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Short Communication

Identification of Mutations in Gene *BRCA1/2* in Breast Cancer Cases from Balochistan, Pakistan

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Authors' Contributions
AY executed the experimental work. NA and SN provided help in Wet Lab. SD helped in molecular techniques. ML, K, NS, AH, SZ, ZR and MMJ helped in sampling. MM, MA and JA helped in data analysis. JA supervised the work.

Key words
Breast cancer, Missense mutation, Nonsense mutation, Heterozygous, Gene *BRCA1/2*.

ABSTRACT

*BRCA*1/2 genes are highly susceptible genes for both breast and ovarian cancer and account for 15-20% of all the hereditary breast cancer cases. Present study was aimed at identifying mutation in *BRCA1/2* in breast cancer patients in Balochistan. Blood samples of 100 subjects including 50 breast cancer cases and 50 normal subjects were used to determine genetic variants in *BRCA1* and *BRCA2*. Nine variants were identified in *BRCA1* and four were identified in *BRCA2*. In case of *BRCA1*, six missense substitutions (p.Asp343Tyr, p.Gly393Asp, p.Ser561Phe, p.Ser616Phe, p.Pro871Leu and p.Ser1613Gly) two frameshift (p.Ser423fs and p.Gly1770fs) and nonsense mutation (p.Glu1250X) was identified. In case of gene *BRCA2*, all the four variants were missense substitutions (p.Glu58Lys, p.Asp99Asn, p.Asp104Asn and p.Pro999Gln). The data reported here will be valuable addition for the future genetic screening of the breast cancer patients of Balochistan.

Breast cancer is the most frequently diagnosed and second leading cause of death accounting for about 25% of all cancer cases among women worldwide (Ferlay *et al*., 2012). 5-10% of the breast cancer cases are considered to be hereditary, out of which 15-25% cases are caused by mutations in *BRCA1* and *BRCA2* genes. *BRCA1/2* gene mutations are most frequently diagnosed in both breast and ovarian hereditary cancers (Narod, 2012; Baloch *et al*., 2014; Esposito *et al*., 2016).

The human gene *BRCA1* is located on the chromosome 17q2.1, from base pairs 41,196,312 to 41,277,500; whereas gene *BRCA2,* cytogenetic location is 13q13.1, ranging from base pairs 32,315,480 to 32,399,672 on chromosome 13. Germ line alterations of the *BRCA1* (MIM113705) or *BRCA2* (MIM600185) genes result in vulnerability to breast and ovarian tumor. Many germ line alterations in both genes have been reported. Most of these mutations are small deletions or insertions that result in frame shift and trimmed protein (Ford *et al*., 1998).

The protein encoded by *BRCA1* is a tumor suppressor nuclear phosphoprotein that plays a critical role with the interaction of the other proteins forming a protein complex in the nucleus of different cells to repair DNA damage (Cao *et al*., 2003; Deng, 2006; Buckley and Mullan, 2012; Dine and Deng, 2013). The BRCA1 protein contains 1863 amino acids and is divided into domains including ring domain at NH_2 terminus, central DNA binding domain and BRCT domain at COOH terminus. Missense and

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truncating mutations in *BRCA1* are most frequently been found in a wide range of breast cancer cases with family history (Dine and Deng, 2013).

An increased incidence of other malignances, such as prostate, pancreatic and colorectal cancer is also observed among *BRCA1/2* mutation carriers. The proportion of described mutations in *BRCA1* relative to *BRCA2* varies between different populations. With the exception of a strong *BRCA2* founder effect in Iceland, however, *BRCA1* mutations are more regularly reported. In the majority (>80%) of families with breast cancer and ovarian cancer, the disease has been linked to the *BRCA1* gene.

Present study was performed on 50 breast cancer cases and 50 normal subjects. The prime objective of the current study was to analyze germline mutations in *BRCA1/2* gene in the patients of breast cancer and to compare it with normal population. Current study revealed 13 different genetic variants including missense, nonsense and frameshift mutations in gene *BRCA1/2* in breast cancer cases from Balochistan.

