RESEARCH ARTICLE

Identification of Germline *BRCA1* Mutations among Breast Cancer Families in Northeastern Iran

Mohammad Mahdi Kooshyar¹, Mohammadreza Nassiri^{2,3*}, Morteza Mahdavi³, Mohammad Doosti³, Amirreza Parizadeh³

Abstract

Background: The purpose of this study was to evaluate the prevalence of *BRCA1* (MIM: 113705) founder mutations in familial breast cancer (BC) patients with high risks in Iran. *BRCA1* is among the cancer susceptibility genes best known for high penetrance mutations. *BRCA1* genotyping is now used to determine patient counseling, management decisions, and prognosis of this syndrome. <u>Materials and Method</u>: Thirty nine patients with clinical BC and 29 high risk healthy women, related to the patients, participated in the study. DNA from blood samples was extracted and analyzed by PCR and SSCP methods in order to find 185delAG and 5382insC founder mutations. In addition, a 251bp fragment of *BRCA1*'s exon 11 was amplified and analyzed for determination of new mutations. <u>Results</u>: The data indicated the presence of 185delAG and 5382insC founder mutations in both groups studied. Two out of 39 BC patients (5.1%) and one out of 29 relatives (3.4%) were suspected to be carriers of 185delAG mutations. However, we found only one patient (2.6%) to be a carrier of a 5382insC mutation. Also, 2 women (5.1%) of the patient group and 3 n (10.3%) of relatives group were identified as carriers of unclarified mutations in the 251bp fragment of the *BRCA1* gene. The carriers of *BRCA1* founder mutations have a high lifetime risk of breast cancer. <u>Conclusions</u>: Therefore, these data are useful in counseling of individuals with a significant family history of breast cancer.

Keywords: BRCA1 - hereditary breast cancer - 185delAG - 5382insC

Asian Pac J Cancer Prev, 14 (7), 4339-4345

Introduction

Breast cancer (BC) is the most common malignancy in women and the leading cause of cancer death in females worldwide, accounting for 23% (1.38 million) of total new cancer cases and 14% (458,400) of the total cancer deaths in 2008. In the USA, the estimated number of new cases of BC in 2012 was 226,870, of which 39,510 resulted in death (American Cancer Society, 2012). The incidence rates of breast cancer vary among geographic locales with North America and Europe considered high risk areas, and Asia and Africa indicated as low risk areas (Ferlay et al., 2010). About half the BC cases and 60% of the deaths are estimated to occur in economically developing countries. Iran constitutes a low risk region for BC. However, Iran is located in the Middle part of Asia, which has the highest incidence of BC among women in the region, generally followed by gastric, esophageal and cervical lesions (Moore et al., 2010). Among Iranian women, BC is the fifth most common cause of death (Akbari et al., 2008) and ranked first among cancers diagnosed (Sadjadi et al., 2005; Mousavi et al., 2009), comprising 24.4% of all neoplasms (Mousavi et al., 2008), with a crude incidence rate and age standardized incidence rate (ASR) of 17.4 and 23.65 per 100,000, respectively in 2004 (Mousavi et al., 2009). Pursuant to Iran death surveys among 18 provinces in 2001 (Naghavi, 2001) and 23 provinces in 2003 (Naghavi, 2003), the BC mortality rate was registered as 2.5 and 2.7 per 100,000 of the female population, respectively. According to Iran's National Cancer Registry data, there is an increasing trend of BC incidence (Harirchi et al., 2000; Shamsa and Mohagheghi, 2002; Mousavi et al, 2007) and mortality (Taghavi et al., 2012). The incidence age of BC in women in Iran is at least one decade less than their western counterparts (Harirchi et al., 2000) with the mean age ranging from 47.1 to 48.8 years (Harirchi et al., 2004). And more than 36% of the tumors occur in women under 40 years old (Mousavi et al., 2006; Harirchi et al., 2011). Breast carcinomas can be classified as sporadic, familial and/or hereditary. Hereditary BC (HBC) has genetic heterogeneity; is transmitted vertically in an autosomal dominant pattern; and is characterized by dominant inheritance, early onset, severity of the disease and bilaterality (Claus et al., 1994). Familial clustering

¹Department of Hematology-Oncology, Mashhad University of Medical Sciences, ²Department of Agricultural and Animal Biotechnology, Institute of Biotechnology, Ferdowsi University of Mashhad, ³Animal Science Department, Ferdowsi University of Mashhad, Mashhad, Iran *For correspondence: nassiryr@um.ac.ir

