BRCA 1 & 2 mutations in Sudanese secondary school girls with known breast cancer in their families.

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Abstract:

Objective: Breast cancer is a major cause of morbidity and mortality in women worldwide. In Sudan, it is the most commonly diagnosed cancer. This study assesses the prevalence of BRCA1 and BRCA2 mutations among female students with a family history of breast cancer, in secondary schools of Marawi Locality, Northern State, Sudan.

Methods: From a survey of 2370 students, 67 cases (47 with family history and 20 controls) were analyzed for BRCA1 and BRCA2 mutations with a single-stranded conformation polymorphism (SSCP) mutation detection method applied to peripheral blood. Eighteen subjects knew of first degree female relatives with breast cancer, 23 with second degree female family members affected and 6 with related male sufferers. Twenty randomly selected girls from the remainder of the survey population with no known family history were also tested.

Results: The breast cancer susceptibility genes BRCA1 and BRCA2 accounted, respectively, for 1.21% of responders or 51% of those claiming a family history. Mutations were found in 20% of the group selected with no family history. Only 2 BRCA 2 mutations were found, both in girls with no known afflicted relatives. Six girls knew of male relatives with breast cancer; five of these girls carried mutant BRCA 1. Most of the BRCA1- mutations located to exon 11 fragments 11.9 and 11.1.

Conclusion: The study indicates a high prevalence of genetically associated breast cancer in the Marawi locality suggesting a need to focus on the two mutation sites in developing screening protocols for at least this area of Sudan.

Keywords: Breast cancer, BRCA 1, BRCA 2, Sudan, Schoolgirls

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Introduction

Mutations in BRCA1 and BRCA2 have been reported in African-American women, but the extent of the contribution of BRCA1 and BRCA2 to breast cancer burden in Africa is uncertain. Limited financial resources lead to suboptimal cancer data collection, as well as delayed diagnosis and treatment of many African breast cancer patients.[1] Also breast cancer incidence is low among African American, Asian American, Hispanic and American Indian women compared with White women.[2] However, the death rate from breast cancer is higher among African/African American women than among White women.[3] The advanced stage at diagnosis is a major factor leading to high mortality rates. Information about the distribution and clinical relevance of BRCA1/2 in non-European populations is only beginning to be understood.[4] In a report from a commercial laboratory only 3% of individuals undergoing BRCA1/2 testing are self-reported to be African American.[5] There are striking similarities between BRCA1 associated breast cancer and breast cancer in young African and African American women. Both cancers are often poorly differentiated, hormone receptor negative and have increased S-phase fraction. [6] Therefore, the current study is aimed to detect the prevalence of BRCA1 and BRCA2 mutations among female in secondary schools at Marawi Locality Northern State Sudan.

In Sudan, cancer of breast is the most commonly diagnosed type of cancer. According to a statistical report from the Radiation and Isotopes center in Khartoum, approximately 836 women developed breast cancer in 2007 and 895 in 2008. This accounts for more than 30% of all cancers in women in Sudan and is estimated as 17.2% of all types of cancers in 2007 and 17.9% in 2008.[6]Awadelkarim et al. characterized germline BRCA1/2 mutations in a series of 34 female and 1 male patient from central Sudan over a period of one year, selected by diagnosis at under 40 years of age or male gender. Variants detected included 5 truncating mutations, one of which (in BRCA2) was in the sole male patient. This result suggests that in Central Sudan BRCA1/2 represents an important etiological factor of breast cancer in young women and possibly males.[7]In this study, school girls were taken to represent a cross section of their community and were offered a questionnaire relating to breast cancer in their families. All those who knew of afflicted relatives, plus a sample of the majority who did not have a known family history, were subjected to genetic analysis as described below.

Materials and methods

Subjects and sample collection

A total of 2,370 subjects were recruited from the female students in high secondary schools of the Marawi locality, Northern state of Sudan. Interviews and questionnaires were used to collect demographic data: age, sex, ethnicity (tribe) and family history. Ethical approval was obtained for the project and written informed consent was given by each participant. Of the participants, 2.3% (n=47 in 1892) had first and/or second degree relatives with breast cancer. These constitute the theme of this study. Furthermore, a control panel of 20 participants was also selected from among those who knew of no relatives with a history of breast cancer.