Materials and methods

Breast cancer cases (n=50) and normal subjects (n=50), all females, belonging to different ethnic groups, were enrolled in current study. Informed consent forms were signed from the volunteers of both breast cancer patients and normal subjects. History of the disease including the family history of breast or other cancers, onset and date of diagnosis was enquired from the patients. Clinical history including histopathology was obtained from the patient files enrolled for chemotherapy and/or radiotherapy in CENAR (Center for Nuclear Medicine and Radiotherapy). Venous blood (5 ml) was collected in 15 ml tubes containing EDTA as an anticoagulant agent.

DNA was extracted from the blood samples through an inorganic method. Primers were designed for all the coding exons of *BRCA1/2* genes using the Prime3 software. *BRCA1/2* exons were amplified through PCR by using the primers designed for *BRCA1/2* and visualized through gel documentation system after gel electrophoresis. All the amplified products were sequenced on 3100 ABI prism DNA sequencer using the Big Dye Terminator Cycle Sequencing Kit. The results of DNA sequences were analyzed for the genetic variants by comparing the sequences of the exons with the normal sequences of *BRCA1/2* genes (ENST00000357654.7) through ENSEMBLE genome browser.

Results and discussion

All the coding exons and intron-exon boundaries of *BRCA1/2* gene were analyzed. From among 50 breast cancer patients, nine genetic variants in *BRCA1* and four in *BRCA2* were recorded. In the case of *BRCA1*, six missense substitutions (p.Asp343Tyr, p.Gly393Asp, p.Ser561Phe, p.Ser616Phe, p.Pro871Leu and p.Ser1613Gly) two frameshift (p.Ser423fs and p.Gly1770fs) and one nonsense mutation (p.Glu1250X) was identified (Table I). In the case of gene *BRCA2*, all the four variants were missense substitutions (p.Glu58Lys, p.Asp99Asn, p.Asp104Asn and p.Pro999Gln) (Table II). Sequence data of all exons of *BRCA1/2* were compared with normal sequences of *BRCA1/2* cDNA sequences and genomic data from public databases.

Table I.- List of genetic variants identified in gene *BRCA1*.

Region	Variant	Mutation type	Effect on protein
Exon 11	c.1033G>T	Missense substitution	p.Asp343Tyr
Exon 11	c.1181G>A	Missense substitution	p.Gly393Asp
Exon 11	c.1267insAT	Frameshift	p.Ser423fs
Exon 11	c.1682C>T	Missense substitution	p.Ser561Phe
Exon 11	c.1847C>T	Missense substitution	p.Ser616Phe
Exon 11	c.2612C>T	Missense substitution	p.Pro871Leu
Exon 11	c.3748G>T	Nonsense/Stop codon	p.Glu1250X
Exon 16	c.4837A>G	Missense substitution	p.Ser1613Gly
Exon 21	c.5308insG	Frameshift	p.Gly1770fs

Table II.- List of genetic variants identified in gene *BRCA2*.

Region	Variant	Mutation type	Effect on protein
Exon 3	c.172G>A	Missense substitution	p.Glu58Lys
Exon 3	c.295G>A	Missense substitution	p.Asp99Asn
Exon 3	c.310G>A	Missense substitution	p.Asp104Asn
Exon 11	c.2996C>T	Missense substitution	p.Pro999Gln

Among the Asian states, Pakistan is considered to have the highest incidences of breast cancer (Liede *et al*., 2002). *BRCA1* and *BRCA2* are the most susceptible breast cancer genes. Proteins encoded by both genes play an important role to protect the genome. *BRCA1* encodes a pleiotropic protein in response to DNA damage that functions to activate cell cycle checkpoint and DNA repair (Gudmundsdottir and Ashworth, 2006; Roy *et al*., 2012; Almeer *et al*., 2018).

