RESEARCH ARTICLE

Mutation Spectra of BRCA Genes in Iranian Women with Early Onset Breast Cancer - 15 Years Experience

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Abstract

Breast cancer is the most common cancer in Iran. In the recent years an upward trend has been observed in the Iranian population. Early detection by molecular approaches may reduce breast cancer morbidity and mortality. We provided consultation to 3,782 women diagnosed with early onset breast cancer during the past 15 years (1999-2014). To establish a data set for BRCA gene alterations of the Iranian families at risk, two hundred and fifty four women who met our criteria were analyzed. A total number of 46 alterations including 18 variants with unknown clinical significance (39.1%), 18 missense mutations (39.1%), 7 Indels (15.2%) and 3 large rearrangement sequences (6%) were identified. Further scanning of affected families revealed that 49% of healthy relatives harbor identical causative mutations. This is the first report of comprehensive BRCA analysis in Iranian women with early onset breast cancer. Our findings provide valuable molecular data to support physicians as well as patients for the best decision making on disease management.

Keywords: Breast cancer - BRCA1 - BRCA2 - genetic testing - mutation - disease management

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Introduction

Breast cancer is the most frequent invasive cancer comprising 16% of all cancers in women in both developed and under developed countries. Being a woman is an enormous contributing factor to develop BC with a life time risk of 10% in the general population (Easton 1997). Internationally BC is more frequent in developed countries due to the predominance of risk factors such as age and reproductive fashion, social economy, and diet (Parkin et al., 1997). It is the leading cause of cancer death in women with an estimate of 1.67 million (25% of all cancers) new cases diagnosed in 2012 (IARC 2012).

Data revealed a rapid upward trend in BC incidence in traditionally low frequency Asian countries as a consequence of altered living standards and adaptation of western lifestyles (Wilson et al., 2004). Over half of the breast cancer cases are estimated to occur in more industrialized countries, 788,200 (ASR=73.4/100,000) compared to less developed countries with 882,949 (ASR= 31.3/100,000). In general BC rate is higher in North America, Australia and Europe with mean average of 91.6, 86 and 84.8 per 100,000 respectively and moderate in South America (Mean= 51.6) and South Asia (Mean= 45.9) whereas Africa and central Asia show the least level of BC incidence with mean of 35.6 and 37.3 respectively (IARC, 2012). The increase of BC incidence in western countries observed in late 1980s and 1990s is likely to be the result of changes in lifestyle and reproductive factors (Althuis et al., 1973-1997). In contrast, mortality rate of BC has been decreasing in North America and several European countries over the past two decades as a result of early detection and improved treatments (Jemal et al., 2010). Death rates, however, have been rising in many African and Asian countries with changes in reproductive pattern, physical activity and obesity (Parkin et al., 2005) (Figure 1). Breast cancer incidence is globally on the rise and it is swiftly striking less wealthy countries as a result of adoption of lifestyle changes that impact the prevalence of the disease (McTiernan 2003). Breast Cancer is the most common female cancer in the Middle East increasing up to 5% each year (Kahan et al., 1997; Anderson 2006). The incidence of BC is roughly lower in Iranian population with 28.1 affected per 100,000 in comparison to the breast cancer in Asia and neighboring countries; 39.1 per 100,000 in Turkey and 50.3 per 100,000 in Pakistan (IARC 2012) (Table 1), however its rapid upward trend has become a national burden.

According to the CDC reports in Iran, breast cancer strikes women with a mean age of 48.8 years (Harirchi et al., 2004) where over 30% of malignancies occur below the age of 40 (Mousavi et al., 2006; National Cancer

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Registry report, 2004). Studies have shown that religious influence on lifestyle behaviors such as reproductive style, early age at first full-term pregnancy, higher number of births and more years of breast-feeding can extremely reduce the rate of female BC incidence (Merrill and Folsom, 2005; Sadjadi et al., 2009). Asians constitute 60% of the world population; hence, determination of risk of BC and mutation spectrum of the high penetrance genes can contribute to the better understanding and adoption of effective genetic testing (Kwong et al., 2015). The present retrospective cohort study aimed to identify the spectrum of BRCA mutations/variants in Iranian women with early onset BC during the past 15 years.

