

Short communication

DETECTION OF A NOVEL MUTATION IN EXON 20 OF THE BRCA1 GENE

ABHIJIT CHAKRABORTY¹, ATUL KATARKAR², KEYA CHAUDHURI²,
 ASHIS MUKHOPADHYAY³ and JAYASRI BASAK^{1,*}

¹Department of Molecular Biology, Netaji Subhas Chandra Bose Cancer
 Research Institute (NCRI), Kolkata, India, ²Molecular & Human Genetics
 Division, Indian Institute of Chemical Biology (IICB), Kolkata, India,
³Department of Oncology, Netaji Subhas Chandra Bose Cancer Research
 Institute (NCRI), Kolkata, India

Abstract: Hereditary breast cancer constitutes 5-10% of all breast cancer cases. Inherited mutations in the BRCA1 and BRCA2 tumor-suppressor genes account for the majority of hereditary breast cancer cases. The BRCA1 C-terminal region (BRCT) has a functional duplicated globular domain, which helps with DNA damage repair and cell cycle checkpoint protein control. More than 100 distinct BRCA1 missense variants with structural and functional effects have been documented within the BRCT domain. Interpreting the results of mutation screening of tumor-suppressor genes that can have high-risk susceptibility mutations is increasingly important in clinical practice. This study includes a novel mutation, p.His1746 Pro (c.5237A>C), which was found in BRCA1 exon 20 of a breast cancer patient. *In silico* analysis suggests that this mutation could alter the stability and orientation of the BRCT domain and the differential binding of the BACH1 substrate.

Key words: Breast cancer, BRCA1, BRCT repeat, BACH1, Cancer predisposition, DNA sequencing, Docking, Exon 20, *In silico* analysis, Missense mutation

* Author for correspondence. E-mail: hmcwt@dataone.in;
ncri.molecularbiology@gmail.com; abhijit.drems@gmail.com tel.: +91-33-2229 1049/5628,
 fax: +91-33-2226 4704

Abbreviations used: BACH1 – BRCA1-associated C-terminal helicase; BIC – Breast Cancer Information Core; BRCA1 – breast cancer gene one; BRCT – BRCA 1 C-terminal domain; DGG – free energy change, HGMD – Human Gene Mutation Database; HGVS – Human Genome Variation Society; MIM – Mendelian Inheritance in Man; NCBI – National Center for Biotechnology Information; OC – ovarian cancer; RMSD – root mean-square deviation

INTRODUCTION

Breast cancer is the most common neoplastic disease in women. It is significantly influenced by hereditary factors. In India, it is the second most common malignant cancer among women [1, 2]. India's National Health Profile 2010 predicted that breast cancer will have overtaken cervical cancer as the most common type of cancer by 2020 [3]. In West Bengal, a state in Eastern India, one in 14 women are at risk of developing breast cancer before they reach 64 years of age. Hereditary breast cancer constitutes 5-10% of all breast cancer cases. Major genetic factors, such as predisposing mutations in the BRCA1 and BRCA2 genes, are generally considered an indicator of genetic susceptibility in the case of early onset [4, 5]. Women with a BRCA1 (MIM# 113705) or BRCA2 (MIM# 600185) germline mutation are at high risk of developing breast and/or ovarian cancer [6, 7].

The mutations in the tumor-suppressor BRCA1 gene are associated with hereditary breast cancer [8, 9]. The BRCA1 protein plays an essential role in maintaining the genomic stability associated with several cellular processes, including DNA repair, cell cycle checkpoints, transcriptional regulation, and protein ubiquitination [10-12]. The C-terminus of BRCA1 has a complex structure consisting of two BRCT repeats connected by a 23-amino acid linker. The BRCT domain seems to function as a protein-protein interacting moiety and directly recognizes the phosphorylated Ser 990 site of BACH1. The significance of these interactions provides clues to the molecular basis that underlies cell-cycle regulation and DNA repair [13].

In this study, 103 samples were collected from clinically confirmed breast cancer patients attending the oncology department of the Netaji Subhas Chandra Bose Cancer Research Institute (NCRI), Kolkata, India, for treatment between May 2010 and December 2012. Our study on the identification of c.5263_5264insC among these patients was published previously [14]. This study excludes the 7 patient samples bearing the c.5263_5264insC mutation. We sequenced the remaining 96 samples exclusively for exon 20 of the BRCA1 gene. This report provides mutation analysis of the BRCA1 gene of a special case with novel mutation and functional effect using an *in silico* and docking study.

MATERIALS AND METHODS

Blood samples

After obtaining written informed consent and family history status from the patients, 5 ml of peripheral blood was collected in EDTA vials from each of them. The median age of the patients is 42.60 ± 3.59 years.