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of cancer has been recognized since Roman times while autosomal dominant hereditary BC has been recognized as a syndrome since an early description by Lynch and Krush (1971). Twin studies estimate that around 27% of BC is because of hereditary factors (Peto et al., 2000). However, 5-10% of BC has a strong inherited component and 4-5% is due to high penetrance genes transmitted in an autosomal dominant pattern (Newman et al., 1988; Hall et al., 1990; Miki et al., 1994). It is twice as common in first-degree relatives of affected women compared with the general population (Newman et al., 1988), indicating that BC risk has a substantial inherited component (Newman et al., 1988). The genetic basis of HBC is usually an inherited germline mutation in one allele of high penetrance genes and subsequent loss of heterozygosity in somatic tissues (ACOG Committee on Practice Bulletins, 2009). Approximately 20-25% of this risk is explained by two high-penetrance susceptibility genes: BRCA1 (17q21) (MIM: 113705) and BRCA2 (13q12 13) (MIM: 600185) (Diez et al., 2011). The linkage of BRCA1 and BRCA2 to early-onset hereditary BC was discovered in 1990 and 1994, respectively (Hall et al., 1990; Wooster et al., 1994; Wooster et al., 1995). BRCA1 is located on chromosome 11q21 and BRCA2 on 13q12-13. About 5-10% of BCs are inherited, one-third of which are caused by dominant BRCA gene mutations (Xu et al., 2012). BRCA1 or BRCA2 mutations are detected in almost 25% of cases presenting with familial cancer (van der Groep et al., 2006). The cloning of two major BC susceptibility genes, BRCA1 and BRCA2, in 1994 and 1995 (Futreal et al., 1994; Miki et al., 1994; Wooster et al., 1994; 1995) and the subsequent development of commercial genetic testing has brought hereditary cancer genetics into the public eye. BRCA1 mutations are uniformly more frequent than BRCA2 mutations (Malone et al., 2006; Hall et al., 2009; Kurian et al., 2010) with some exceptions (Nelson-Moseke et al., 2004) and a number of studies have reported an extremely rare case of familial BC with deleterious germline mutations in both BRCA1 and BRCA2 genes (double heterozygosity) (Lavie et al., 2011; Nomizu et al., 2012). The major role of BRCA1 appears to be DNA repair including homologous recombination and nucleotide excision repair (Roy et al., 2012). BRCA1 comprises 22 coding exons spanning 80kb of genomic DNA that encodes a 7.8kb transcript into a protein of 1,863 amino acids. Over 1,700 unique BRCA1 mutations have been reported to the Breast Cancer Information Core Database (BIC) (Irwin, 2008). Of these mutations, 858 have been confirmed as being "clinically significant". The BIC reports that the most common BRCA1 mutations identified are 185delAG (16.5%), 5382insC (8.8%) and the missense mutation C61G (1.8%). In one meta-analysis, a systematic review on the frequency and distribution of common BRCA1 and BRCA2 mutations found they were associated with BC risk in 29 published epidemiological studies; 20 common founder germline mutations were identified and 4 BRCA1 mutations (5382insC, 185delAG, 3819del5 and 4153delA) were found to be the most common. The overall frequency of these mutations was 0.09, 0.07, 0.02 and 0.06, respectively (Wang et al., 2012). The prevalence and phenotype of the BRCA mutation

varies according to country and race; specific BRCA1 and BRCA2 mutations have been associated with certain ethnic populations, and founder mutations identified in several groups (Neuhausen, 2000; Liede and Narod, 2002; Nagy et al., 2004). However, 185delAG and 5382insC in BRCA1 and 174delT in BRCA2 are estimated in 2.5% of the worldwide populations (Roa et al., 1996; Struewing et al., 1997; Fodor et al., 1998) and account for 98-99% of the identified mutations (Frank et al., 2002; Phelan et al., 2002). While the screening for specific founder mutations is practical in several other countries, due to a lack of BRCA mutation data in BC patients in Iran and other racial and geographic differences, it is not possible to apply the data from western countries. Therefore, there is a need for further investigation of BRCA mutations in Iran (Schwartz et al., 2008; Balmana et al., 2010). Once a mutation is known within a family, predictive genetic testing becomes available. This then allows the identification of mutation carriers with the potential for targeted screening and intervention. These data are useful in the counseling of individuals with a significant family history of breast or ovarian cancer. Through the identification of women at high risk, it is hoped that cases of breast and ovarian cancer will be prevented. The present study attempts to identify the most frequent BRCA1 mutations in familial breast cancer patients.

Materials and Methods

Study subjects and data collection

The samples recruited for the study comprised the following groups. The first group was comprised of 39 patients with BC confirmed by medical tests and pathological reports, each from a different family. The families were from different parts of the states located in Khorasan province and these families were not related to each other. The second group included 29 first degree relatives of patients. The relatives were free of BC. All the patients included in this study were treated at the Hematology and Oncology Ward, Emam Reza Hospital, Mashhad, Iran. Complementary data about subjects were collected from medical and pathological reports, and when needed, complemented by a telephone interview. A genetic counselor explained the aims and objectives of this study to the patients and written informed consent sheets were obtained from the participants. Sample collections were carried out on the subject cases and were referred to the pathologic laboratory between February 2010 and February 2012.

