BRCA1 and BRCA2 mutations in breast cancer patients from Saudi Arabia

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ABSTRACT

Objectives: The aim of this pilot study was to screen the major segments of the BRCA1 and BRCA2 genes for disease-associated mutations in Arab and Asian women with breast cancer from the Kingdom of Saudi Arabia.

Methods: Deoxyribonucleic acid samples from 29 Arab women and 11 Asian women, with unilateral breast cancer were investigated for BRCA1 and BRCA2 mutations. For this purpose single strand conformation polymorphism and direct nucleotide sequencing techniques were employed. This study was carried out at King Fahad Hospital of the University, Al-Khobar, Kingdom of Saudi Arabia, during the time frame March 2000 through to August 2001.

Results: One novel BRCA2 truncating mutation, the frame-shift mutation 2482delGACT, was uncovered in an Arab patient of Palestinian descent. This mutation is a 4-nucleotide deletion that creates a stop signal at codon 770 of the BRCA2 transcript. The BRCA1 disease-associated mutation Arg841Trp was detected in another Arab patient from Egypt. The clinical presentation in the 2 heterozygous carriers of these 2 mutations is described here. In addition the unclassified BRCA1 variant Phe486Leu combined with Asn550His, and the unclassified BRCA2 variant Asp1420Tyr, were identified in Arab patients. Five BRCA1 polymorphisms and 6 BRCA2 polymorphisms were detected at different allele frequencies in both mutation carriers and patients with normal genotype.

Conclusion: We conclude that BRCA1 and BRCA2 mutations are likely to contribute to the pathogenesis of familial breast cancer in female patients from the Kingdom of Saudi Arabia.

Keywords: BRCA1, BRCA2, breast cancer.

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Intense research work on the inherited susceptibility to breast cancer in the last decade has cumulated in the identification of BRCA1 and BRCA2 genes as autosomal dominant susceptibility genes for familial breast cancer. Mutations in these tumor suppressor genes have been shown to account for a high proportion of the inherited type of breast cancer.[1,2] Work is still continuing for elucidation of the mutation spectrum of BRCA1 and BRCA2 in specific populations[3] and for identification of their potential function.[4] A classical example for population-specific mutations is the report that the founder mutations 185delAG and 5382insC in BRCA1 and 6174delT in BRCA2 appear in 60% of ovarian cancer and 30% of early-onset breast cancer patients among Ashkenazi women.[5] Another example is the recent study determining the frequency of deleterious mutations; 15 germline mutations in BRCA1 and 21 in BRCA2, in Japanese breast cancer families. In this study, the cumulative frequency of BRCA1 and BRCA2 mutations amounted to a similar range as observed in Caucasian breast cancer families.[6]

The role of BRCA1 and BRCA2 gene mutations in breast/ovarian tumorigenesis and evaluation of the

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benefits and limitations of molecular testing for these cancers have drawn the attention of many researchers in recent years.[1,2,7,8] Genetic testing for predisposition to malignancy seems to be particularly useful in families where high risk of cancer is known. In these families, testing for the molecular lesions in the 2 genes is favoured as it generates useful information for genetic counselling and it bears potential diagnostic value and potential clinical benefits. The majority of the defined pathologic mutations are frame-shift and nonsense mutations that result in premature truncation of proteins.[9] These mutations may substantially increase the risk for breast and ovarian cancer, but a precise risk estimate for each mutation is difficult to determine due to diversity of BRCA1 and BRCA2 mutations and lack of general population data. Generally, families with multiple affected first-degree relatives and patients with early onset disease have been found to harbor mutations at a higher frequency than in the general population. It is estimated that 3% to 8% of all women with breast cancer will be found to carry a mutation in either BRCA1 or BRCA2 gene.[10] Depending on the familial context, the risk of breast cancer associated with carrying a mutation has been estimated to range from 50% to 85%, whereas the role of these genes in sporadic cancer remains less defined.[10] The penetrance of BRCA1 and BRCA2 mutations may be modified by environmental factors. At the cellular level, BRCA1 and BRCA2 genes code for large proteins that appear to act as tumour suppressor genes, and play a role in the maintenance of genome integrity.[3] Expression and activity of BRCA1 and BRCA2 in human breast cancer cells is regulated by deoxyribonucleic (DNA)-damaging agents such as topoisomerase inhibitors, ionising radiation, and ultraviolet radiation.[3]