Genotype analysis

DNA was extracted from blood samples following the method of Miller *et al.* [15]. PCR amplification of exon 20 in the BRCA1 gene was performed using the forward primer: 5'-ATATGACGTGTCTGCTCCAC-3' and the reverse primer:

5'-GGAATCCAAATTACACAGC-3' using the methods of Dufloth *et al.* [16]. The PCR products were used for direct sequencing from both directions using a Big Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer) in a model 377 Automated DNA Sequencer (Applied Biosystems). Mutation was only recorded when detected in both the forward and reverse sequences. The sequence was compared with the sequences available at BIC, HGMD, HGVS and NCBI.

***In silico* analysis**

Residues 1649-1859 in a complex with BACH1 peptide (PDB ID 1T15) were retrieved from the PDB database. The mutant p.His1746 Pro was built using Modeller9v6. The energy minimization was performed using a NOMAD-Ref server (<http://lorenz.immstr.pasteur.fr/>). The root mean square deviation (RMSD) was calculated to check the deviation from the native structure using the TM-align server (<http://zhanglab.ccmb.med.umich.edu/TM-align/>). The prediction of the functional effect on p.His1746Pro mutation was done using PolyPhen-2 (<http://genetics.bwh.harvard.edu/pph2/>), I-Mutant Suite (<http://gpcr2.biocomp.unibo.it/cgi/predictors/I-Mutant3.0/I-Mutant3.0.cgi>) and SClde (<http://www.enzim.hu/scide/>). The docking study was performed using AUTODOCK Vina [17]. An eight-residue peptide (STpSPTFNK) of BACH1, used as a ligand for the docking analysis, was extracted from the PDB ID 1T15. A docking grid with a size of 18 x 26 x 20 Å³ was used around the active site of the mutant model and centered on the coordinates x = -4.539, y = 26.1, z = 35.791. All of the structures, docking pose visualization and images were generated using PYMOL Software.

RESULTS

We analyzed the samples sequenced with both the forward and reverse primers and identified a novel mutation, A5237C, in codon 1746 (Fig. 1), which is located in exon 20 of the BRCA1 gene. The sample was from a 34-years old patient who had breast surgery 5 years previously and had received routine chemotherapy and radiotherapy. She had a strong family history: her mother died of breast cancer, her maternal aunt is currently suffering from ovarian cancer, and her maternal grandfather had colon cancer. Her histology report revealed that it was an ER positive (ER+), PR negative (PR-), Grade II tumor.

Substitution of nucleotide c.5237A>C at codon 1746 (CAC→CCC) results in the replacement of histidine by proline (NCBI Reference U14680.1). To confirm the functional effect of the c.5237A>C mutation in BRCA1, we performed an *in silico* analysis.

The functional effect of the BRCA1 p.His1746Pro mutation

The potential energies for native 1T15 and the mutant p.His1746 Pro model protein were respectively -3.48124×10^6 and -3.47936×10^6 kJ/mol. The final RMSD between the native and mutant p.His1746 Pro was 1.42 Å. It is observed

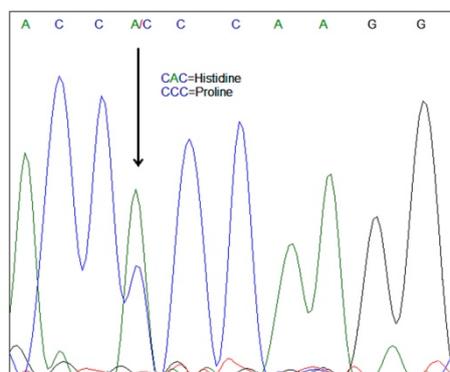


Fig. 1. Sequence analysis of a 401-bp PCR product covering the whole sequence of exon 20 of the BRCA1 gene from a breast cancer patient (# BRCA 24). A point mutation (substitution) was identified at codon 1746 (CAC→CCC), which is indicated by an arrow.

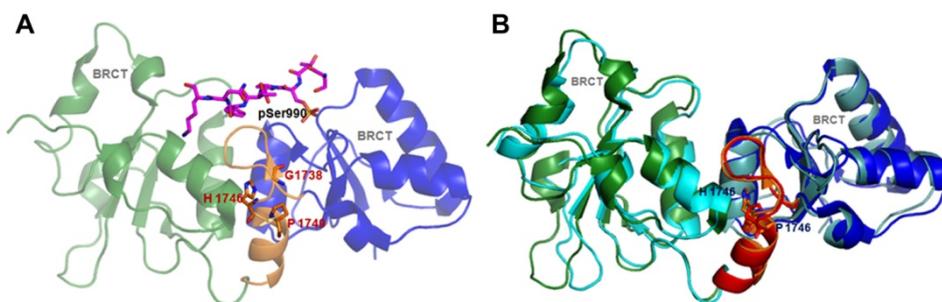


Fig. 2. Superimposition and position of the mutation in the inter-domain linker. A – Representation of the superimposition of native 1T15 (BRCT 1 is blue, BRCT 2 is green and the linker is orange) with the p.His1746Pro mutant (BRCT 1 is gray, BRCT 2 is cyan and the linker is red). B – Representation of native 1T15 showing the position of Gly1738, His1746 and Pro1749 mutations in the inter BRCT domain linker, along with BACH1 phosphopeptide binding to the active site.