DNA extraction and PCR analysis

Blood samples from the subjects' brachial vein were drawn into tubes containing 6% EDTA (Ethylenediaminetetraacetic Acid). Genomic DNA was extracted from 200μ l peripheral blood lymphocytes using a commercial DNA isolation kit, AccuPrep Genomic DNA Extraction Kit (Bioneer, South Korea) and in accordance with the manufacturer's protocols. After measuring the DNA concentration and its purity by Nanodrop 2000 spectrophotometer (Thermo Scientific, USA), the genomic DNA was diluted to a final concentration of 50 ng/µl in

Table 1. List of Primers Used for Identification of the5382insC Mutations in the Gene

Sequences	Primer name
F-5`-CAGCATGATTTTGAAGTCAGAGGAG-3	3` 5382insC
R-5`-CGAGACGGGAATCCAAATTACAC-3`	
F-5`-CTCTTCGCGTTGAAGAAGTAC-3`	185delAG
R-5°-GAGTGGATGGAGAACAAGGA-3°	
F-5'-CATAGCACCGTTGCTACCGAGT-3`251bp fragment of exon 11	
R-5`-TGATGCCTCATTTGTTTGGAAGA-3`	

dH₂O and stored at 4°C until subsequent analysis. For investigation of 5382insC and 185delAG mutations plus a 251bp fragment of the BRCA1 gene, 330, 255 and 251bp fragments in BRCA1 possibly containing mutations, respectively, were amplified by PCR with the primer sets described in Table 1. Although mutations are scattered throughout the BRCA1 gene, a putative 251bp coding fragment of BRCA1 was chosen for several reasons; it has been shown that exons 11 have a significant role in protein function and belonged to the BRCT domain of BRCA1 protein (Wu et al., 1996). Moreover, exons 11 cover a large segment of the gene and many putative mutations have been reported in these regions. PCR amplification was accomplished in a thermal cycler T-Personal (Biometra, Germany) in a final volume of 25μ l. The PCR mix contained a 2.5µl PCR buffer 10X, 1.5mM MgCl₂, 2mM dNTPs, and 10pM from each primer, 25-50ng genomic DNA, 1u Taq DNA polymerase (CinnaGen, Iran) and ddH₂O. PCR conditions for all fragments were 95°C for 5 min (94°C for 1 min, annealing for 30 seconds, and 72°C for 1 min), for 33 cycles, and final extension at 72°C for 10 min. PCR conditions for all fragments were identical, except that the annealing was at 50, 56 and 53°C for the 5382insC, 185delAG and 251bp fragments of BRCA1, respectively. Detection of the mutations within the amplification products of BRCA1 genes was carried out by using the single-strand conformation polymorphism (SSCP) gel electrophoresis method (Orita et al., 1989) as described below. The PCR-SSCP method is based on the fact that the electrophoretic mobility of a singlestranded molecule of DNA depends on the conformation of analyzed DNA fragments (Rusc et al., 2007; Chu et al., 2008). For SSCP analysis, 4μ L of PCR products were denatured by heating at 95°C for 5 min with 14µl of SSCP dye (95% formamide, 0.05% bromphenol blue and 0.05%xylene cyanol) and immediately plunged into ice, for 10 min to generate heteroduplexes. Thereafter, the samples were subjected to electrophoresis on 10% polyacrylamide gel and were run at a constant voltage of 250 volt at 6°C for 8 hours and were visualized with silver staining according to the Zhu et al. (2006) procedure.

Results

In the present study, we screened 68 individuals from 39 families for common polymorphisms in the *BRCA1* gene. Most of the patients (83%) were affected with ductile carcinoma. Lobular carcinoma was reported in two patients (5.5%) and a woman had modular carcinoma (5.5%). One case involved metastatic BC and all tumors were unilateral. The age distribution of all patients ranged

from 23 to 72 years with a mean of 49.3 years. Out of the 37 patients with age records, 1 patients was less than 25 years of age (2.7%), 5 patients were in the age group of 25-40 years (13.5%) and 31 patients were above 40 years (83.8%). In the BC subjects that were analyzed in the present study, two out of these 39 BC patients (5.1%)and one out of 29 relatives (3.4%) were suspected to be carriers of the 185delAG mutation. However, we detected only one patient (2.56%) and no one from relatives group to be carriers of the 5382insC mutation. These rates of polymorphisms in the 251bp region of the BRCA1 gene amplified, were 2 (5.1%) and 3 (10.3%), respectively. These mutations at mentioned fragments can't be verified or the type and position of these mutations cannot be clarified until sequencing samples are obtained. Also, in some families, both the patient and her relative had mutations, but in the others, just one of them had mutation. In other words, some mutation in other carriers was only found in one individual in the family (patient or relative).

