NWFP, upper Punjab	36-60	81%
	Karachi	30-66	91%
	Karachi	40-49	37%
Invasive intraductal carcinoma	Karachi	31-53	94%
Ductal carcinoma in situ	Karachi	48	16.25%
	Karachi	15-80	2.40%
	Karachi	48-95	1%
	Hyderabad, Jamshoro, Sindh	33-45	90%
Mucinous carcinoma	Peshawar	40-59	2.17%
	Karachi	15-18	12%
	Karachi	48	0.52%

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Infiltrating lobular carcinoma	Peshawar	40-59	6.50%
	Karachi	48	0.34%
	Karachi	15-80	1.20%
Papillary carcinoma	Peshawar	40-59	4.35
	Karachi	48	0.17
Invasive lobular carcinoma	Peshawar	40-59	6.50%
Medullary carcinoma	Peshawar	40-59	0.34%
	Karachi	15-80	6%
Benign lumps	**Karachi**	15-80	39.70%
	Rawalpindi, Islamabad, NWFP, upper Punjab	36-60	30.91%
	Karachi	48-95	92.10%
Total Breast cancer	**Baluchistan**	31-50	19%
	Lahore	>18	45.41%

Source: (Basra *et al.,* 2016)

1.3. Most Dominant *BRCA1/2* mutations in Pakistan

1.3.1. *BRCA1* mutations reported from Federal Capital

Previously, three genetic studies had been reported showing alterations in the *BRCA1* (Majeed*et al*.,2020).OnestudywhichwasconductedinFederalCapitalTerritoryinvolved 5000 women. 348 women were diagnosed with breast cancer and reported 14 types of mutations. 2 were novel frameshift mutations, 6 missense mutations out of which 3 were recurrentandtheother3werenovel,4synonymousmutationsand2mutationsintheintron variants (Table 2). Probably the introns nucleotide are involve in the regulation of the expression of exonicregions.

Type of mutation	Alteration in Nucleotide	Protein	Recurrent/Novel
Frameshift	c.932_933insC	Gly312Trpfs*8	Novel
Frameshift	c.964_965insG	Ala322Glyfs*4	Novel
Missense	c.1084G>A	Glu362Lys	Novel
Missense	c.1952A>G	Lys651Arg	Novel
Missense	c.2077G>A	Asp693Asn	Recurrent
Missense	c.2612C>T	Pro871Leu	Recurrent
Missense	c.3400G>A	Glu1134Lys	Novel
Missense	c.3548A>G	Lys1183Arg	Recurrent
Synonymous	c.981A>G	p.Thr327Thr	Novel
Synonymous	c.2082C>T	p.Ser694Ser	Novel
Synonymous	C.2311T>C	p.His771His	Novel
Synonymous	c.3405G>A	p.Gln1135Gln	Novel
Intron Variant	g.75407T>C		Novel
Intron Variant	g.75401_75401delT		Novel

Table 2: Types of mutations in *BRCA1* found in Federal Capital Territory by (Majeed *et al.,* 2020)

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The information identified by authors is very economical as one can screenforthe13recurrent mutationsof*BRCA1*and2of*BRCA2*inspecificethnicgroups. Nonsensemutation,4627C→A, is identifiedasthe mostcommonmutationin *BRCA1*and reported in five families and 22% of patients. Besides 4627C→A three other mutations, 185insA, 185delAG, and 5622 C →T, are also found to be recurrent representing 52% of all *BRCA1* mutations. Another researcher Malik et al., also observed 2 types of changes in *BRCA1*, one is nonsense mutations and the other is an addition mutation (Table 3 and 4). Mutational investigation of every one of these observations underlines the importance of genetictestingforgermlinemutationsin*BRCA1*forPakistanifamilieswithdifferentbreast or ovarian malignant cases Rashid *et al*.,.4184del4, *BRCA1* mutation appears in 15% of patients and ranks the second most dominant mutation in Pakistan (Figure4).
Mutational analysis of *BRCA2* uncovered that 2 mutations, 5057delTG, and 3337C →T are equally recurrent and common with each appearing 50% in breast cancer patients.

Table 3: Summary of the mutations found different ethnics group in *BRCA1*.