Finger-prick blood samples were collected from each participant onto FTA cards. These cards keep blood dry and can be stored. Dried specimens were kept at room temperature from March 2009 to January 2010.

Single-Strand Conformation Polymorphism

DNA analysis for BRCA1 and BRCA2 involved the Single-Strand Conformation Polymorphism (SSCP) test, performed at Cairo University National Cancer Institute (NCI) Egypt during the period from May 2009 to January 2010.

The primer sequences were selected and chosen in the Cancer Biology Department, National Cancer Institute (NCI), Cairo University according to previous studies in BRCA1and BRCA2 mutation detection(8). The primers are Exon11 fragments 11.0 to 11.18 for BRCA1 and Exon14 fragment 14B for BRCA2. The primers and reagents are produced by Promega Corporation (USA). Of the BRCA1 primers, ten primers showed

mutations (See Table-1). In contrast, one mutationinExon14 fragment, 14 B (F: 5'GTGTACTAGTCAATAAAC-3'; 5'RCATCACACAAATTGTCAT-3')was detected in BRCA2.

Table 1: primersselected for BRCA1 gene mutation

Exon 11 fragment	primers	Product size (bp)
11.1	F: AGAGGCATCCAGAAAAGTATCAGG R: GGGAGTCCGCCTATCATTACAT	239
11.2	F: ACAGCCTGGCTTAGCAAGGAG R: CCCCATCATGTGAGTCATCAGA	278
11.3	F: AGAAACTGCCATGCTCAGAGAATC R: ATGAGGATCACTGGCCAGTAAGTC	245
11.6	F: CAAACGGAGCAGAATGGTCA R: GCCTGGTAGAAGACTTCCTCCTC	244
11.7	F: TCCACAATTCAAAAGCACCTAAAA R: CTCTGGGAAAGTATCGCTGTCAT	299
11.8	F: GCAACTGGATCCAAGAAGTAAC R: TTTGCAAAACCCTTTCTCCACTTA	256
11.9	F: TTGTCAATCCTAGCCTTCCAAGAG R: TTTTGCCTTCCCATGAGTGCTAAC	224
11.11	F: ACATTAGGGAAACAAGCATAGAA R: TTTGGCATTATCAACTGGCTTATC	314
11.18	F:CAGGGAGGTTGGTCTGAGTGAC R: GCTCCCCAAAAGCATAAAC	181

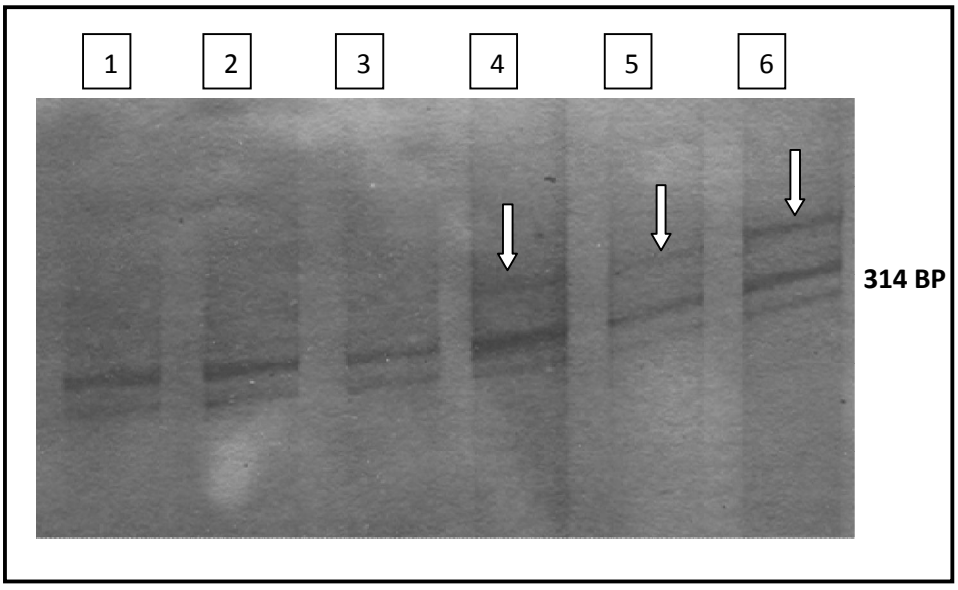
This table details the base sequences of the nine primer pairs (F=forward, R=reverse) that showed BRCA1 mutations in this study.