Discussion

To our knowledge, this is the first genetic study on BRCA1 gene mutations conducted in BC patients from northeastern Iran. To date, very few reports have been published about the spectrum of BRCA1 sequence variants in the Iranian population and several studies have evaluated the frequency and risk associated with BC with the selected mutations in BRCA1 in other geographic regions, except northeastern Iran. So, we are unable to draw direct comparisons with other regional studies. Moreover, results from these studies showed conflicting evidence. Similar to our findings, in Tehran the mean age of the patients was 51.3 and 20% of the patients were under 40 years old (Mousavi et al., 2006). In another study on 2358 cases from the Tehran cancer registry data, the mean age of patients was 51.3 and 31.4% of them were under 40 years old (Mousavi et al., 2008). In a recent study to estimate familial BC prevalence in 400 patients from 24 different provinces, 28.2% of the patients were younger than 40 years (Sabokbar et al., 2012). The first description of the BRCA1 185delAG mutation was in a patient of Iranian Jewish descent (Levy-Lahad et al., 1997; Bar-Sade et al., 1998). In other studies on the BRCA1 185delAG mutation in 50 patients with BC from the Fars province in Iran, none of the patients had this mutation (Ghaderi et al., 2001). In 83 early-onset BC patients under the age of 45 in Tehran BRCA1 exons 2, 3, 5, 11, 13 and 20 mutations were analyzed, and two BC patients had the 185delAG mutation (2.5%) and none of the subjects had the 5382insC mutation (Yassaee et al., 2002). Pietschmann et al. (2005) also found no mutation in the BRCA1 gene. Also, in one investigation on 400 Iranian patients, the 5382insC mutation carrier of the BRCA1 gene were not found whereas, two (0.5%) 185delAG mutation carriers were found (Mehdipour et al., 2006). Fattahi et al. (2009) investigated the frequency of 185delAG and a 5382insC mutation in familial and sporadic cases of BC in five southern provinces, 250 women with sporadic BC, 55 women with a familial history of BC and their first degree relatives, and 200 healthy women formed the studied

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groups. The 5382insC or 185delAG were not detected. Keshavarzi et al. (2012) investigated mutations of BRCA1/2 in 85 patients from high risk Iranian families. The entire coding sequences and each intron/exon boundary of BRCA1/2 genes were screened by direct sequencing. They could detect the 20 novel mutations in BRCA1. The majority of detected variants (80%) were located within the BRCA1 gene and only four in BRCA2 (20%). However, only five pathogenic BRCA1 mutations and one pathogenic BRCA2 mutation were detected in 85 index cases and none had 185delAG and 5382insC. Salehgohari et al. (2012) considered nine different mutations of exon 2 and a partial region of exon 11 BRCA1 from 30 patients, living in Kerman province. They found no carrier of 185delAG (Saleh-gohari et al., 2012). For the BRCA1, overall worldwide frequency of 5382insC was 0.09 (95% CI 0.06-0.12) and the frequency of 185delAG was 0.07 (95% CI 0.01-0.13) (Wang et al., 2012). A low prevalence of 5382insC and 185delAG in BRCA1 has been reported from a province of Iran (Mehdipour et al., 2005). It founds that penetrance or prevalence of BRCA1 mutations is lower in Iran (Pietschmann et al., 2005) and suggested that the pattern of mutations seen in the BRCA genes among Iranians might be different from other populations (Mehdipour et al., 2006). This finding is not specific to Iran as there are some differences in BC prevalent mutations throughout the world. For example, in Iceland, BRCA1 mutations are rare and 0.4-0.6% of the Icelandic population carries a single BRCA2 founder mutation, 999delTCAAA. The 999del5 BRCA2 founder mutation is responsible for the vast majority of ovarian and/or BC families caused by these genes (Johannesdottir et al., 1996; Thorlacius et al., 1996). The c.5266dupC mutation is also found in other eastern European populations particularly in Poland (Lalloo and Evans, 2012). Another example from this region is the high frequency of the 5382insC BRCA1 mutation as well as the presence of other common mutations, which makes targeted screening possible in several Eastern European countries including Russia, Poland and Hungary (Van Der Looij et al., 2000; Menkiszak et al., 2003; Sokolenko et al., 2007). In Norway the BRCA1 1675delA and 1135insC mutations are particularly common, accounting for 3% of ovarian cancer cases and it has been suggested they could be offered in population-based clinical testing (Dorum et al., 1999). In Sweden, six mutations, including 3172ins5 and 1201del11 in BRCA1, accounted for 75% of mutations in a clinical screening unit (Einbeigi et al., 2007). Finally, in Denmark, the 2594delC mutation has been shown to account for 18% of all BRCA1 mutations (Sogaard et al., 2008) and 7 mutations account for 35% of carriers. Most family physicians believed that identifying familial cancer risk in their patients was important, but did not feel confident doing so (Freedman et al., 2003). Management of BC patients with a BRCA mutation should be established based on the clinicopathologic status as well as the results of genetic testing. If a mutation is found in an unaffected woman, she can be offered the intervention of prophylactic surgery and/or more intense screening to detect the earliest, treatable signs of the disease. For women who are negative for a known mutation in their family, there