4	11	4184del4	frameshift and truncation	3 Punjabi 1 Sindhi	(Liede *et al.*, 2002)

Mutational statistics of *BRCA1* in Pakistan					
No	Exon no.	Point Mutation	Mutation	Ethnicity	Reference
Type of Mutation: Deletion					
1	2	185delAG	frameshift and truncation	1 Punjabi 2 Pathan	(Liede *et al.*, 2002; Rashid *et al.*, 2006)
2	11	1616delAAAT	frameshift and truncation	1 Muhajir	(Liede *et al.*, 2002)
3	12	4284delAG	frameshift and truncation	2 Muhajir	(Liede *et al.*, 2002)
5	11	1476delG	frameshift and truncation	1 Punjabi	(Liede *et al.*, 2002)
6	11	3889delAG	frameshift and truncation	2 Punjabi	(Liede *et al.*, 2002)
7	11	2388delG	frameshift and truncation	2 Muhajir	(Liede *et al.*, 2002; Rashid et al., 2006)
8	11	894delG	frameshift and truncation	1 Muhajir	(Liede *et al.*, 2002)
9	11	1956delA	frameshift and truncation	1 Punjabi	(Liede *et al.*, 2002)
10	11	1127delA	frameshift and truncation	1 Punjabi	(Liede *et al.*, 2002)
11	11	2266delG	frameshift and truncation	1 Punjabi	(Liede *et al.*, 2002)

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12	7	550delA	frameshift and truncation	1 Multiracial	(Muhammad U. Rashid *et al.,* 2006)
13	8	589delCT	frameshift and truncation	1 Punjabi	(Rashid *et al*., 2006)
14	17	5149delCTAA	frameshift and truncation	1 Punjabi	(Rashid *et al.,* 2006)
15	11	1013delTG	frameshift and truncation	1 Muhajir	(Liede *et al*., 2002)
Type of Mutation: Insertion					
16	11	2080insA	frameshift and truncation	3 Pathan	(Liede *et al.,* 2002)
17	11	2041insA	frameshift and truncation	2 Punjabi	(Liede *et al*., 2002)
18	2	185insA	frameshift and truncation	4 Punjabi	(Liede *et al.,* 2002; Rashid *et al.,* 2006)
19	11	1770insT	frameshift and truncation	1 Balouchi	(Liede *et al.,* 2002)
20	11	3812insT	frameshift and truncation	1 Multiracial	(Rashid *et al.,* 2006)
21	20	5376insA	frameshift and truncation	1 Multiracial	(Rashid *et al.,*2006)
22	13	4356insA	frameshift and truncation	3 Not identified	(Malik *et al.,* 2008)

Type of Mutation: Nonsense (Protein Change)					
23	15	4627C→A, S1503X	Stop 1503	6 Punjabi	(Liede *et al.,* 2002; Rashid *et al*., 2006)
24	11	1590 C→T, Q491X	Gln to stop	1 Punjabi	(Rashid *et al.,* 2006)
25	11	1731C→T, Q531X	Gln to stop	1 Multiracial	(Rashid *et al.,* 2006)
26	12	4302C→T, Q1395X	Gln to stop	1 Punjabi	(Rashid *et al.,* 2006)
27	24	5622C→T, R1835X	Arg to stop	2 Punjabi	(Rashid *et al.,* 2006)
28	11	1912T→G	Gln to stop	1 Muhajir	(Liede *et al.,* 2002)
Missense Mutations (Protein Change)					
29	13	4305	Serine changed	3 Not specified	(Malik et al., 2008)
30	11	3405C→T	C→T	1 Muhajir	(Liede *et al.,* 2002)

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31	11	2722C→G	C→G	1 Kashmiri	(Liede *et al.*, 2002)

Mutational statistics of *BRCA2* in Pakistan					
No	Exon no.	Point Mutation	Mutation	Ethnicity	Reference
Type of mutation: Deletion					
1	22	9140delA	frameshift and truncation	1 Muhajir	(Liede *et al.*, 2002)
2	11	3913delG	frameshift and truncation	1 Sindhi	(Liede *et al.*, 2002)
3	11	5950delCT	frameshift and truncation	1 Punjabi	(Liede *et al.*, 2002)
4	11	6696delTC	frameshift and truncation	1 Punjabi	(Liede *et al.*, 2002)
5	11	2674delG	frameshift and truncation	1 Muhajir	(Liede *et al.*, 2002)
Splice site					
32	Intron 14	IVS14-1G→A		2 Punjabi 1 Pathan	(Liede *et al.*, 2002)
33	Intron 4	IVS4-1G→T		1 Punjabi	(Rashid *et al.*, 2006)
34	Intron 20	IVS20-1G→C		1 Multiracial	(Rashid *et al.*, 2006)