The dried specimens provided templates for amplification of genomic DNA according to the manufacturer instructions (Whatman UK). PCR amplification of the samples was carried out directly from FTA cards.[9] The extracted DNA from the sample was amplified using Promega protocols.[10]The PCR conditions for BRCA1 and BRCA2 were performed according to the method inPromega™ protocols[11] as follows: (i) one cycle of denaturation at 95 C° for 5minutes (ii) 19 cycles from denaturation at 92 C° for 30 seconds, annealing at 57 C° for 40 seconds and extension at 70 C° for 30 seconds (iii) one cycle of denaturation at 95 C° for 5minutes (iv)19 cycles (from denaturation at 92 C° for 30 seconds, annealing at 60 C° for 40 seconds and extension at 70 C° for 30 seconds) (v) the last extension at 72 C° for 10 minutes.

The PCR conditions for BRCA2 was also performed according to thePromega™ protocols [11] as follows: (i) One cycle of denaturing at 95 C° for 5minutes, (ii) 19 cycles (from denaturing at 92 C° for 30 seconds, annealing at 58 C° for 40 seconds and extension at 70 C° for 30 seconds) (iii) one cycle of denaturing at 95 C° for 5minutes (iv) 19 cycles from denaturing at 92 C° for 30 seconds and annealing at 60 C° for 40 seconds and extension at 70 C° for 30 seconds (v) the last extension at 72 C° for 10 minutes.

The products of PCR were run using 10% polyacrylamide gel according to SSCP methods.[12] Six lanes from one gel showing bands representing mutant BRCA1 are reproduced in Figure 1.

Figure 1



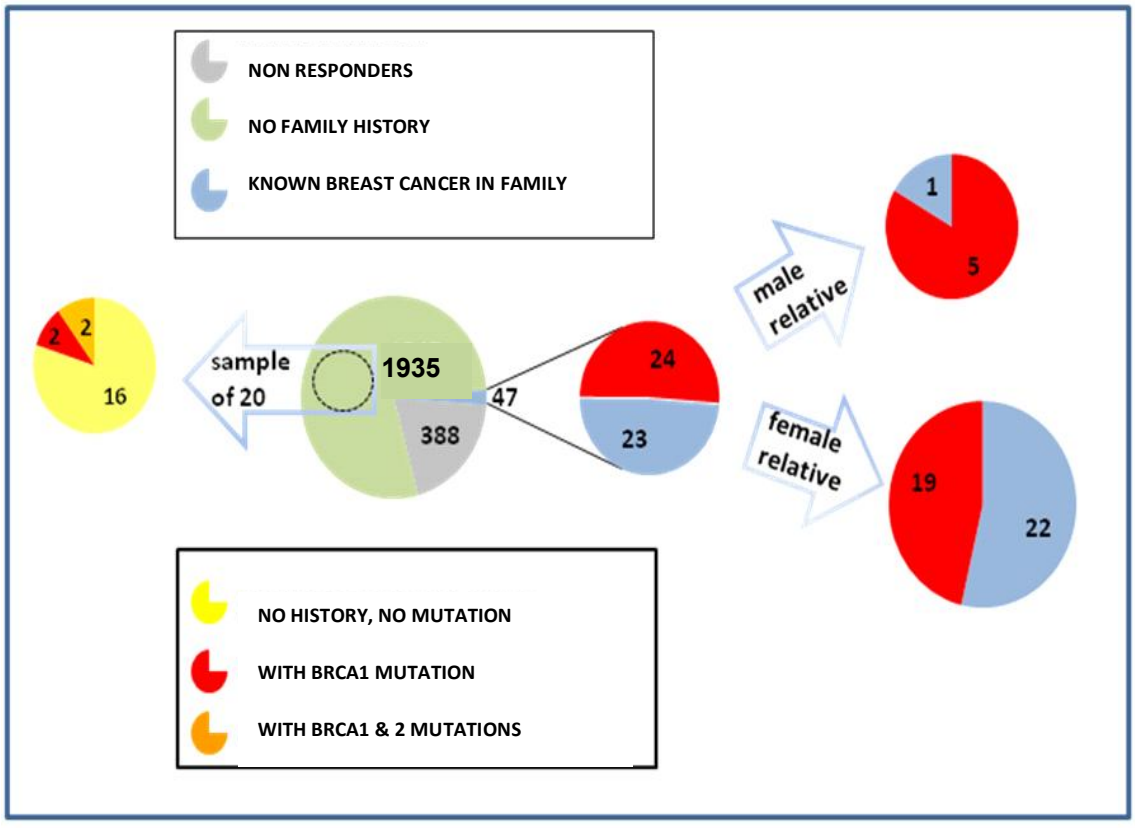
Polyacrylamide gel detection of BRCA1 mutations in exon 11.11 in channels 4-6. The mutant product (arrowed) appears above the normal bands common to all six lanes at 314 base-pair size.