is a level of reassurance that they are not at the highest risk of developing BC. In Iran, specific screening measures such as founder mutation detection should be implemented because the vast majority of BC has been diagnosed in advanced stages (Harirchi et al., 2011). Thus, health education programs to rectify the lack of women awareness among women about BC signs and effective screening are urgently needed (Taghavi et al., 2012). Identification of BRCA1 germline mutations of fetal cells from chorionic villus sampling (11th weeks) or amniocentesis (16th week) is technically possible (Quinn et al., 2009; Sagi et al., 2009). Therefore, these tests can make mothers or BC susceptible women aware of preventive approaches. It is likely that an increase in educational level followed by awareness about cancer can lead to early detection and treatment of the disease (Hussain et al., 2008; Hajian-Tilaki et al., 2012). There is evidence that BRCA1 associated cancers are not inevitable (Levin et al., 2012). Chemoprevention and prophylactic surgery (bilateral mastectomy and bilateral salpingooophorectomy) can decrease cancer risk in carriers of BRCA1 mutations (Fisher et al., 1998; Bevers, 2007; Kauff and Barakat, 2007; Visvanathan et al., 2009; Lostumbo et al., 2010; Goss et al., 2011). Guidelines are available to identify individuals to consider for genetic testing for BRCA1 mutations (Daly, 2010). Genetic counseling and genetic testing to identify BRCA1 gene mutations in high risk patients is widely available and commonly employed in the US and Europe (Frank et al., 2002; Robson, 2007). Nationally, physicians have a need for guidelines and better evidence about how to manage familial cancer susceptibility. However, studies, specifically, describing the clinicopathologic features, stages, and age distributions of BC in Iran are limited (Harirchi et al., 2000; 2004; 2010; Rezaianzadeh et al., 2009); thus, it is difficult to predict the present and future patterns of BC and carry out the most appropriate preventive and therapeutic measures to decrease the burden of the disease in Iran (Harirchi et al., 2004). By studying and establishing a defined registry system, we can overcome the difficulties and deficiencies in the patient's history making. In addition, given the different prevalence of this cancer in various races, environmental agents and life styles in different areas of Iran, at definition of localized criteria for suitable screening will be necessary in the future (Sabokbar et al., 2012). These mutations seem to have a much lower frequency in Iranian BC patients and there might be other genes that contribute more significantly to familial breast carcinoma in this population than BRCA or it is possible that common founder mutations remain to be identified in some populations, because many studies have not screened for large rearrangement mutations (Ramus et al., 2007). A complete BRCA1 gene sequence analysis might be required for identification of specific mutations in Iran, a country with an ethnically diverse population (Pietschmann et al., 2005). Also, the data in high risk BC patients without family a history of breast or ovarian cancer did not accurately reflect the prevalence of the BRCA1 mutation in Iranian BC patients because of the very small sample size. A large-scale multicenter study is needed to evaluate the prevalence of BRCA mutations among Iranian BC patients nationwide.

In conclusion, over the past two decades, significant advances have been made in our understanding of cancer genetics; however, we are only now beginning to appreciate the true role of genetic factors in cancer susceptibility. The identification of *BRCA1* mutation carriers is important in the clinical management of BC in families with BC. It is one of the few successes of clinical intervention for breast cancer in recent years, and screening for *BRCA1* mutations is now offered routinely in clinical practice. As further targeted therapies are developed, it will be critically important for close collaborations to exist between academics, pharmaceutical companies and regulatory agencies to ensure that these agents are used appropriately.

References

- Akbari ME, Harrirchi I, Khadivi R, Khosravi Z (2008). Ten year breast cancer screening and follow up in 52200 women in Shahre-Kord, Iran (1997-2006). *Iranian J Cancer Prevention*, **1**, 1-2.
- ACOG Committee on Practice Bulletins (2009). Hereditary breast and ovarian cancer syndrome. *Gynecol Oncol*, **113**, 6-11.
- American Cancer Society (2012). Cancer facts and figures. Atlanta: American Cancer Society, 3.
- Balmaña J, Diez O, Rubio I, Castiglione M (2010). BRCA in breast cancer: ESMO clinical practice guidelines. Ann Oncol, 21, 20-2.
- Bar-Sade RB, Kruglikova A, Modan B, et al (1998). The 185delAG BRCA1 mutation originated before the dispersion of Jews in the diaspora and is not limited to Ashkenazim. Human Molecular Genetics, 7, 801-5.
- Bevers TB (2007). The STAR trial: evidence for raloxifene as a breast cancer risk reduction agent for postmenopausal women. J Natl Comprehensive Cancer Network, 5, 817-22.
- Chu Q, Sun D, Yu Y, Zhang Y (2008). Identification of complex vertebral malformation carriers in Chinese Holstein. J Veterinary Diagnostic Investigation, 20, 228-30.
- Daly MB (2010). Genetic/familial high-risk assessment: breast and ovarian. J Natl Compr Canc Netw, 8, 562-94.
- Einbeigi Z, Bergm an A, Meis-Kindblom JM, et al (2007). Occurrence of both breast and ovarian cancer in a woman is a mark er for the BRCA gene mu tations: a populationbase d study from western Sweden. *Fam Cancer*, **6**, 35-41.
- Freedman AN, Wideroff L, Olson L, et al (2003). US physicians' attitudes toward genetic testing for cancer susceptibility. *Am J Med Genet*, **120**, 63-71.
- Ferlay J, Shin HR, Bray F, et al (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer, 127, 2893-917.
- Claus EB, Risch N, Thompson WD (1994). Autosomal dominant inheritance of early-onset breast cancer: implication for risk prediction. *Semin Oncol Nurs*, **73**, 643-51.
- Diez O, Pelegri A, Gadea N, et al (2011). Novel *BRCA1* deleterious mutation (c.1949_1950delTA) in a woman of Senegalese descent with triple-negative early-onset breast cancer. *Oncology letters*, **2**, 1287-9.
- Dorum A, Hovig E, Trope C, Inganas M, Moller P (1999). Three percent of Norwe gian ovarian cancers are cause d by *BRCA1* 1675delA or 1135ins A. *Eur J Cancer*, **35**, 779-81.
- Fisher B, Costantino JP, Wickerham DL, et al (1998). Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Project P-1 Study. J