Table 4: Mutational statistics of *BRCA2* in Pakistan

6	11	5057delTG	frameshift and truncation	1 Punjabi 1 Parsi	(Liede *et al.*, 2002; Rashid *et al.*, 2006)
7	11	3179delA	frameshift and truncation	1 Muhajir	(Liede *et al.*, 2002)
8	10	1993delAA	frameshift and truncation	1 Multiracial	(Rashid *et al.*, 2006)
9	11	4052delTAGA	frameshift and truncation	1 Multiracial	(Rashid *et al.*, 2006)
10	25	9658delT	frameshift and truncation	1 Multiracial	(Rashid *et al.*, 2006)
Type of Mutation: Insertion					

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11	11	5302insA	frameshift and truncation	1 Punjabi	(Liede *et al.,* 2002)
12	11	6679insAA	frameshift and truncation	1 Punjabi	(Liede *et al.,* 2002)
Type of Mutation: Nonsense (Protein Changed)					
13	11	3337C→T	Gln to stop	2 Memon	(Liede *et al.,* 2002)
14	10	2083C > T, Q619X	Gln to stop	1 Punjabi	(Rashid *et al.,* 2006)
15	11	3218T > G, L992X	Leu to stop	1 Multiracial	(Rashid *et al.,* 2006)
16	11	5962G > T, E1912X	Glu to stop	1 Multiracial	(Rashid *et al.,* 2006)

An investigation in 2011 from Aga Khan Hospital further provides us with a study that identifies a common *BRCA1* polymorphism c.4837A > G in exon 16 (Table 5). The other findings of the study reported 3 new mutations never found in the population before were c.271T > G, c.5231 delG and c.1123 T > G in exon 6, 20, and 11 respectively (Moatter *et al.,* 2011).

Table 5: Mutations found in 2011 in *BRCA1* by (Moatter *et al*., 2011)

Exon (BRCA1)	Change in Nucleotide	Amino acid change	Novel	Type of Mutation	Ethnicity
11	c.1123T > G	stop at 374	Novel	Frameshift	Pashtun
20	c.5231delG	Open reading Frame	Novel	Frameshift	Mohajir
16	c.4837A > G	S1613G	Reported	Polymorphism	Mohajir
6	c.271T > G	C91W	Novel	Missense	Mohajir

1.4.*BRCA1/2* prevalence in different ethnicities in Pakistan

Allthe4provincesofPakistanhavedifferentethnicpercentages.Punjabi'sarethebiggest ethnic group in the nation and ranks first with 44.15 percent. Pashtuns are 15.42 percent, Sandhi'shave14.1%,Seraiki's10.53%,Muhajir's7.57%,Balochi'sare3.57%,andother ethnic groups comprised of 4.66% in Pakistan population. (Farooq *et al.,*2011).

The nation has a blend of different ethnicities moved in this locale during migration in 1947andthemigrationofAfghanrefugees,particularlyinurbanareas.That'swhyPakistan has incredibly diverse hereditary variations for the breast and ovarian cancerinheritance.

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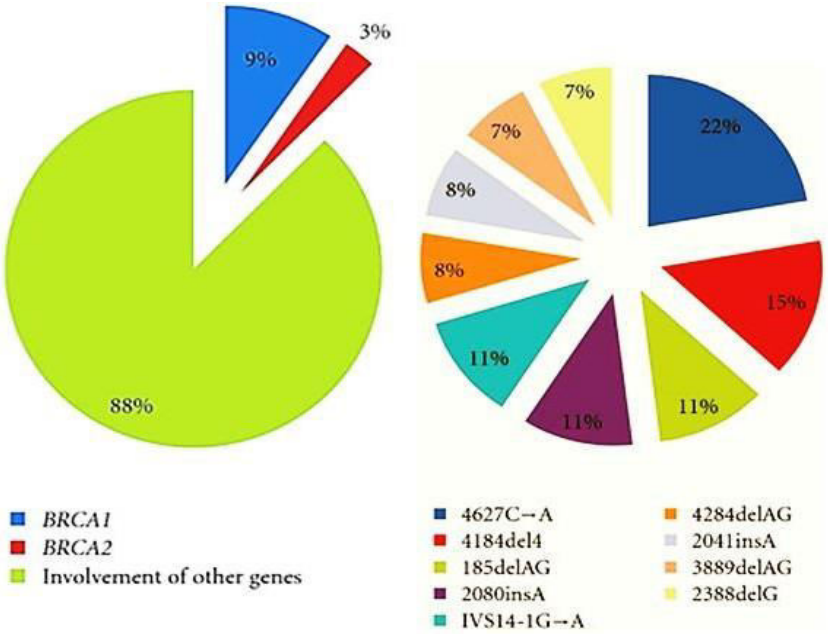

Figure4: Percentageof*BRCA 1/2*involveinBreastandOvari ancancerpatientsisshown inleftgraphwhile right graph shows most prevalent *BRCA1* mutations in Pakistani population. Adopted from (Farooq *et al.,* 2011).