Material & Methods

Subjects

Our previous study led to identification of BRCA germline mutations in 39 Iranian women with early onset breast cancer (Yassaee et al., 2002). Here we have expanded our investigations to obtain a BRCA data set of alterations in Iranian women with familial BC. During the past 15 years (1999-2014). 3,782 women who were diagnosed with breast and/or ovarian cancer (HBOC) referred to our center from hospitals throughout the country for consultation. Two hundred and fifty four patients who met the criteria were selected for BRCA analysis. The selection criteria included age ≤ 45 with a history of at least two affected first degree relatives, bilateral BC, male BC in the family and BC followed by ovarian cancer. The study was conducted in accordance to the NICE guidelines and was approved by ethical committee of deputy of research affairs of Shahid Beheshti University of Medical Sciences (SBUMS) (grant number 9293SBMU-931485). Following the declaration of Helsinki, whole blood samples were obtained from the subjects after informed and written consent was acquired. Genetic test results were provided to the patients upon request.

Genetic Analysis

Genomic DNA was prepared and BRCA analysis were performed for all coding exons and flanking intron and UTR sequences by single strand conformation polymorphism (SSCP), protein truncation test (PPT), multiplex ligation-dependent probe amplification (MLPA), and Sanger sequencing techniques. Results were analyzed by Finch TV 1.4 software. Primer sequences and PCR conditions are available on request.

Results

A total of 46 BRCA alterations were identified in this cohort study including 18 variants with unidentified clinical significance (39.13%), 18 missense mutations (39.13%) and 7 indel mutations (15.21%). Fifty BRCA negative patients were examined for LGR using MLPA whereby 3 of them showed a large deletion (Tables 2 and 3).

Table 1 . Incidence of Breast Cancer in the World in All Age Groups (GLOBOCAN 2012, IARC)

Region	Country	Case	ASI rate per 100,000	Mean
North America	USA	256,222	91.6	91.6
	Canada	23,420	79.8	51.6
	Mexico	20,444	35.4	
South America	Colombia	8,686	35.7	
	Peru	3,952	28	
	Brazil	67,316	59.5	
	Argentina	19,386	71.2	
Europe	UK	52,399	95	84.8
	Iceland	225	96.3	
	Spain	25,215	67.3	
	Sweden	6,624	80.4	
	Finland	4,477	89.4	
	Norway	2,887	73.1	
	Germany	71,623	91.6	
	Italy	50,658	91.3	
	France	48,763	89.7	
	Denmark	5,224	105	
	Belgium	10,337	111.9	
	The Netherland	13,895	99	
	Poland	17,259	51.9	
	Belarus	3,781	45.9	
	Russian Federation	57,502	45.6	37.3
Asia	Kazakhstan	6,252	63	
	Turkmenistan	656	26.8	
	Turkey	15,230	39.1	
	India	144,937	25.8	
	China	187,213	22.1	
	Iran	9,795	28.1	
	Pakistan	34,038	50.3	
	Afghanistan	3,108	35	
	Saudi Arabia	2,791	29.6	35.6
Africa	Egypt	18,660	49.5	
	Sudan	3,439	27.8	
South Asia	Korea	17,140	52.1	45.9
	Japan	55,710	51.5	
	Philippine	18,327	47	
	Malaysia	5,410	38.7	
	Indonesia	48,998	40.3	
Australia	Australia	14,710	86	86

ASI (ASR) indicates age standard rate per 100,000. Rates are standardized in accordance with the world population. Highlighted countries depict incidence of BC in comparison to Iran.

Table 2. Germline Alterations in BRCA1/2 Genes

Location	cDNA Nomenclature NM_007294.3	Nucleotide Change	AA change NP_009225.1	Variation class	SNP (dbSNP)
Exon 2	c.66_67delAG	Del AG	p.Glu23Valfs*17	FS	----
	c.441+36_441+38delCTT	Del CTT	----	Intronic	rs147,856,441
Intron 7	c.441+52_441+63 delCTTTTTTTTTTT	Del CTTTTTTTTTTT			rs536,390,258
Exon 11	c.1067A>G	A>G	p.Gln356Arg	MS	rs1,799,950
Intron 10	c.671-248_671-246dup	Dup AGG	----	Intronic	----
Exon 11	c.1568delT	Del T	p.Leu523Trpfs*9	FS	----
Exon 11	c.1684-1685 dup A	Dup A	p.Ile562Asnfs*9	FS	
Exon 11	c.2082C>T	C>T	p.Ser694=	Silent	rs1,799,949
Exon 11	c.2216_2217delAA	Del AA	p.Lys739Serfs*3	FS	----
Exon 11	c.2311T>C	T>C	p.Leu771=	Silent	rs16,940
Exon 11	c.2612C>T	C>T	p.Pro871Leu	MS	rs799,917
Exon 11	c.2649_2650insGGCA	Ins GGCA	p.Thr884Glyfs*20	FS	----
Exon 11	c.3113A>G	A>G	p.Glu1038Gly	MS	rs16,941
Exon 11	c.3548A>G	A>G	p.Lys1183Arg	MS	rs16,942
Exon 11	c.3748G>A	G>A	p.Glu1250Gln	MS	rs28,897,686
Intron 12	c.4097-141A>C	A>C	----	Intronic	rs799,916
Exon 13	c.4308T>C	T>C	p.Ser1436=	Silent	rs1,060,915
Exon 15	c.4609C>T	C>T	p.Gln1537X	NS	----
Exon 16	c.4837A>G	A>G	p.Ser1613Gly	MS	rs1,799,966
Intron 17	c.5074+65G>A	G>A	----	Intronic	rs8,176,235
Intron 17	c.5074+284C>A	C>A	----	Intronic	rs11,654,396
Intron 18	c.5152+66G>A	G>A	----	Intronic	rs3,092,994
Intron 20	c.5277+59_5277+60 dup.GTATTCCACTCC	dup.GTATTCCACTCC	----	Intronic	rs572,766,355

