Analysis of novel mutations in *BRCA1* in Iranian families with breast cancer

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In Iran and the rest of the world, breast cancer (BC) is the most common malignancy in women. Familial history and age are significant risk factors for the development of this disease in Iran. Most hereditary BCs are associated with inherited mutations in the *BRCA1* and *BRCA2 genes.* Some recent studies demonstrated that *BRCA1* mutations are seen in high-risk women with family histories of BC. In this report we investigated all *BRCA1* exons from 40 female patients with family histories of BC and one BC twin, and report a novel mutation in this gene in one patient. As controls, *BRCA1* exons from 100 normal women and the BC-free twin of the BC twin were also examined for this mutation. None of the women in the normal group harbored the mutation. Whether this variation is specific for the Iranian population or for special subgroups remains to be determined.

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Breast cancer (BC) is the most frequent cancer (Ariad et al. 2011) and most pernicious form of malignancy among women (Anderson and Jakesz 2008), accounting for 16% of cancer deaths worldwide (World Health Organization 2008). It occurs most commonly in women of 40 to 50 years of age. It is a noxious disease that causes large numbers of deaths not only in developed countries such as the United Kingdom, United States of America and Canada, but also in underdeveloped and developing countries (Singh et al. 2011).

Most BC cases are considered sporadic in nature because they are not associated with extensive family histories of BC. Familial BC, often seen in families with high incidences of BC, has been associated with a number of susceptibility genes (El-Tamer et al. 2004; Brekelmans et al. 2006; Carroll et al. 2008; Ellsworth et al. 2010). These genes can be roughly divided into 'high-risk' and 'low to moderate risk' BC susceptibility groups. The high-risk BC susceptibility genes that generally are more prevalent in younger versus older women include *BRCA1*, *BRCA2*, *PTEN*, *TP53*, *LKB1/STK11* and *CDH1*, while *CHEK2*, *TGFβ1*, *ASP8* and *ATM* belong to the 'low to moderate-risk' BC susceptibility group (Oldenburg et al. 2007; Palacios et al. 2008). Of these genes, germline mutations in *BRCA1* and *BRCA2* are generally 'caretaker' genes that participate in DNA damage repair. Consequently, their inactivation allows other genetic defects to accumulate and leads to genetic instability (Kinzler and Vogelstein. 1997; Breivik 2005).

BRCA1 is known to be a potential tumor suppressor gene, located on the long arm of chromosome 17q21, with a length of nearly 100 kb, consisting of 24 exons encoding 1863 amino acids (Miki et al. 1994). The product of exons 2, 3 and 5 constitutes a zinc finger motif, which may be necessary for the transcriptional activation of other genes. The middle part of the protein, encoded by exon 11, can interact with the Rad51 molecule (the human counterpart of bacterial RecA protein) (Kijima et al. 1998). Most hereditary BCs can be accounted for by inherited mutations in *BRCA1* and *BRCA2* (Lynch et al. 2008). A new study demonstrated that *BRCA1* is seen in high-risk women with family histories of breast cancer (Kamal et al. 2011).

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The incidence of specific mutations is strongly dependent on the study population and its ethnic and geographic origin (Peelen et al. 1997; Balman et al. 2010). The number of germline mutations identified within *BRCA1* and *BRCA2* is growing and most of them are unique to each high-risk family (Fattahi et al. 2009). In Iran, breast cancer is ranked first among malignancies among women (Sadjadi et al. 2005; Mousavi et al. 2009), comprising 24.4% of all neoplasms (Goya 2007) with a crude incidence rate of 17.81 (Mousavi et al. 2009).

In the present study we analyzed *BRCA1* and report a novel mutation in this gene in Iranian breast cancer patients.

MATERIAL AND METHODS

Case selection

After genetic counseling of 200 families, the life time risk and the heterozygote risk were analyzed with Cyrillic 2.1 software. Women from 40 BC families with life time risks to 80% and heterozygote risks to 25% were analyzed in hot spot exons of *BRCA1*. In these 40 families, one woman with breast cancer had undergone two surgeries at 27 and 41 years of age (Fig. 1). Another patient was a monozygotic twin who had been diagnosed with BC at 24 years of age. Her twin was BC free. Our control group contained 100 families with no BC in three generations.