A s ge of Breast cancer patients. Further dig ximately 46% cases of breast cancer. Sec ara, Sahiwal, Faisalabad and Gujrat (Ta

Table 6: Percentages of Breast cancer patients in Punjab and statistical data of mutations in *BRCA1* and *BRCA2* in different ethnic groups.

%age of breast cancer Patients in different areas of Punjab	
Lahore	46 %
Gunjranwala	7%
Sialkot	6%
Sargodha	4%
Shiekhupura	4%
Kasur	3%
Okara	3%
Sahiwal	3%
Faisalabad	3%
Gujrat	3%

Mutations in *BRCA1*		Mutations in *BRCA2*	
Ethnicity	%	Ethnicity	%
Punjabi	57	Punjabi	33
Muhajir	17	Multiracial	28
Pathan	13	Muhajir	17
Sindhi	1.8	Memon	11
Balouchi	1.8	Parsi	5.5
Mutiracial	7.4		
Source: (Arshad *et al.,* 2019; Farooq *et al.,* 2011)			

According to the studies most dominant missense mutation 4627C →A is seen to be more frequent in Punjabis.

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4184del4 is also found in 3 Punjabis' and 1 Sindhi. *BRCA1* 185insA has 4 and 5622 C →T has 2 carriers.

Findings in the data suggest that there are some variants which are more common in the specific ethnic group. Like Pahktuns or Pathans have more *BRCA1* carriers of 2080insA. Muhajirs have 2 carriers of 4284delAG (Liede *et al.,* 2002; Rashid *et al.,* 2006). 3 more novel mutations are also reported in Mohajir ethnic group (Moatter *et al.,* 2011). Duringthescreeningof*BRCA2*variants,Liede*et al*.,havefound2Memonstohave3337C→T recurrent variant, depicting the chances of same variant in this ethnic group.Another *BRCA2*genemutation5057delTGwasthefindingofbothLiede*et al*andRashidet al, thedifference isjustthatLindefinditinaParsi(minorityethnicgroup)whowassuffering from ovarian cancer while Rashid *et al.,*find it in aPunjabi.

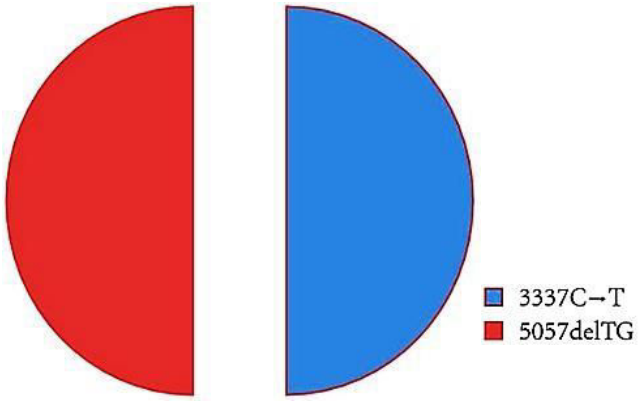

Figure 5: Two most common*BRCA2* mutations in Pakistani population. Adopted from (Farooq *et al.,* 2011).

Veryfewstudieshavereportedthetypeofmutations intheBalochiethnicgroup.In2019, a study reported 9 mutations in *BRCA1* gene and 4 variants in *BRCA2* in Balochi people. (Yousafzai et al., 2019). The details of the finding are given in Table07.

If we summarized all the data at the national level in Pakistan then we can look for a specific common mutation in our targeted ethnic groups. There are many evidences in Liede *et al.,*2002; Muhammad U. Rashid *et al.,*2006 &2016studiesthatsomemutationsarestronglyorprobablyhaveaweakassociationwith a particular group inPakistan.

Table 7
Identified mutations in *BRCA1/2* in Baloch ethnic group are shown.