Analysis of recent data on breast/ovarian cancers among the female population of the Kingdom of Saudi Arabia (KSA) has shown that these malignancies are characterized by low onset age and a tendency of diagnosis at late stages of malignancy.[7] Breast cancer is the most common malignancy among women living in KSA reaching 18.8% of the total cancer cases.[8] The aim of this pilot study was to analyse the major segments of BRCA1 and BRCA2 genes in the search for specific mutations associated with breast cancer in women from the KSA. The topic of genetic predisposition to breast cancer in the Saudi community still remains unstudied. However, the patterns of allelic loss at the BRCA1 locus in Arabic women with breast cancer have recently been investigated. By determining loss of heterozygosity (LOH) and microsatellite instability (MSI), it was suggested that the proportion of BRCA1 aberrations could be higher in Arab women than in other populations studied to date.[11] To the best of our knowledge the present report is the first to demonstrate the presence of BRCA1 and BRCA2 mutations in patients with breast cancer from the KSA.

Methods. The patients of this hospital based pilot investigation represent an unselected cohort of patients, as they were routinely seen in a clinic at King Fahad Hospital of the University (Al-Khobar, KSA). This study was carried out during the period March 2000 through to June 2001. These were 29 Arab women (from KSA, Republic of Yemen, Bahrain, Sudan, Egypt, Palestine, Morocco and Lebanon), and 11 Asian women (from Pakistan, India, Sri Lanka, Philippines, and Indonesia) who had been diagnosed to have unilateral breast cancer. The carcinomas were at either the localised (30 patients) or regional (10 patients) stage of malignancy, as investigated by mammography, fine needle aspiration cytology and histopathology. The mean age of patients at diagnosis was 40.6 years (33 of 40 women were below 50 years), in consistence with the previously observed age at diagnosis in the general population of this country.[7] The medical history, and disease presentation was recorded. Nine of the patients had at least one blood relative with breast cancer, and 14 had relatives having other cancers.

Genomic DNA was extracted from leukocytes according to a standard salting-out procedure. Exon 11 of BRCA1 and exons 10 and 11 of BRCA2 genes were amplified by polymerase chain reaction (PCR) using different primers and applying different PCR conditions. The primer sequences have been published elsewhere.[12] Polymerase chain reaction products of 150 to 400bp in size, cut by restriction enzymes where necessary, were analysed by single strand conformation polymorphism (SSCP) analysis.[13] The PCR products with aberrant migration on SSCP gels were further analysed by nucleotide sequencing. An ABI d-Rhodamine Terminator Cycle Sequence Kit (Applied Biosystems, United States of America, USA) and suitable sequencing primers were used to perform the sequencing reactions. The products were purified and then analyzed using an ABI 310 Genetic Analyzer (Applied Biosystems). Details of the analysis techniques are described by one of our group elsewhere.[12]

Results. ***BRCA1 and BRCA2 mutations.*** By nucleotide sequencing of BRCA1 and BRCA2 we were able to identify in one patient a novel BRCA2 (exon 11) frame-shift mutation 2482delGACT. This mutation is a 4-nucleotide deletion that creates a stop signal at codon 770 of the BRCA2 transcript **(Figure 1).** The breast cancer-associated mutation Arg841Trp of BRCA1 (exon 11) was detected in another patient. The BRCA1 unclassified variant Phe486Leu combined with the variant Asn550His, and BRCA2 unclassified variant Asp1420Tyr were also detected