that though the RMSD is within the range ($< 2\text{\AA}$) of similar folding, the BRCT repeat of the mutant p.His1746 Pro protein model showed significant deviations from its native structure. The superimposed structures of the native protein 1T15 with mutant p.His1746 Pro are shown in Fig. 2A. The PolyPhen-2-predicted p.His1746 Pro mutation was possibly damaging, with a score of 0.834 (sensitivity 0.84 and specificity 0.93). I-mutant predicted a DGG value for the p.His1746 Pro mutation of -0.96 kcal/mol, interpreted as a decrease in protein stability. SCide results show a total of 57 stabilization centers in the native protein and indicate the His1746 residue as involved in the stabilization of two centers: Ala1843 and Leu1844. The mutant p.His1746 Pro model had 69 stabilization centers and no Pro 1746 residue involved in stabilization (Fig. 3). The information from I-mutant and SCide server suggests that the BRCA1 p.His1746 Pro mutation would reduce protein stability.

Docking study

Docking studies using AutoDock Vina were performed to analyze the binding mode of BRCT-phosphopeptide. As previously reported, the Gly1738 and Pro1749 mutation are located at the BRCT1-BRCT2 interface beneath the molecular surface (Fig. 2B) and their effect is reconciled through an alteration in the relative orientation of the tandem BRCT motif. Each pose is predicted to bind the active site with the free energy of binding values at -5.3 kcal/mol or lower, suggesting a different binding pattern of phosphopeptide with the mutant p.His1746 Pro protein. Poses 1, 3 and 7 were predicted to be optimal binding poses (binding energy -5.8 kcal/mol, -5.6 kcal/mol and -5.4 kcal/mol, respectively; Fig. 4) compared to the native structure. The residue involved in hydrogen bond interaction with the BACH1 peptide is shown in Fig. 4E. This suggested that the BACH1 binding mode is considerably different to its native form.

DISCUSSION

The mutation found in codon 1746 at nucleotide c.5237A>C (CAC→CCC) has not yet been reported, as evidenced by the Human Genome Variation Society (HGVS) or Breast Cancer Information Core (BIC). Myriad genetics had reported a mutation in the same amino acid in codon 1746. There, the nucleotide position was c.5236C>A (CAC→AAC) and the amino acid changed was p.His1746Asn. The functional study related to this mutation has not yet been reported. That previous report and our finding of a mutation at the same position suggest that amino acid 1746 may have an important molecular role in breast tumorigenesis and familial breast cancer. The result that we obtained suggests that mutation p.His1746 Pro disrupts the structure, stability and function of the BRCA1 protein. Several missense mutations reported in BRCT repeats of the BRCA1 gene are responsible for the malfunction of tumor-suppressor activity, as evidenced from several clinical reports. It is reported that most cancer-associated BRCT missense mutations impart their effect through structural consequences, such as a change in the orientation of the BRCT repeat interface, alteration of the surface of the protein and BRCT global folding [18]. Functionally, BRCT mutation eliminates the capacity to function in DNA repair and interactions with histone deacetylases and BACH1. Most of these mutations are present over the phosphopeptide surface and directly or indirectly influence the function of BRCA1. *In silico* analysis revealed that this p.His1746 Pro mutation probably alters the structure and reduces the protein stability of BRCA1. Docking analysis suggested that the difference in the binding mode of BACH1 peptide on the BRCT interface of the mutant model can change the orientation of the BRCT domain and accelerate breast cancer.

This novel finding can help to identify cancer patients that are in a high-risk group. This positive result can serve as an indicator for intensive secondary malignancy screening programs. Early gynecological and oncological detection programs need to be implemented to identify asymptomatic mutation carriers

who are at high risk of developing breast cancer and ovarian cancer. Mass screening and haplotype analysis is required to identify the frequency and origin of this mutation among Eastern Indian breast cancer patients.

Conflict of interest. The authors declare no potential conflicts of interest.

Acknowledgment. We are grateful to the Indian Council of Medical Research (ICMR), Government of India, New Delhi, India for their financial assistance (File No. 54/07/2011/HUM-BMS; Project Number: 2011-03270). We owe our sincere thanks to the patients and their family members for their extensive cooperation with us. We would also like to thank all the staff at our hospital who helped to accomplish the study successfully.