Mutations identified in *BRCA1* in Balochi ethnicity			
Exon	Variant	Type of Mutation	Change in protein
11	c.1033G>T	Missense	p.Asp343Tyr
11	c.1181G>A	Missense	p.Gly393Asp
11	c.1267insAT	Frameshift	p.Ser423fs
11	c.1682C>T	Missense	p.Ser561Phe
11	c.1847C>T	Missense	p.Ser616Phe
11	c.2612C>T	Missense	p.Pro871Leu
11	c.3748G>T	Nonsense	p.Glu1250X
16	c.4837A>G	Missense	p.Ser1613Gly

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21	c.5308insG	Frameshift	p.Gly1770fs
Mutations identified in *BRCA2* in Balochi ethnicity			
Exon	Variant	Type of Mutation	Change in protein
3	c.172G>A	Missense	p.Glu58Lys
3	c.295G>A	Missense	p.Asp99Asn
3	c.310G>A	Missense	p.Asp104Asn
11	c.2996C>T	Missense	p.Pro999Gln

Source: (Yousafzai *et al.,* 2019)

1.5. Initiatives by Pakistan's government

ThegovernmentofPakistaniscurrentlyworkingonsomeinitiativesinordertocontrolthe current situation of breast cancer in the country. One such step taken by the governmentis PHRC. Pakistan National Health Research Council which is working under the Ministry of National Services, Regulation and Co-ordination. Its main head office is in Islamabad and its 12 research centers are working all across the country. ("PHRC," n.d.) It is serving as a bridge between all private and government healthcare facilities and is aim is to build a cancer registry database. It conducts awareness programs and strolls on breastcancer. Almost 30 cancer hospitals are currently working in Pakistan. Sindh has 12 cancer hospitals,PunjabandKPKhas7,CapitalIslamabadhas3hospitalswhileGilgit,Baltistan, and Baluchistan each has 1 cancer hospital (Arshad *et al*.,2019). Additionally, the International Association of Cancer Registry is working in the country since 1966 ("International Association of Cancer Registries," n.d.). KCR, GICR, and ENCR are also working on cancer registries.

Shukat Khanum memorial hospital and research center, SKMHRC in Lahore has another cancer registry database. The archive is following all the WHO policies regarding the coding the cancer database. They share this information with health care experts in the country and trying to found the reason behind cancer in children. As Punjab is ranking on the top for its more breast cancer cases, PCR or Punjab cancer registry established in 2005 is working on a local mass levels to deal with the situation. In 2008 it spreads its work in Faisalabad, Kasur, Sheikhupura, and Nankana Sahib. Its main office is in Shakut Khanum Memorial Hospital. In 2016, PCR expands its work in Sialkot andNarowal.NCCPorNationalCancerControlprogramisalsolooking forwardtosetup ways to detect cancer timely and working on its management andcure. Pakistan has many institutions that are working and can help the Government of Pakistan inmakingpoliciesaboutthealarmingbreastcancersituationinthenation.Especiallythey canworktodrillthetruthbehindcancerinchildren.Thus, it'saneedofanhourtoconduct national level molecular analysis among the BC patients across different regions of the country and add towards statistics that can help making betterpolicies. Pakistanshouldlookandconnectwiththosepatientswhodon'tlookforclinicaltreatment. Cancer treatment is very expensive and just hardly any medical clinics have figured out how to give free of cost treatment plans, follow-ups and pharmaceuticaldrug. Hospitals like SKMCH, Children cancer Foundation, and Bait-ul-Sukoon Cancer Hospital doofferfreeorsupportedtreatments.However,zakatsponsorsorfreechannelshavebroad and repetitive procedures that applicants have tofollow.

Conclusion

Breast cancer is a disease that has both genetic and environmental factors. Its number of patients are increasing day by day and it is huge issue in developed as well as developing countries. Except Israel, Pakistan has the highest number of breast cancer patients in Asia. And icing on the cake is that 60% of the population found to be on advanced stages when diagnosedwithbreastcancer.Thetrendandprevalenceofbreastcancerisalsoduetolack of awareness and facilities. It is estimated that women between ages 30-34 would account for a 130.6% increase in incidence by 2025. There would be an estimated 23.1% increase from 2015 in 2020 and 60.7% in 2025 (Zaheer *et al.,* 2019). Dozensofgeneswithhigh,moderateandlowpenetrancareinvolvedinbreastandovarian cancer. Among such genes *BRCA1/2* has pivotal roles in suppressing tumor. Researchers are exploring new dimensions about cancergenetics. More studies regarding *BRCA1* and *BRCA2* mutations in the specific population could be immensely helpful. Study of breast cancer molecular issues, development, physiology, anatomy, lymphatics including sentinel nodes, and blood supply followed bytumor suppressor and DNA repair genes, role of steroid receptors, benign breast diseases, risk

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factors, malignant lesions, pathologyandstagesofbreastcancerstudiescouldaddfurtherandtakeustonewhorizons in curing the disease. All this information can reduce fear which in spite of hundreds of years is stillthere.