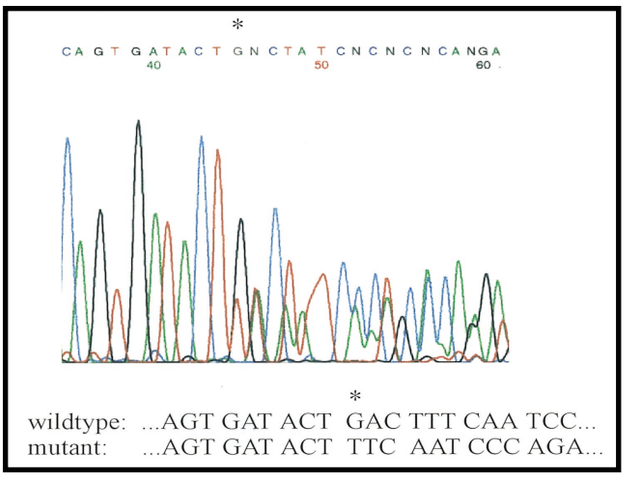

Figure 1 - Identification of the new frame-shift 2482delGACT mutation, a 4 nucleotide deletion in exon 11 of the BRCA2 gene, detected in a breast cancer patient in the heterozygous state. Wildtype and mutant sequences are written below the chromatogram. An asterisk marks the start of the deletion. A - adenine, G - guanine, T - thymine, C - cytosine.

in 2 further patients. These mutations and variants were detected in the heterozygous state in patients of Arabic origin. In addition, 5 BRCA1 frequent polymorphisms (2201C-->T, 2430T-->C, 2732C-->T, 3232A-->G and 3667A-->G) and 6 BRCA2 polymorphisms (1093A-->C, 1342A-->C, 3199A-->G, 3624A-->G, 4035T-->C and 5973C-->T) were detected in both Arab and Asian patients at similar allele frequencies **(Table 1).**

Clinical presentation of the heterozygous carrier of the BRCA2 truncation 2482delGACT. This is an Arab woman, of Palestinian descent, 68 years of age at diagnosis, a mother of 7 children whom she had breast fed for 2 years each. She occasionally used oral contraceptives. The family history was free from breast or any other type of cancer as reported by the patient. She presented with a right breast mass that had started as a painless lump, increased in size with time and persisted for one and half years prior to investigation. Change of skin colour was noticed with time but this was not associated with either nipple discharge or systemic complications. At investigation the mass was 6x5 cm, occupying the lower outer quadrant with evidence of skin infiltration. Two palpable mobile axillary nodes were observed during examination. The tumor was classified as a stage 3 loco-regional malignancy, and the histopathology results confirmed the diagnosis of a ductal epithelial carcinoma. The patient underwent mastectomy and axillary clearance.

Clinical presentation of the heterozygous carrier of the BRCA1 mutation Arg841Trp. This is an Arab woman, of Egyptian descent, 45 years at diagnosis, and a mother of 4 children whom she lactated for approximately 2 years each. Oral contraceptives were occasionally ingested. The patient's mother was diagnosed with breast cancer at the age of 65 years; her father had cancer of the prostate at the age of 78 years. The patient presented with left breast mass that had lasted for 2 months prior to investigation. At investigation the mass was 3x4cm, occupying the upper outer quadrant with no

Table 1 - BRCA1 and BRCA2 gene alterations in women from the Kingdom of Saudi Arabia with unilateral breast cancer.

Location	Nucleotide alteration	Amino acid change	Allele frequency Arab	Asian	Classification of variant	Reference
BRCA1 exon 11	2640C-->T	Arg841Trp	0.02	-	BC-associated mutation	Barker et al[15]
	1575C-->T	Phe480Leu	0.02	-	Unclassified	Myriad Genetics
	1767C-->T	Asn550His	0.02	-	variants	(BIC Database)
	2201C-->T	None	0.31	0.32	Polymorphism	Friedmann et al[16]
	2430T-->C	None	0.31	0.32	Polymorphism	Friedmann et al[16]
	2732C-->T	Pro871Leu	0.31	0.32	Polymorphism	Durocher et al[17]
	3232A-->G	Glu1038Gly	0.31	0.32	Polymorphism	Friedmann et al[16]
	3667A-->G	Lys1183Arg	0.31	0.32	Polymorphism	Neuhausen et al[18]
BRCA2 exon 10	1093A-->C	Asn289His	0.07	0.18	Polymorphism	Osorio et al[19]
	1342A-->C	Asn372His	0.02	0.09	Polymorphism	Tavtigian et al[20]
BRCA2 exon11	2482delGACT	Stop770	0.02	-	Truncating mutation	This study
	4486G-->GT	Asp1420Tyr	0.02	-	Unclassified varaint	Wagner et al[21]
	3199A-->G	Asn991Asp	0.02	0.09	Polymorphism	Tavtigian et al[20]
	3624A-->G	None	0.09	-	Polymorphism	Teng et al[22]
	4035T-->C	None	0.14	0.14	Polymorphism	Tavtigian et al[20]
	5973C-->T	Thr1915Met	0.03	0.05	Polymorphism	Couch et al[23]

evidence of skin infiltration, lymph node involvement or systemic complications. Both axillae were free from malignancy. The histology confirmed the diagnosis of ductal epithelial carcinoma of stage 2 (local). She received neoadjuvant chemotherapy that was followed by breast conserving surgery with prophylactic axillary clearance and radiotherapy.