DNA isolation and mutation analysis

Genomic DNA was extracted from peripheral whole blood samples using standard protocols (Qiagen mini kit, catalogue no. 51304).

PCR

All *BRCA1* exons were amplified by PCR using exon-specific primers. Primers were designed using the mutation discovery system and the BCC-Consortium in Germany. PCR was performed in a 50 µl volume containing 2.5 mM of each dNTP, 1 U of Taq DNA polymerase (GenetBio), 2.5 µl of 10 × PCR buffer, 0.5 mM of each primer, and 50 ng of genomic DNA.

Direct sequencing

DNA was sequenced with both forward and reverse primers. Sequencing was performed according to the standard protocol provided with the BigDye Terminator Kit® V 1.1 (ABI 3700 Genetic analyzer). The reaction products were purified with the Qiagen DyeEx® spin kit. The data was analysed with SeqScape 2.5 software (Applied Biosystems). The sequence data obtained was compared with the NCBI/GenBank data base using BLAST and with our own data base using BioEdit and Mega.

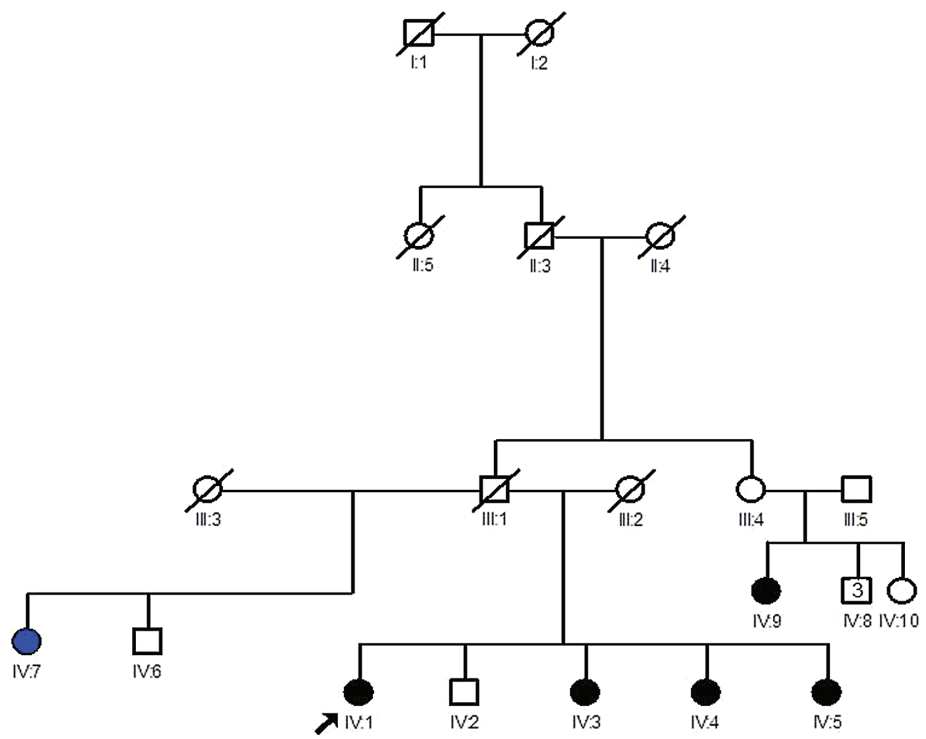

Fig. 1. Family pedigree for the patient whose BRCA gene contained the novel mutation (c.2286 T > A or p.Arg762Ser).

RESULTS

We performed PCR on 22 *BRCA1* exons (2, 3, 5, 6, 7, 8, 9, 10b, 11A, 11B, 11C, 11D, 11E, 11F, 11G, 11H, 11I, 11J, 11K, 11L, 11M, 12, 13, 14, 15, 16a, 17, 18a, 19, 20, 21, 22, 23 and 24) from patients and healthy women. We found some polymorphisms and a novel mutation in exon 11F of *BRCA1*. DNA samples from 100 control and 40 women from high-risk breast cancer families and one twin pair were analyzed for the novel mutation on exon 11F (c.2286 T > A or p.Arg762Ser) by direct sequencing (Fig. 2). The results confirmed that the novel mutation was not present in the control families or BC-free twin.