Despite the fact, that many cancer registries have been created and are useful yet they appear to be in their initial stages for planning the treatment of malignancy and are not working on their full potential. With the help of the statistics and awareness provided by these archive institutions, it will be a lot easier to prevent the disease in its early stages all over Pakistan in different ethnicities. The requirement for bigger synergistic investigations between molecular and clinical experts and scholars can't be underscored enough. This is fundamental for increasing further understanding of the broad mutational spectrum and distribution of mutations in diverse ethnic groups. We can make screening strategies. The high prevalence level of *BRCA1* variations in breast and ovarian cancer cases, underline the requirement for improvinggeneticcounseling.Genetictestingshouldbeapartofthescreeningapproaches. Themoreinformationwehaveonthebreastcancergenomicswithrespecttothe Pakistani populace, the earlier a hereditary fix for that particular populace could befound.

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References

1. Arshad,S.,Rehman,M.U.,Abid,F.,&Yasir,S.(2019).*Currentsituationofbreast cancer in Pakistan withthe available interventions*. (June). https://doi.org/10.12692/ijb/14.6.232-240
2. Basra, M. A. R., Saher, M., Athar, M. M., & Raza, M. H. (2016). Breast Cancer in Pakistan a Critical Appraisal of the Situation Regarding Female Healthand Where the Nation Stands. *Asian Pacific Journal of Cancer Prevention*, *17*(7),3035–3041. https://doi.org/10.14456/apjcp.2016.50/APJCP.2016.17.7.3035
3. Bland, Copeland, Klimberg, G. (2017). *The Breast comprehensive management of benign and malignant diseases* (5th editio; K. I. E. M. C. S. K. J. G. Bland, Ed.). Elsevier.
4. Bollati, V., Fabris, S., Pegoraro, V., Ronchetti, D., Mosca, L., Deliliers, G. L., … Neri, A. (2017). BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. *Carcinogenesis*, *30*(8), 1330–1335.https://doi.org/10.1093/CARCIN
5. Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A.(2018). Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and MortalityWorldwidefor36Cancersin185Countries.*CACANCERJCLIN*,*68*(6). https://doi.org/10.3322/caac.21492
6. Cable, P. L., Wilson, C. A., Calzone, F. J., Rauscher, F. J., Scully, R.,Livingston, D. M., … Afshari, C. A. (2003). Novel consensus DNA-binding sequence for BRCA1 protein complexes. *Molecular Carcinogenesis*, *38*(2), 85–96. https://doi.org/10.1002/mc.10148
7. Clark, S. L., Rodriguez, A. M., Snyder, R. R., Hankins, G. D. V., & Boehning, D. (2012,April1).Structure-functionofthetumorsuppressorBRCA1.*Computational and Structural Biotechnology Journal*, Vol. 1, p. e201204005. https://doi.org/10.5936/csbj.201204005
8. Claus, E. B., Risch, N., & Thompson, W. D. (1991). Genetic analysis of breast cancer in the cancer and steroid hormone study. *American Journal of Human Genetics*, *48*(2),232–242.
9. Cohen, S. M., & Ellwein, L. B. (1990, August 31). Cell proliferation in carcinogenesis. *Science*, Vol. 249, pp. 1007–1011. https://doi.org/10.1126/science.2204108
10. Collins, N., McManus, R., Wooster, R., Mangion, J., Seal, S., Lakhani, S. R., … Stratton,M.R.(1995).Consistentlossofthewildtypealleleinbreastcancersfrom a family linked to the BRCA2 gene on chromosome 13q12-13. *Oncogene*, *10*(8), 1673–1675. Retrieved fromhttps://europepmc.org/article/med/7731724
11. Economopoulou, P., Dimitriadis, G., & Psyrri, A. (2015, January 1). Beyond BRCA: New hereditary breast cancer susceptibility genes. *Cancer Treatment Reviews*, Vol. 41, pp. 1–8.https://doi.org/10.1016/j.ctrv.2014.10.008
12. Farooq, A., Naveed, A. K., Azeem, Z., & Ahmad, T. (2011). Breast and Ovarian Cancer Risk due to Prevalence of BRCA1 and BRCA2 Variants in Pakistani Population: A Pakistani Database Report. *Journal of Oncology*, *2011*, 8. https://doi.org/doi.org/10.1155/2011/632870