- Natl Cancer I, 90, 1371-88.
- Fodor FH, Weston A, Bleiweiss IJ, et al (1998). Frequency and carrier risk associated with common *BRCA1* and *BRCA2* mutations in Ashkenazi Jewish breast cancer patients. *Am J Hum Genet*, **63**, 45-51.
- Frank TS, Deffenbaugh AM, Reid JE, et al (2002). Clinical characteristics of individuals with germline mutations in *BRCA1* and *BRCA2*: analysis of 10,000 individuals. *J Clin Oncol*, **20**, 1480-90.
- Fattahi MJ, Mojtahedi Z, Karimaghaee N, et al (2009). Analysis of *BRCA1* and *BRCA2* mutations in southern Iranian Breast cancer patients. *Archives of Iranian medicine*, **12**, 584-7.
- Futreal PA, Liu Q, Shattuck-Eidens D, et al (1994). BRCA1 mutations in primary breast and ovarian carcinomas. Science, 266, 120-2.
- Ghaderi A, Talei A, Farjadian S,et al (2001). Germline *BRCA1* mutations in Iranian women with breast cancer. *Cancer Letters*, **165**, 87-94.
- Goss PE, Ingle JN, Ales-Martinez JE, et al (2011). Exemestane for breast-cancer prevention in postmenopausal women. *New Engl J Med*, **364**, 2381-91.
- Hajian-Tilaki K, Kaveh-Ahangar T, Hajian-Tilaki E (2012). Is educational level associated with breast cancer risk in Iranian women? *Breast cancer (Tokyo, Japan)*, **19**, 64-70.
- Hall MJ, Reid JE, Burbidge LA, et al (2009). *BRCA1* and *BRCA2* mutations in women of different ethnicities undergoing testing for hereditary breast-ovarian cancer. *Cancer*, **115**, 2222-33.
- Harirchi I, Karbakhsh M, Montazeri A, et al (2010). Decreasing trend of tumor size and downstaging in breast cancer in Iran: results of a 15-year study. *Eur J Cancer Prev*, **19**, 126.
- Harirchi I, Ebrahimi M, Zamani N, Jarvandi S, Montazeri A (2000). Breast cancer in Iran: a review of 903 case records. *Public Health*, **114**, 143-5.
- 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**, 93-7.
- Harirchi I, Karbakhsh M, Kashefi A, Momtahen AJ (2004). Breast cancer in Iran: results of a multi-center study. Asian pacific J cancer prevention, 5, 24-7.
- Hussain SK, Altieri A, Sundquist J, Hemminki K (2008). Influence of education level on breast cancer risk and survival in Sweden between 1990 and 2004. *Int J Cancer*, **122**, 165-9.
- Irwin JJ (2008). Community benchmarks for virtual screening. *J Comput Aided Mol Des*, **22**, 193-9.
- Johannesdottir G, Gudmundsson J, Bergthorsson JT, et al (1995). High prevalence of the 999del5 mutation in Icelandic breast and ovarian cancer patients. *Cancer Res*, **56**, 3663-5.
- Kauff ND, Barakat RR (2007). Risk-reducing salpingooophorectomy in patients with germline mutations in *BRCA1* or *BRCA2*. J Clin Oncol, 25, 2921-7.
- Keshavarzi F, Javadi GR, Zeinali S (2012). BRCA1 and BRCA2 germline mutations in 85 Iranian breast cancer patients. Familial Cancer, 11, 57-67.
- Kurian AW (2010). BRCA1 and BRCA2 mutations across race and ethnicity: distribution and clinical implications. Curr Opin Obstet Gyn, 22, 72-78.
- Lalloo F, Evans DG (2012.) Familial breast cancer. *Clin Genet*, **82**, 105-14.
- Lavie O, Narod S, Lejbkowicz F, Dishon S, et al (2011). Double heterozygosity in the *BRCA1* and *BRCA2* genes in the Jewish population. *Ann Oncol*, **22**, 964-6.
- Levin B, Lech D, Friedenson B (2012). Evidence that *BRCA1*/2 associated cancers are not inevitable. *Mol Med (Cambridge, Mass)*, **18**, 1327-37
- Levy-Lahad E, Catane R, Eisenberg S, Kaufman B, et al (1997).

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Founder *BRCA1* and *BRCA2* mutations in Ashkenazi Jews in Israel: frequency and differential penetrance in ovarian cancer and in breast-ovarian cancer families. *Am J Hum Genet*, **60**, 1059-67.