REFERENCES

1. Gajalakshmi, C.K., Shanta, V., Swaminathan, R., Sankaranarayanan, R. and Black, R.J. A population-based survival study on female breast cancer in Madras, India. **Br. J. Cancer** 75 (1997) 771-775.
2. Pestonjamas, P.H. and Mitra, I. Analysis of BRCA1 involvement in breast cancer in Indian women. **J. Biosci.** 25 (2000) 19-23.
3. Murthy, N.S., Chaudhry, K., Nadayil, D., Agarwal, U.K. and Saxena, S. Changing trends in incidence of breast cancer: Indian scenario. **Ind. J. Cancer** 46 (2009) 73-74.
4. Armaou, S., Pertesi, M., Fostira, F., Thodi, G., Athanasopoulos, P.S., Kamakari, S., Athanasiou, A., Gogas, H., Yannoukakos, D., Fountzilias, G. and Konstantopoulou, I. Contribution of BRCA1 germ-line mutations to breast cancer in Greece: a hospital-based study of 987 unselected breast cancer cases. **Br. J. Cancer** 101 (2009) 32-37.
5. Newman, B., Austin, M.A., Lee, M. and King, M.C. Inheritance of human breast cancer: evidence for autosomal dominant transmission in high-risk families. **Proc. Natl. Acad. Sci. USA** 85 (1988) 3044-3048.
6. Begg, C.B., Haile, R.W., Borg, A., Malone, K.E., Concannon, P., Thomas, D.C., Langholz, B., Bernstein, L., Olsen, J.H., Lynch, C.F., Anton-Culver, H., Capanu, M., Liang, X., Hummer A.J., Sima, C. and Bernstein, J.L. Variation of breast cancer risk among BRCA1/2 carriers. **JAMA** 299 (2008) 194-201.
7. Stratton, M.R. and Rahman, N. The emerging landscape of breast cancer susceptibility. **Nat. Genet.** 40 (2008) 17-22.
8. Rosen, E.M., Fan, S., Pestell, R.G. and Goldberg, I.D. BRCA1 gene in breast cancer. **J. Cell Physiol.** 196 (2003) 19-41.
9. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P.A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L.M., Ding, W., Bell, R., Rosenthal, J., Hussey, C., Tran, T., Melody, M., Frye, C., Hattier, T. *et al.* A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. **Science** 266 (1994) 66-71.

10. Korlimarla, A., Bhandary, L., Prabhu, J.S., Shankar, H., Sankaranarayanan, H., Kumar, P., Remacle, J., Natarajan, D. and Sridhar, T.S. Identification of a non-canonical nuclear localization signal (NLS) in BRCA1 that could mediate nuclear localization of splice variants lacking the classical NLS. **Cell. Mol. Biol. Lett.** 18 (2013) 284-296.
11. Huen, M.S., Sy, S.M. and Chen, J. BRCA1 and its toolbox for the maintenance of genome integrity. **Nat. Rev. Mol. Cell Biol.** 11 (2010) 138-148.
12. O'Donovan, P.J. and Livingston, D.M. BRCA1 and BRCA2: breast/ovarian cancer susceptibility gene products and participants in DNA double-strand break repair. **Carcinogenesis** 31 (2010) 961-967.
13. Yu, X., Chini, C.C., He, M., Mer, G. and Chen, J. The BRCT domain is a phospho-protein binding domain. **Science** 302 (2003) 639-642.
14. Chakraborty, A., Mukhopadhyay, A., Bhattacharyya, D., Bose C.K., Choudhuri, K., Mukhopadhyay, S. and Basak, J. Frequency of 5382insC mutation of BRCA1 gene among breast cancer patients: an experience from Eastern India. **Fam. Cancer** 12 (2013) 489-495.
15. Miller, S.S., Dykes, D.D. and Polesky, H.F. A simple salting out procedure for extracting DNA from human nucleated cells. **Nucleic Acids Res.** 16 (1988) 12152.
16. Dufloth, R.M, Carvalho, S., Heinrich, J.K., Shinzato, J.Y., Santos C.C., Zeferino, L.C. and Schmitt, F. Analysis of BRCA1 and BRCA2 mutations in Brazilian breast cancer patients with positive family history. **Sao Paulo Med. J.** 123 (2005) 192-197.
17. Trott, O. and Olson, A.J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. **J. Comput. Chem.** 31 (2010) 455-461.
18. Williams, R.S. and Glover, J.N. Structural consequences of a cancer-causing BRCA1-BRCT missense mutation. **J. Biol. Chem.** 278 (2003) 2630-2635.