ISSN- 2394-5125 VOL 08, ISSUE 03, 2021

13. Hall, J. M., Lee, M. K., Newman, B., Morrow, J. E., Anderson, L. A., Huey, B., & King, M. C. (1990). Linkage of early-onset familial breast cancer to chromosome 17q21. *Science*, *250*(4988), 1684–1689.https://doi.org/10.1126/science.2270482
14. Hollis, R. L., Churchman, M., & Gourley, C. (2017). Distinct implications of different BRCA mutations: Efficacy of cytotoxic chemotherapy, PARP inhibition andclinicaloutcome inovariancancer. *OncoTargetsandTherapy*,*10*,2539–2551 https://doi.org/10.2147/OTT.S102569
15. InternationalAssociationofCancerRegistries.(n.d.).RetrievedJuly8,2020,fromhttp://www.iacr.com.fr/index.php?option=com_content&view=article&id=111&Itemid=435
16. Johannesdottir, G., Gudmundsson, J., Bergthorsson, J. T., Arason,A., Agnarsson, B. A., Eiriksdottir, G., … Barkardottir, R. B. (1996). High Prevalence of the 999del5 Mutation in Icelandic Breast and Ovarian Cancer Patients. *Cancer Research*, *56*(16).
17. Khokher, S., Riaz, M. U. Q. M., Akhtar, N., & Saleem, A. (2012). Clinicopathologic Profile of Breast Cancer Patients in Pakistan: Ten Years Data of aLocalCancerHospital.*AscianPacificJournalofCancerPrevention*,*13*(2),693–698.https://doi.org/10.7314/apjcp.2012.13.2.693
18. Liede, A., Malik, I. A., Aziz, Z., De los Rios, P., Kwan, E., & Narod, S. A. (2002). Contribution of BRCA1 and BRCA2 mutations to breast and ovarian cancer in Pakistan. *American Journal of Human Genetics*, *71*(3), 595–606. https://doi.org/10.1086/342506
19. Loeb, L. A. (1991). Mutator phenotype may be required for multistage carcinogenesis.*Undefined*.
20. Majeed, A. I., Ullah, A., Jadoon, M., Ahmad, W., & Riazuddin, S. (2020). Screening, diagnosis and genetic study of breast cancer patients in Pakistan. *Pakistan Journal of Medical Sciences*, *36*(2), 16–20. https://doi.org/10.12669/pjms.36.2.1059
21. Malik,F.A.,Ashraf,S.,Kayani,M.A.,Jiang,W.G.,Mir, A.,Ansar,M.,…Sadiq, R. (2008). Contribution of BRCA1 germline mutation in patients with sporadic breast cancer. *International Seminars in Surgical Oncology*, *5*(1), 1–5. https://doi.org/10.1186/1477-7800-5-21
22. Mamoon,N.,Sharif,M.A.,Mushtaq,S.,Khadim,M.T.,&Jamal,S.(2009).Breast carcinoma over three decades in northern Pakistan - Are we getting anywhere? *Journal of the Pakistan Medical Association*, *59*(12), 835–838. Retrieved from https://europepmc.org/article/med/20201176
23. Menhas,R.,&Umer,S.(2015,April1).BreastcanceramongPakistaniwomen *Iranian Journal of Public Health*, Vol. 44, pp. 586–587. Iranian Journal of Public Health.
24. Miki,Y.,Swensen,J.,Shattuck-Eidens,D.,Futreal,P.A.,Harshman,K.,Tavtigian, S.,…Skolnick,M.H.(1994).Astrongcandidateforthebreastandovariancancer susceptibility gene BRCA1. *Science*, *266*(5182), 66–71. https://doi.org/10.1126/science.7545954
25. Moatter, T., Aban, M., Khan, S., Azam, I., & Pervez, S. (2011). BRCA1 status in Pakistani breast cancer patients with moderate family history. In *Journal of the College of Physicians and Surgeons Pakistan* (Vol. 21). Retrieved from http://ecommons.aku.edu/pakistan_fhs_mc_pathol_microbiol/6
26. Parsons, R. (2007). Breast Cancer. In D. F. Roses (Ed.), *CardiovascularImaging* (2nd ed.). Philadelphia: Elsevier Churchill Livingstone.
27. Pasche,B.(2008).Recentadvancesinbreastcancergenetics.In*AdvancesinBreast cancer management* (2nd ed., p. 10). Retrieved from http://www3.utsouthwestern.edu/cancergene.
28. PHRC.(n.d.).RetrievedJuly8,2020,fromhttp://phrc.org.pk/research-centres.html
29. Raponi, M., Smith, L. D., Silipo, M., Stuani, C., Buratti, E., & Baralle, D. (2014). BRCA1 exon 11 a model of long exon splicing regulation. *RNA Biology*, *11*(4), 351–359.https://doi.org/10.4161/rna.28458