Liede A, Narod SA (2002). Hereditary breast and ovarian cancer in Asia: genetic epidemiology of *BRCA1* and *BRCA2*. *Hum Mutat*, **20**, 413-24.

Lostumbo L, Carbine NE, Wallace J (2010). Prophylactic mastectomy for the prevention of breast cancer. *Cochrane Database Syst Rev*, **11**, 2748.

Lynch HT, Krush AJ (1971). Carcinoma of the breast and ovary in three families. *Surg Gynecol Obste*, **133**, 644-8.

Malone KE, Daling JR, Doody DR, et al (2006). Prevalence and predictors of *BRCA1* and *BRCA2* mutations in a populationbased study of breast cancer in white and black American women ages 35 to 64 years. *Cancer Res*, **66**, 8297-308.

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 Mol*, **2**, 123-7.

Menkiszak J, Gronwa ld J, Gorski B, et al (2003). Hereditary ovarian cancer in Poland. *Int J Cancer*, **106**, 942-5.

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.

Moore MA, Eser S, Igisinov N, et al (2010). Cancer epidemiology and control in North-Western and central Asia-past, present and future. *Pancreas*, **4**, 0.7-1.9.

Mousavi SM, Gouyam M, Ramazani R, et al (2009). Cancer incidence and mortality in Iran. *Ann Oncol*, **20**, 556-63.

Mousavi SM, GoyamM, Ramazani R, et al (2008). National Cancer Registry Report 2005-2006. Tehran, Iran: Ministry of Health, Deputy for Health Directory, CDC Cancer Office.

Mousavi SM, Mohagheghi MA, Mousavi-Jerrahi A, et al (2008). Outcome of breast cancer in Iran: a study of Tehran cancer registry data. *Asian Pac J Cancer Prev*, **9**, 275-8.

Mousavi SM, Mohaghegghi MA, Mousavi-Jerrahi A, et al (2006). Burden of breast cancer in Iran: a study of the Tehran population based cancer registry. *Asian Pac J Cancer Prev*, 7, 571-4.

Mousavi SM, GoyamM, Ramazani R, et al (2007). National Cancer Registry Report 2004-2005. Tehran, Iran: Ministry of Health, Deputy for Health Directory, CDC Cancer Office.

Naghavi M (2003). Epidemiology of Mortality in 18 Provinces of Iran 2001. Tehran: Ministry of Health, Deputy to Health Directory, Research and development office.

Naghavi M (2005). Epidemiology of Mortality in 23 Provinces of Iran 2003. Tehran: Ministry of Health, Deputy to Health Directory, Research and development office.

Nagy R, Sweet K, Eng C (2004). Highly penetrant hereditary cancer syndromes. *Oncogene*, **23**, 6445-70.

Nelson-Moseke AC, Jeter JM, et al (2012). An unusual BRCA mutation distribution in a high risk cancer genetics clinic. *Familial Cancer*, **12**, 83-7.

Neuhausen SL (2000). Founder populations and their uses for breast cancer genetics. *Breast Cancer Research*, 2, 77-81.

Newman B, Austin MA, Lee M, et al (1998). Inheritance of human breast cancer: Evidence for autosomal dominant t ransmission in high-risk families. *Proc Natl Acad Sci USA*, 85, 3044-8.

Nomizu T, Matsuzaki M, Katagata N, et al (2012). A case of familial breast cancer with double heterozygosity for *BRCA1* and *BRCA2* genes. Breast cancer (Tokyo, Japan).

Orita M, Suzuki Y, Sekiya T, Hayashi K (1989). Rapid and sensitive detection of point mutations and DNA polymorphisms using the polymerase chain reaction. *Genomics*, **5**, 874-9.

Pietschmann A, Mehdipour P, Mehdipour P, et al (2005).

Mutation analysis of *BRCA1* and *BRCA2* genes in Iranian high risk breast cancer families. *J Cancer Res Clin*, **131**, 552-8.

- Peto J, Mack TM (2000). High constant incidence in twins and other relatives of women with breast cance. *Nat Genet*, **26**, 411-4.
- Phelan CM, Kwan E, Jack E, et al (2002). A low frequency of non-founder *BRCA1* mutations in Ashkenazi Jewish breastovarian cancer families. *Hum Mutat*, 20, 352-7.

Quinn GP, Vadaparampil ST, Bower B, et al (2009). Decisions and ethical issues among BRCA carriers and the use of preimplantation genetic diagnosis. *Minerva Med*, 100, 371-83.

Roa BB, Boyd AA, Volcik K, et al (1996). Ashkenazi Jewish population frequencies for common mutations in *BRCA1* and *BRCA2*. Nat Genet, 14, 185-7.

Ramus SJ, Harrington PA, Pye C, et al (2007). Contribution of BRCA1 and BRCA2 mutations to inherited ovarian cancer. Hum Mutat, 28, 1207-15.

Rezaianzadeh A, Peacock J, Reidpath D, et al (2009). Survival analysis of 1148 women diagnosed with breast cancer in Southern Iran. *BMC Cancer*, **9**, 168-73.