DISCUSSION

Breast cancer is the most prevalent and pernicious malignancy among women (Parkin et al. 2005). According to a report of the Iranian Ministry of Health and Medical Education (Goya 2007), BC has become the most general primary female cancer in Iranian women. Although the incidence of this cancer is relatively low compared with western countries (23.65 per 100 000 in Iran vs 140.8 per 100 000 Caucasian women in the United States), the number of patients with newly-diagnosed BC is increasing (Harirchi et al. 2011).

Molecular diagnostics for families with hereditary BC is a rapidly evolving field. The Breast Cancer Information Core (BIC) database demonstrated that three founder mutations, 185delAG and 5382insC in *BRCA1* and 6174delT in *BRCA2*, have been observed in different populations, especially in Ashkenazi Jewish patients. But studies in Iran have generally indicated that these three mutations are less frequent in Iranian BC patients than in patients from other areas (Mehdipour et al. 2006, Fattahi et al. 2009).

Breast cancer patients in Iran are generally young, and the findings suggest that family histories of BC, and marital status, are risk factors (Pharoah et al. 1997); however, few studies concerning *BRCA1* and *BRCA2* alterations in the Iranian population have been published (Yassaee et al. 2002, Pietschmann et al. 2005, Keshavarzi et al. 2011).

In this study, all *BRCA1* exons from Iranian high-risk breast cancer woman of non-Jewish origin were analyzed. Many polymorphisms from the entire *BRCA1* gene have been identified, and one putative novel mutation was detected. The novel mutation was a missense mutation and previously unclassified variant (UV). The impact of limited changes with missense substitutions or in-frame exon deletions is often not clear, and presents a challenge in the clinical setting. Approximately 30% of *BRCA1* and 60% of *BRCA2* variants are labeled as unclassified variants (UVs) (National Human Genome Research Institute). We studied this novel mutation in 100 women with no family history of BC for three generations, and women from 40 families at high risk for breast cancer (life-time risk to 80% and heterozygote risk to 25%). The novel *BRCA1* mutation was not found in any normal women. Pathologic information for patient who had two tumors at ages 27 and 41 (patient 1) and the twin who had a tumor at age 24 (patient 2) is presented in Table 1. The tumor characteristics were different between the two patients. We examined the twin patient