ISSN- 2394-5125 VOL 08, ISSUE 03, 2021

30. Rashid, Muhammad U., Zaidi, A., Torres, D., Sultan, F., Benner, A., Naqvi, B., … Hamann, U. (2006). Prevalence of BRCA1 and BRCA2 mutations in Pakistani breastandovariancancerpatients.*InternationalJournalofCancer*,*119*(12),2832– 2839.https://doi.org/10.1002/ijc.22269
31. Rashid, Muhammad Usman, Muhammad, N., Bajwa, S., Faisal, S., Tahseen, M., Bermejo, J. L., … Hamann, U. (2016). High prevalence and predominance of BRCA1 germline mutations in Pakistani triple-negative breast cancer patients. *BMC Cancer*, *16*(1), 673. https://doi.org/10.1186/s12885-016-2698-y
32. Rosenthal, C. K. (2012). BRCA2 in abscission. *Nature Cell Biology*, *14*(8), 792– 792.https://doi.org/10.1038/ncb2559
33. Szabo,C.I.,&King,M.C.(1997).PopulationgeneticsofBRCA1andBRCA2.

    American Journal of Human Genetics, Vol. 60, pp. 1013–1020.

    Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1712447/
34. Thorlacius, S., Struewing, J. P., Hartge, P., Olafsdottir, G. H., Sigvaldason, H., Tryggvadottir,L.,…Eyfjörd,J.E.(1998).Population-basedstudyofriskofbreast cancer in carriers of BRCA2 mutation. *Lancet*, *352*(9137), 1337–1339. https://doi.org/10.1016/S0140-6736(98)03300-5
35. Wooster,R.,Bignell,G.,Lancaster,J.,Swift,S.,Seal,S.,Mangion,J.,…Stratton, M. R. (1995). Identification of the breast cancer susceptibility geneBRCA2. *Nature*, *378*(6559), 789–792. https://doi.org/0.1038/378789a0
36. Xu, X., Weaver, Z., Linke, S. P., Li, C., Gotay, J., Wang, X. W., … Deng, C. X. (1999). Centrosome amplification and a defective G2-M cell cycle checkpoint induce genetic instability in BRCA1 exon 11 isoform-deficient cells. *Molecular Cell*, *3*(3), 389–395.https://doi.org/10.1016/S1097-2765(00)80466-9
37. Yousafzai, A., Luqman, M., Ahmed, N., Arbab, M., Murad, M., Sajjad, N., … Ahmad, J. (2019). Identification of mutations in gene BRCA1/2 in breast cancer cases from Balochistan, Pakistan. *Pakistan Journal of Zoology*, *51*(4), 1579–1582. https://doi.org/10.17582/journal.pjz/2019.51.4.sc3
38. Zaheer, S., Shah, N., Maqbool, S. A., & Soomro, N. M. (2019). Estimates of past and future time trends in age-specific breast cancer incidence among women in Karachi,Pakistan: 2004-2025.*BMC Public Health*. https://doi.org/10.1186/s12889-019-7330-z
39. Zhang,J.,Fackenthal,J.D.,Huo,D.,Zheng,Y.,&Olopade,O.I.(2010).Searching for large genomic rearrangements of the BRCA1 gene in a Nigerian population. *Breast Cancer Research and Treatment*, *124*(2), 573–577. https://doi.org/10.1007/s10549-010-1006-9
40. Zhou, X., Han, S., Wang, S., Chen, X., Dong, J., Shi, X., … Shen, H. (2009). Polymorphisms in HPV E6/E7 protein interacted genes and risk of cervical cancer in Chinese women: A case-control analysis. *Gynecologic Oncology*, *114*(2), 327– 331.https://doi.org/10.1016/j.ygyno.2009.05.011