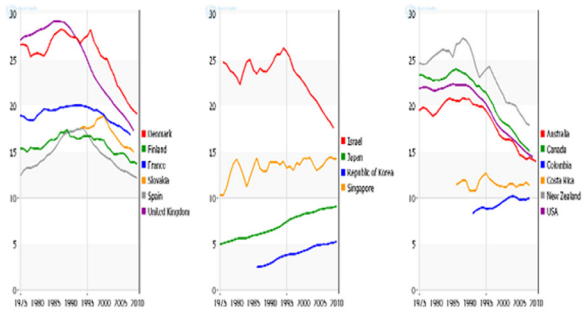

Figure 1. Trends in Female Breast Cancer Mortality Rates Per 100,000 (GLOBOCAN 2012, (IARC)

Discussion

Although the incidence of BC is lower in Iranian women compared to other Asian countries, based on data reported by the National Cancer Center, it is still a considerable health issue with an annual growth of 10%. Incidence of breast cancer in Iran has risen since 1999 and currently it is the most adequate malignancy and the fifth most common form of death among Iranian women. In addition to living standards and environmental factors, genetic factors also significantly contribute to the odds of developing cancer. Women with family history of BC are at an estimated two fold risk of developing breast cancer whereby the risk progressively increases with the number of affected relatives. Majority of hereditary BC cases are associated to two faulty BRCA1 and BRCA2 genes with 55-65% and 45% risk respectively (Easton 2002). When a deleterious alteration in one of the genes is inherited, women will have increased risk of developing breast and ovarian cancer (Antoniou A et al., 2003). Deleterious mutations in BRCA1 confer 60-85% lifetime risk of BC and 46-60% risk of ovarian cancer (OC) while BRCA2 mutations account for 40-85% lifetime risk of breast cancer (Lalloo and Evans, 2012). Thus far, over 500 types of various mutations have been defined in the Asian population with breast cancer (Kwong et al., 2015). The most prevalent mutations reported in the BRCA1 gene include c.68-69delAG, c.390C>A, c.470-471delCT and c.981-982delAT and c.7480C>T, c.1399A>T and c.3744-3747delTGAG in the BRCA2 gene. The genetic aberrations have been widely observed in Chinese, Japanese, Pakistani and Korean populations (Kwong et al., 2015). Despite significant advances in screening techniques, breast cancer evades conventional therapies generating disease relapse and claiming the lives of women worldwide.

To evaluate the risk of breast and ovarian cancers in Iran, we analyzed BRCA genes in patients who