Results

Questionnaires were handed out to 2,370 secondary school girls in the Marawi district of Northern Sudan. In all, 1982 (83.6%) of these questionnaires were completed, some with assistance from a teacher. Of these, 1436 (72.4%) were between fourteen and seventeen years old; Eighty-six (4.4%) were less than fourteen and 122 (6.2%) were over seventeen. The girls displayed a reasonable level of knowledge of the existence and importance of cancer, 64% knowing something about breast cancer, 37.7% leukemia, and 37.1% lung cancer while only 15.2% said they had no knowledge of cancer.

Forty seven girls knew of relatives who had had breast cancer, 20 first degree female relatives and 27 second degree female relatives; 6 had male relatives with the condition (2 first-, 4 second degree). These girls were selected for genetic analysis, as was a random sample of 20 girls from the majority with no known family history. The results of this analysis are displayed graphically in **Figure 2**and recorded below in narrative fashion with percentages where appropriate and added detail.

Figure -2



This flow chart describes how the cases tested derived from the original survey population (green pie) and the incidence of BRCA1 and 2 mutations within each sub-population. Absolute numbers of cases are shown in each segment.

The percentage of girls reporting known breast cancer in their family was 2.37% (47 in 1982) of responders. The incidence of any BRCA I or II mutation was 1.21% of responder families or 51% of those with a family history. In the sample group with no known family history the incidence of any BRCA mutation was 20%.The relative incidence of mutation in girls with affected female relatives is shown in **Table 2.**

Table -2

Mutation in	Affected families n=47	Sample of unaffected families n=20
BRCA I	51.1% (24)	20.0% (4)
BRCA II	0	10.0% (2)

Tabulation of mutations in all students reporting breast cancer affecting their family cancer and the random sample of 20 with unaffected families. Absolute numbers in parentheses

Of the six girls with a male relative affected in the family, 5 had BRCA I mutations, two in first and three in second degree relatives.

The commonest mutations were in the BRCA I gene, accounting for all of those found in the family history group. Details of the location of these mutations are given in **Table 3**.

Table- 3

Exon 11 Fragment	1st degree Female n=18	2nd degree Female n=23	1st degree Male n=2	2nd degree Male n=4	No History n=20
11.1	1		2		3
1.2		3			
1.3		1			
1.6	1	1			
1.7		1			
1.8		1			1
1.9	6	6	2		
1.11		1		1	
1.18	1			1	
Total	9	14	4	2	4

Breakdown of sites of mutations found in BRCA1, with indication of closest relation involved. Four subjects had affected female relatives with double mutations: one 1st degree relative (11.9 & 11.18) and three 2nd degree relatives (11.3 & 11.6, 11.8 & 11.9, 11.9 & 11.11)

By far the commonest site of mutation was Exon 11fragment 11.9 (12 instances) followed by 11.2 (3) and 11.6 (2). BRCA mutations at 11.1 occurred in 2/6 girls with male relatives affected but only 1/41 in those with affected female relations. One girl with a first-degree affected relative and three with second degree relatives had two mutations in Exon 11. The two instances of BRCA II mutation recorded were both in the control sample with no known family history. Both of these blood samples also gave positive results for BRCA I mutation.

Discussion

67 blood samples were analyzed for BRCA1 and BRCA 2 single-strand conformation polymorphism (SSCP) mutations. Twenty of these had been randomly selected from the 1982 girls who responded with a negative family history of breast cancer. With hindsight this sample was too small; nevertheless the mutation rate of 20% in that group was half that of the girls declaring a family history (51%).