The identified missense mutations was studied by bioinformatics software Polyphen-2 that showed that most of the missense mutations were benign (Fig. 1); while

the other nonsense mutation and frameshift mutations may have been the case of breast cancer in the patients (Fig. 2). Studies suggest that *BRCA1* and *BRCA2* are most predisposing genes to hereditary breast and ovarian cancers. Germline mutations in both *BRCA1* and *BRCA2* genes may increase the risk of developing breast cancer up to about 80% throughout the life time of an individual (Roy *et al*., 2012; Narod, 2012). In Pakistan, consanguineous marriages are very common. In current study, parents of 7 patients were reported with consanguinity. Hashmi (1997) and Hussain and Bittles (1998) reported in their study that Pakistan has the highest rate of consanguinity globally with 60-70% consanguineous marriages, which may play major role in spreading the hereditary cancers. Liede *et al*. (2002) reported five *BRCA1* mutations (2080insA, 3889delAG, 4184del4, 4284delAG, and IVS14-1A→G) and one *BRCA2* mutation (3337C→T) in multiple case subjects in Pakistani population. Rashid *et al*. (2006) identified thirty deleterious germ-line mutations in the 176 families (17.0%), including 23 in *BRCA1* and 7 in *BRCA2*. Four mutations, 185delAG, 185insA, S1503X and R1835X, were recurrent; these accounted for 52% of all identified *BRCA1* mutations.

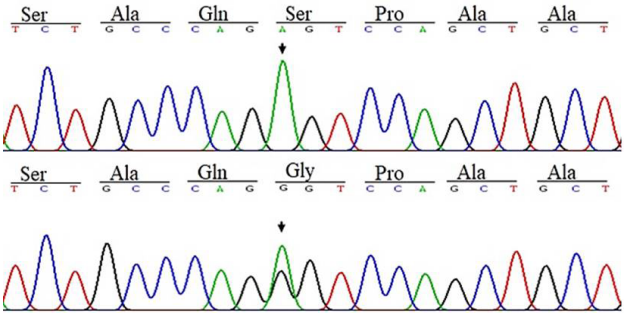

Fig. 1. Missense substitution in Exon 16 of gene *BRCA1*.

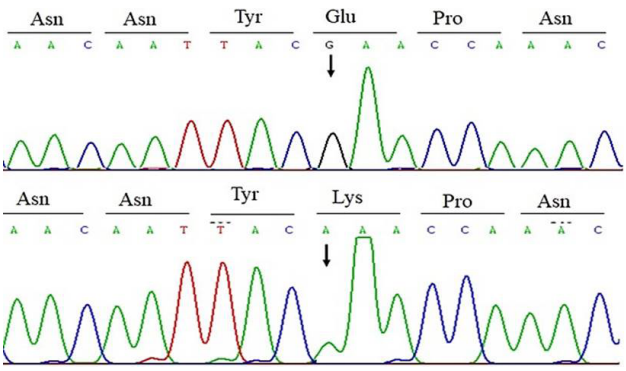

Fig. 2. Missense substitution in Exon 3 of gene *BRCA2*.

The data reported here will be valuable addition for the future genetic screening of the breast cancer patients either with a family history or not and for the other family members of the patients who are at risk.

Conclusion

Current study revealed 13 different genetic variants including missense, nonsense and frameshift mutations in gene *BRCA1/2* in breast cancer cases from Balochistan.

Statement of conflict of interest

The authors declare no conflict of interest.

References

Ahmad, J., Le Calvez-Kelm, F., Daud, S., Voegele, C., Vallee, M., Ahmad, A., Kakar, N., McKay, J.D., Gaborieau, V., Leone, M. and Sinilnikova, O., 2012. *Clin. Genet*, **82**: 594-598. https://doi.org/10.1111/j.1399-0004.2012.01869.x

Almeer, R., Alqarni, A., Alqattan, S., Abdi, S., Alarifi, S., Hassan, Z. and Semlali, A., 2018. *Pakistan J. Zool.,* **50**: 1999-2007. http://dx.doi.org/10.17582/journal.pjz/2018.50.6.1999.2007

Baloch, A.H., Daud, S., Raheem, N., Luqman, M., Ahmad, A., Rehman, A., Shuja, J., Rasheed, S., Ali, A., Kakar, N. and Naseeb, H.K., 2014. *Mol. Biol. Rep.,* **41**: 1103-1107. https://doi.org/10.1007/s11033-013-2956-x

Borg, A., Haile, R.W., Malone, K.E., Capanu, M., Diep, A., Törngren, T., Teraoka, S., Begg, C.B., Thomas, D.C., Concannon, P. and Mellemkjaer, L., 2010. *Hum. Mutat.*, **31**: E1200-E1240. https://doi.org/10.1002/humu.21202