Table 3. Germline Alterations in the BRCA2 Gene

Location	cDNA Nomenclature NM_000059.3	Nucleotide Change	AA change NP_000050.2	Variation class	SNP (dbSNP)	Biological significance
5'UTR	c.-26G>A	G>A	----	----	rs1,799,943	1-Neutral
Exon 4	c.331A>G	A>G	p.Asn111Asp	MS	----	3-Neutral
Intron 9	c.793+64 delT	Del T	----	Intronic	----	3-Neutral
Exon 10	c.865A>C	A>C	p.Asn289Asp	MS	rs766,173	1-Neutral
Exon 10	c.1,114A>C	A>C	p.Asn372His	MS	rs144,848	1-Neutral
Exon 11	c.2,971A>G	A>G	p.Asn991Asp	MS	rs1,799,944	Polymorphism
Exon 11	c.3,396A>G	A>G	p.Lys1132=	Silent	rs1,801,406	1-Neutral
Exon 11	c.3,807T>C	T>C	p.Val1269=	Silent	rs543,304	Polymorphism
Exon 11	c.3,751dup	Dup A	p.Thr1251Asnfs*14	FS	----	NR (causative)
Exon 11	c.5,081G>T	G>T	p.Arg1694Ile	MS	----	NR
Exon 11	c.5,555T>A	T>A	p.Val1852Asp	MS	rs483,352,930	NR
Exon 11	c.5,972C>T	C>T	p.Ala1991Val	MS	rs80,358,829	NR
Exon 11	c.6,261_6,262insGT	Ins GT	p.Thr2088Valfs*32	FS	----	NR (causative)
Exon 11	c.6,513G>C	G>C	p.Val2171=	Silent	rs206,076	1-Neutral
Intron 11	c.6,841+80_6841+83 delTTAA	Del TTAA	----	Intronic	rs11,571,661	3-UV
Exon 14	c.7,242A>G	A>G	p.Ser2414=	Silent	rs1,799,955	1-Neutral
Exon 14	c.7,397C>T	C>T	p.Ala2466Val	MS	rs169,547	1-Neutral
Intron 16	c.7,806-14T>C	T>C	----	Intronic	rs9,534,262	1-Neutral
Exon 18	c.8,117A>G	A>G	p.Asn2706Ser	MS	----	3-UV
Exon 20	c.8,490G>A	G>A	p.Trp2830X	NS	----	NR (causative)
Exon 22	c.8,851G>A	G>A	p.Ala2951Thr	MS	rs11,571,769	1-Neutral
Exon 23	c.9,266C>T	C>T	p.Pro3089Leu	MS	----	NR
Exon 27	c.9,827G>A	G>A	p.Arg3276Lys	MS	----	NR
Exon 27	c.9,976A>T	A>T	p.Lys3326X	NS	----	NR (causative)

MS, Missense Mutation; NS, Nonsense Mutation; FS, Frameshift mutation; NR, Not Reported in UMD; Classification of BRCA1/2 alterations based on formerly UMD mutation database; Class 1, Neutral, neutral polymorphism; Class 2, Likely neutral, Contradictory neutral/UV; Class 3, Unknown/unclassified variant (UV); Class 4: Likely deleterious, Contradictory deleterious/UV; Class 5: Deleterious mutation

referred to our clinic. The alterations consisting indel/duplication lead to large genomic rearrangement (LGR) undetectable by common screening assays. Therefore, MLPA was implemented as a robust and sensitive method for detection of the LGRs in patients negative for BRCA mutations asserted by Sanger sequencing. Among 254 patients who met the increased risk criteria, mutations in BRCA1/2 were detected in 18% of the patients comprising 39.13% missense, 15.21% indel and 39.13% unknown clinical significant alterations (Table 2 a, b). We have also demonstrated that LGRs encompass a significant fraction of 6% of all substantial mutations in the BRCA1 gene (Yassaee et al., 2013). These findings suggest that early onset and high risk clinical history were efficient criteria for investigation of patients possibly harbor faulty genes. Germline mutations in BRCA1/2 are significant prognosticators of breast and ovarian cancers presenting variable prevalence and penetrance among ethnic groups (Fackenthal and Olopade, 2007). Mutations in the BRCA genes are expected to increase the risk of breast cancer in women. However, BRCA negative women from BRCA families are at higher risk of developing breast cancer than the general population as a result of SNP variations that are associated with breast cancer (Evans et al., 2013). It is noteworthy to accentuate that mere dependence on BRCA mutations is a poor judgment of a woman's future cancer risk while she may still have a significant risk of developing a new primary cancer (Pohlreich et al., 2005). Risk assessment of BC for family members, in addition to a three generation pedigree also confides to the integration of new genomic techniques and prognostic profiles for early detection and accurate diagnosis of BC in terms of suitable treatment and better quality of life. Management of hereditary/familial BC varies from sporadic tumors. In 2004, UK National Institute for Clinical Excellence (NICE) issued a protocol for managing hereditary/familial breast cancer which aims to classify women on the basis of high, moderate and average risk of BC (McIntosh et al., 2004). The amalgamation of clinical features and molecular techniques has led to the stratification of breast cancer types. These approaches can complement the present diagnostic tests and aid in better understanding of molecular basis of BC.

In summary, incidence of BC is globally divergent among countries and various ethnic groups. The present study represents the largest BRCA1/2 mutation analysis

in Iranian women with early onset BC. The targeted patient cohorts comprise probabilities of carrying a wide range of mutations. We documented a variety of alteration demonstrating the framework for assessing the overall importance of BRCA1/2 analysis. Our findings not only provide a data set of BRCA alterations in high risk Iranian families with breast cancer, but also update and contribute to the present BC data set reported by Kwong et al in 2015. The overall knowledge of BRCA1/2 mutations will assist in genetic counseling and ultimately in the disease management of patients who need them the most.

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