Discussion. The cohort of patients in this pilot study comprised Arab and Asian patients living in KSA though originating from different countries. The apparent heterogeneity of the patients' origin is a reflection of the fact that the Saudi community includes a high percentage of expatriates reaching 26% of the population as reported by (Central Department of Statistics, 1992, KSA). Since the patients usually seen at our clinics comprise Arab and Asian women from different countries it was considered medically relevant to include all patients who consented to the study, despite their heterogeneous ethnic background. The fact that the topic of genetics of familial breast cancer in Arab and Asian populations has not been adequately studied to date represents an added reason for the random selection of patients from different countries.

It is observed that the mean age of onset of breast cancer among this group of patients (41 years) is identical to the figure previously reported for the Saudi community.[8] However, this age is much lower as compared with the onset age (63 years) of Caucasian patients with breast cancer as reported by Markus and co-workers.[14] Some support was obtained for the concept that the mean onset age in Caucasian patients with the familial type of the disease was lower compared with that of patients with the sporadic type.[14] It would be interesting to investigate existence of a similar trend among the female population of the Saudi community.

The novel frame-shift mutation 2482delGACT is a 4-nucleotide deletion that creates a stop signal at codon 770 of the BRCA2 transcript. This mutation is certainly an etiologically significant lesion since frame-shift mutations generating truncated proteins have been observed to be the most common types of pathogenic BRCA2 mutations (Breast Cancer Information Core database (**http://www.nhgri.nih.gov/Intramural-Research/Lab Transfer/Bic/**). However, the family history as reported by the heterozygous carrier of this mutation did not reveal another blood relative with breast carcinoma. A segregation analysis of this mutation in the family of the patient was not feasible due to the family members not being available for testing. Although direct linkage to breast cancer of the missense mutation Arg841Trp of BRCA1 has not yet been proven to be directly causative for breast cancer, Barker and colleagues[15] have obtained strong epidemiological evidence for its involvement with familial breast cancer among female American patients. This mutation has been detected here in the heterozygous state in an Egyptian woman. The relatively young age (45 years) at diagnosis of the patient may favor the likelihood of a familial type of the disease. Similar to the previous mutation, segregation analysis of this mutation deserves to be investigated.

The 2 unclassified variants Phe486Leu and Asn550His (BRCA1) were detected in a Yemeni patient (45 years) who has a malignancy-free family history. Both these substitutions have been detected in breast cancer patients from the USA (Myriad Genetics, BIC database), but no evidence has yet been obtained that they are associated with breast cancer, and hence they are still considered as unclassified variants. Furthermore, the BRCA2 variant Asp1420Tyr was detected here in a Saudi patient (34 years) who, likewise, has a cancer-free family history. This substitution was reported as a rare polymorphism (allele frequency=0.03) globally encountered and currently it is categorized as an unclassified variant.[15] The allele frequencies for the other BRCA1 and BRCA2 polymorphisms reported here were comparable in Arab patients from different countries and in patients from Asia with different ethnic origin, and there were no significant differences to published data from European countries.[12]

Although our pilot study was restricted at the present stage to the analysis of the largest exons of BRCA1 and BRCA2 (60% of the coding sequence), and by methodology would fail to detect large genomic rearrangements such as gross deletions in the heterozygous state, it already shows the existence of BRCA1 and BRCA2 mutations as disease-causing factors in the Saudi population. Forthcoming work is in progress by our group to sequence the complete BRCA1 and BRCA2 genes, identify further mutations, and explore the linkage of identified mutations to breast tumorigenesis.

We conclude that BRCA1 and BRCA2 mutations are likely to contribute to the pathogenesis of familial breast cancer in female patients from KSA. Furthermore, the results of this study and similar studies should facilitate the application of genetic testing of predisposition to breast cancer in women of the Saudi community.

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