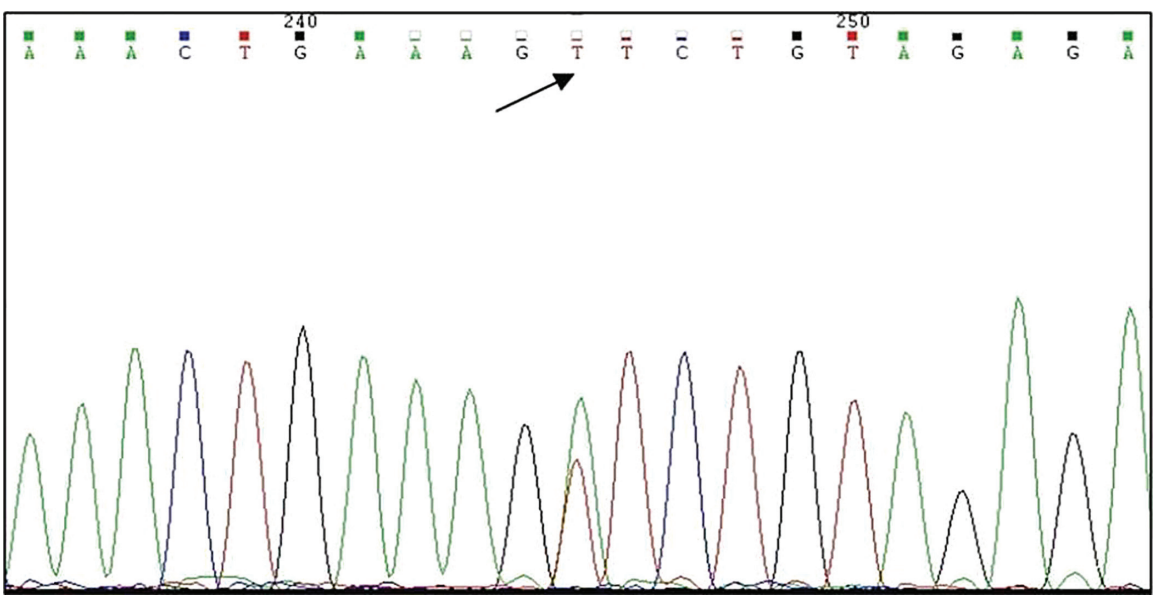

Fig. 2. DNA sequence data of the novel mutation site designated as exon 11F, Arg762Ser amino acid.

Table 1. *Main characteristics of pathology in female patients.*

	Age	Histology	Size	ER•	PR†	Her2‡
Patient 1						
right breast	27	ductal carcinoma invasion/undetermined	4 × 3.5 × 2 cm	T2N2MD	negative	negative
left breast	41	ductal carcinoma invasion comodo type/grade III	4 cm	T2N2MD	negative	negative
Patient 2						
left breast	24	undetermined/grade II	5 × 4.5 × 1 cm	strongly positive (90%)	moderately positive (50%)	negative

•Estrogen receptor. †Progestrone receptor. ‡Epidermal growth factor receptor.

and 39 subjects from the high-risk families, but the mutation was not detected in them. Therefore this novel mutation can be a new unclassified variant in Iran. This amino acid sequence (c.2286 T > A or p.Arg762Ser) that was changed is not described in BRCA1 at this time. We predict that this missense mutation may affect the secondary structure of the protein. We analyzed the character of this mutation with regard to pathogenicity with the PolyPhen-2 prediction of functional effects of human ns SNPs software. This mutation is predicted to be damaging with a score of 0.588 (sensitivity: 0.81; specificity: 0.83).

In summary, one pathogenic and novel mutation in the *BRCA1* has been detected in this study. Whether this mutation is specific for the Iranian population or for special subgroups remains to be determined.

GenBank accession numbers

These sequence variant have already been submitted to GenBank: accession number BankIt1473921 JN686490.

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REFERENCES

Anderson, B. O. and Jakesz, R. 2008. Breast cancer issues in developing countries: an overview of the Breast Health Global Initiative. – World J. Surgical Oncol. 32: 2578–2585.

Ariad, S., Milk, N., Bolotin, A. et al. 2011. Measles virus antigens in breast cancer. – Anticancer Res. 3: 913–920.

Balman, J., Diez, O., Rubio, I. et al. 2010. BRCA in breast cancer: ESMO clinical practice guidelines. – Ann. Oncol. 21: 20–22.

Breivik, J. 2005. The evolutionary origin of genetic instability in cancer development. – Sem. Cancer Biol. 15: 51–60.

Brekelmans, C. T., Seynaeve, C., Menke-Pluymers, M. et al. 2006. Survival and prognostic factors in BRCA1-associated breast cancer. – Ann. Oncol. 17: 391–400.

Carroll, J. C., Cremin, C., Allanson, J. et al. 2008. Hereditary breast and ovarian cancers. – Can. Family Physician 54: 1691–1692.

Ellsworth, R. E, Decewicz, D. J, Shriver, C. D. et al. 2010. Breast cancer in the personal genomics era. – Curr. Genomics 11: 146–161.

El-Tamer, M., Russo, D., Troxel, A. et al. 2004. Survival and recurrence after breast cancer in BRCA1/2 mutation carriers. – Ann. Surgical Oncol. 11: 157–164.

Fattahi, M. J., Mojtahedi, Z., Karimaghaee, N. et al. 2009. Analysis of BRCA1 and BRCA2 mutations in southern Iranian breast cancer patients. – Arch. Iranian Med. 12: 584–587.