Roy R, Chun J, Powell SN (2012). *BRCA1* and *BRCA2*: different roles in a common pathway of genome protection. *Nat Rev Cancer*, **12**, 68-78.

Robson ME, editor (2007). Treatment of hereditary breast cancer. Seminars in Oncology, [New York] Grune & Stratton.

Rusc A, Kaminski S (2007). Prevalence of complex vertebral. malformation carriers among Polish Holstein-Friesian bulls. *J Applied Genetics*, **48**, 247-52.

Sabokbar T, Khajeh E, Taghdiri F, et al (2012). Familial Breast Cancer Registry Program in Patients Referred to the Cancer Institute of Iran. Asian Pac J Cancer Prev, 13, 2675-9.

Sadjadi A, Sadjadi M, Mohagheghi MA (2005). Cancer occurrence in Iran in 2002, an international perspective. *Asian Pac J Cancer Prev*, 6, 359-63.

Sagi M, Weinberg N, Eilat A, et al (2009). Preimplantation genetic diagnosis for *BRCA1*/2-a novel clinical experience. *Prenat Diagn*, 29, 508-13.

Saleh-gohari N, Mohammadi-Anaie M, Kalantari-Khandani B (2012). BRCA1 Gene Mutations in Breast Cancer Patients From Kerman Province, Iran. Iranian J Cancer Prevention, 5, 210-5.

Schwartz GF, Hughes KS, Lynch HT, et al (2008). Proceedings of the international consensus conference on breast cancer risk, genetics, & risk management, April, 2007. *Cancer*, **113**, 2627-37.

Shamsa AZ, Mohagheghi MA (2002). Final report of the National project for cancer registry. Tehran, Iran.

Sokolenko AP, Rozanov ME, Mitiushkina NV, et al (2007). Founder mutations in early-onset, familial and bilateral breast cancer patients from Russia. *Fam Cancer*, **6**, 281-6.

Struewing JP, Hartge P, Wacholder S, et al(1997). The risk of cancer associated with specific mutations of *BRCA1* and *BRCA2* among Ashkenazi Jews. *N Engl J Med*, 336, 1401-8.

Sogaard M, Kruger Kjaer S, Cox M, et al (2008). *BRCA1* and *BRCA2* mutation prevalence and clinical characteristics in an ovarian cancer case population from Denmark clin. *Cancer Research*, **14**, 3761-7.

Taghavi A, Fazeli Z, Vahedi M, et al (2012). Increased trend of breast cancer mortality in Iran. Asian Pac J Cancer Prev, 13, 367-70.

Thorlacius S, Olafsdottir G, Tryggvadottir L, et al (1996). A single *BRCA2* mutation in male and female breast cancer families from Iceland with varied cancer phenotypes. *Nat Genet*, **13**, 117-9.

Van Der Groep P, Bouter A, van der Zanden R, et al (2006).

Distinction between hereditary and sporadic breast cancer on the basis of clinicopathological data. *J Clin Pathol*, **59**, 611-7.

- Van Der Looij M, Szabo C, Besznyak I, et al (2000). Preval ence of founder BRCA1 and BRCA2 mutations among breast and ovarian cancer patients in Hungary. Int J Cancer, 86, 737-40.
- Visvanathan K, Chlebowski RT, Hurley P et al (2009). American society of clinical oncology clinical practice guideline update on the use of pharmacologic interventions including tamoxifen, raloxifene, and aromatase inhibition for breast cancer risk reduction. *J Clin Oncol*, **27**, 3235-58.
- Wooster R, Neuhausen SL, Mangion J, et al (1994). Localizatio<u>100.0</u> of a b reast cancer susceptib ility gene, *BRCA2*, to chromosome 13q12-13. *Science*, **256**, 2088-90.
- Wooster R, Bignell G, Lancaster J, et al (1995). Identification of the breast cancer susceptibility gene *BRCA2*. *Nature*,**75.0 378**, 789-92.
- Wang F, Fang Q, Ge Z, et al (2012). Common *BRCA1* and *BRCA2* mutations in breast cancer families: a meta-analysis from systematic review. *Mol Biol Rep*, **39**, 2109-18. **50.0**
- Wu LC, Wang ZW, Tsan JT et al (1996). Identification of a RING protein that can interact in vivo with the *BRCA1* gene product. *Nat Genet*, **14**, 430-40.
- Xu J, Wang B, Zhang Y, et al (2012). Clinical implications for 25.0 BRCA gene mutation in breast cancer. *Mol Biol Rep*, **39**, 3097-102.

0

- Yassaee VR, Zeinali S, Harirchi I, et al (2002). Novel mutations in the *BRCA1* and *BRCA2* genes in Iranian women with earlyonset breast cance. *Breast Cancer Res Tr*, **4**, 6.
- Zhu X, Niu N, Liu Y, et al (2006). Improvement of the sensitivity and resolution of PCR-SSCP analysis with optimized primer concentrations in PCR products. J Genetics, **85**, 233-5