Buckley, N.E. and Mullan, P.B., 2012. *Stem Cell Rev. Rep.*, **8**: 982-993. https://doi.org/10.1007/s12015-012-9354-y

Cao, L., Li, W., Kim, S., Brodie, S.G. and Deng, C.X., 2003. *Genes Dev.,* **17**: 201-213. https://doi.org/10.1101/gad.1050003

Deng, C.X., 2006. *Nucl. Acids Res.,* **34**: 1416-1426. https://doi.org/10.1093/nar/gkl010

Dine, J. and Deng, C.X., 2013. *Cancer Metast. Rev.*, **32**: 25-37. https://doi.org/10.1007/s10555-012-9403-7

Dodova, R.I., Mitkova, A.V., Dacheva, D.R., Hadjo, L.B., Vlahova, A.I., Hadjieva, M.S.T., Valev, S.S., Caulevska, M.M., Popova, S.D., Popov, I.E. and Dikov, T.I., 2015. *BMC Cancer*, **15**: 523. https://doi.org/10.1186/s12885-015-1516-2

Esposito, M.V., Nunziato, M., Starnone, F., Telese, A., Calabrese, A., D'Aiuto, G., Pucci, P., D'Aiuto, M., Baralle, F., D'Argenio, V. and Salvatore, F., 2016. *Int. J. mol. Sci.,* **17**: 2145. https://doi.org/10.3390/ijms17122145

Ford, D., Easton, D., Stratton, M., Narod, S., Goldgar, D., Devilee, P., Bishop, D., Weber, B., Lenoir, G. and Chang-Claude, J., 1998. *Am. J. Hum. Genet.,* **62**: 676-689. https://doi.org/10.1086/301749

Ferlay, J., Soerjomataram, I., Ervik, M., Dikshit, R., Eser, S., Mathers, C., Rebelo, M., Parkin, D.M., Forman, D., Bray, F. (eds.), 2012. *GLOBOCAN, 2012: Estimated cancer incidence, mortality and prevalence worldwide in 2012*. Available at: http://publications.iarc.fr/Databases/Iarc-Cancerbases/GLOBOCAN-2012-Estimated-Cancer-Incidence-Mortality-And-Prevalence-Worldwide-In-2012-V1.0-2012 (Accessed on 07 March, 2019).

Friedman, L.S., Ostermeyer, E.A., Szabo, C.I., Dowd, P., Lynch, E.D., Rowell, S.E. and King, M.C., 1994. *Nat. Genet.*, **8**: 399-404. https://doi.org/10.1038/ng1294-399

Gudmundsdottir, K. and Ashworth, A., 2006. *Oncogene*, **25**: 5864-5874. https://doi.org/10.1038/sj.onc.1209874

Hashmi, M.A., 1997. *J. Pak. med. Assoc.,* 47: 75-77.

Hussain, R. and Bittles, A.H., 1998. *J. Biosoc. Sci.,* **30**: 261-275. https://doi.org/10.1017/S0021932098002612

Liede, A., Malik, I.A., Aziz, Z., de los Rios, P., Kwan, E. and Narod, S.A., 2002. *Am. J. Hum. Genet.,* **71**: 595-606. https://doi.org/10.1086/342506

Moatter, T., Aban, M., Khan, S., Azam, I. and Pervez, S., 2011. *J. Coll. Physic. Surg. Pak.,* **21**: 680-684.

Narod, S.A., 2012. *Nat. Rev. clin. Oncol.*, **9**: 460-470. https://doi.org/10.1038/nrclinonc.2012.102

Rashid, M.U., Zaidi, A., Torres, D., Sultan, F., Benner, A., Naqvi, B., Shakoori, A.R., Seidel-Renkert, A., Farooq, H., Narod, S., Amin, A. and Hamann, U., 2006. *Int. J. Cancer*, **119**: 2832-2839. https://doi.org/10.1002/ijc.22269

Roy, R., Chun, J. and Powell, S.N., 2012. *Nat. Rev. Cancer*, **12**: 68-78. https://doi.org/10.1038/nrc3181