Goya, M. 2007. Iranian annual cancer registration report 2005/2006. – Ministry of Health and Medical Education, Health Deputy, Center for Disease Control and Prevention (in Persian). Tehran, Iran.

Harirchi, I., Kolahdoozan, S., Karbakhsh, M. et al. 2011. Twenty years of breast cancer in Iran: downstaging without a formal screening program. – Ann. Oncol. 22: 93–97.

Kamal, M., Abu Gabal, K., El-Naggar, S. et al. 2011. BRCA1 gene expression in breast cancer in relation to other prognostic markers in Egyptian women. – Anatol. J. Obstetrics Gynecol. 1: 1

Keshavarzi, F., Eskafi, N., Ayoubyan, H. et al. 2011. Sequence variants of BRCA1 and BRCA2 genes in four Iranian families with breast and ovarian cancer.—– Iranian J. Public Health, Vol. 40, No. 2.

Kijima, G., Murakami,Y., Ohuchi, N. et al. 1998. Non-sense mutation at codon 63 of the BRCA1 gene in Japanese breast cancer patients. – Jpn J. Cancer Res. 89: 837–841.

Kinzler, K. W. and Vogelstein, B. 1997. Cancer-susceptibility genes. Gatekeepers and caretakers. – Nature 386: 761–763.

Lynch, H. T., Silva, E., Snyder, C. et al. 2008. Hereditary breast cancer. Part I. Diagnosing hereditary breast cancer syndromes. – Breast J. 14: 3–13.

Mehdipour, P., Hosseini-Asl, S., Savabi-E, A. et al. 2006. Low frequency of 185delAG founder mutation of BRCA1 gene in Iranian breast cancer patients. —J. Cancer Molecules 2: 123–127.

Miki, Y., Swensen, J., Shattuck-Eidens, D. et al. 1994. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. – Science 266: 66–71.

Mousavi, S. M., Gouya, M. M., Ramazani, R. et al. 2009. Cancer incidence and mortality in Iran. – Ann. Oncol. 20: 556–563.

National Human Genome Research Institute. An open access on-line breast cancer mutation data base. <http://research.nhgri.nih.gov/projects/bic>.

Oldenburg, R. A., Meijers-Heijboer, H., Cornelisse, C. J. et al. 2007. Genetic susceptibility for breast cancer: how many more genes to be found? – Crit. Rev. Oncol. Hematol. 63: 125–149.

Palacios, J., Robles-Frías, M. J., Castilla, M. A. et al. 2008. The molecular pathology of hereditary breast cancer. – Pathobiology 75: 85–94.

Parkin, D. M., Bray, F., Ferlay, J. et al. 2005. Global cancer statistics 2002. – CA: Cancer J. Clinicians 55: 74–108.

Peelen, T., van Vlite, A., Petrij-Bosch, M. et al. 1997. A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families. – Am. J. Human Genet. 60: 1041–1049.

Pharoah, P., Day, N. E., Duffy, S. et al. 1997. Family history and the risk of breast cancer: a systematic review and meta-analysis. – Int. J. Cancer 71: 800–809.

Pietschmann, A., Mehdipour, P., Atri, M. et al. 2005. Mutation analysis of BRCA1 and BRCA2 genes in Iranian high risk breast cancer families. – J. Cancer Res. Clin. Oncol. 131: 552–558.

Sadjadi, A., Nouraie, M., Mohagheghi, M. A. et al. 2005. Cancer occurrence in Iran in 2002, an international perspective. – Asian Pac. J. Cancer Prevention 6: 359–363.

Singh, T., Nagraja, M., Rao, S. et al. 2011. Enhancing image contrast of mammogram and equalization of histograms. – Int. J. Engin. Sci. Technol. 3: 563–572.

World Health Organization 2008. The top 10 causes of death in 2004. Fact Sheet of WHO Report. – WHO.

Yassaee, V. R., Zeinali, S., Harirchi, I. et al. 2002. Novel mutations in the BRCA1 and BRCA2 genes in Iranian women with early-onset breast cancer. – Breast Cancer Res. 4: 1–6.