The risk of breast cancer increases with the number and closeness of family members affected and the earlier the age of onset.[13]Germline mutations of the BRCA1 and BRCA2 genes are estimated to contribute to the majority of breast cancers that have very early disease onset, strong family history and/or association with ovarian cancer.[14, 15]

The alignment of the results presented here with other studies throws up both similarities and differences and can be summarized as follows: A number of studies have involved native or expatriate Spanish populations. Of 102 Spanish breast cancer families, 30% carried BRCA mutations,[16] with all of the 3 cases with affected male relatives involved, albeit with BRCA II mutations, contrasting with the 4/5 BRCA I mutations in the Sudanese population described here. Another study using cohorts of Hispanic origin yielded 34/110 (25 BRCA I, 19 BRCA II) mutations with no BRCA II mutations in probands with male affected relatives.[16] The majority of those individuals were currently suffering breast cancer. Among 54 male breast cancer cases from California only 17% had a family history; no germ-line BRCA I mutations were found and only two BRA II mutations, one with ovarian cancer in his family and one without a family history.

The prevalence and mortality rate of breast cancer in Asian populations vary significantly, Filipino populations being relatively prone, and the incidence lowest in Chinese.[17, 18] Overall the disease is much less common in Asia than in Western countries. Still, studies from Japan[19] and from the Asian Chinese diaspora[16] suggest that BRCA mutations are more common in cancer families. Two publications from China (abstract only in English, with the full text in Chinese) indicate from the abstracts that, in one study [20]9/39 patients with family history vs 3/149 sporadic patients harbored BRCA mutations; in the other [21] patients with early onset cancer or affected relatives yielded just 3 with mutations against 2/426 sporadic breast cancer.

Few studies exist on African populations in Africa. Genomic DNA analysis from breast cancer patients in Algiers indicated that 9.8 % [5/51] of early-onset sporadic and 36.4 % [4/11] of familial cases were associated with BRCA1 mutations.[22] This constituted a much higher frequency in young patients than in French families (10.3%). Results were suggestive of a North African founder mutation. The figures for African Americans are varied and complicated by issues of education and negative perceptions of genetics research including concerns regarding racial discrimination.[23]
In the present study, BRCA I mutations in probands with male breast cancer in the family outnumbered BRCA II mutations; this is a finding inconsistent with published data and there is no obvious explanation for this observation.

There is a high proportion of mutations in the population described here, including those without known familial cancers. In this light, however interesting academically the distribution of BRCA mutations, the important practical questions are: what use should be made of the technique as an indicator for screening and what are the implications of finding a mutation, either in a cancer patient or in a suspected cancer family?

From a mechanistic point of view, it has been argued that as BRCA mutation carriers who are “triple negative” (for estrogen, progesterone or Her2 receptors) have similar survival to the equivalent sporadic cancers using conventional chemotherapy, they may be good candidates for experimental therapies that target the DNA repair mechanisms compromised by the mutant gene product [24] Also, in common with the present work, most BRCA1- mutations were located in exon 11 fragment 11.9 (43.7%) followed by fragment 11.1 (18.7%). The implication is that it is necessary to focus on these fragments both for future academic work or in developing screening protocols for Sudan.

The aggressive or proactive approach is exemplified by those [e.g.[25]] who advocate “multisystem recommendations for risk management” which educates those at very high risk, or [26] who sees a need for “clear-cut recommendations” for the follow up of non-

carrier cancer family members. This group is particularly difficult to manage as they do appear to have an increased risk of cancer but are particularly vulnerable to the psychosocial downside of rigorous genetic monitoring.

Such cultural, educational and social considerations pose difficult choices in assessing and acting upon genetic risk Harou[27] finds that in women with a BRCA mutation who are enrolled for MRI-based screening, a "perception of personal breast cancer risk and a history of breast cancer in a first-degree relative are predictors of the decision to have RRM" (risk reducing mastectomy). That is perhaps a clinician's perspective. In a more cautious tone, Warner [28] explores the issues of concern to patients, particularly those declining RRM, including financial concerns and fear of outcomes, including the impact of the disease on reproductive options. The need is stressed for oncologists to have a low threshold for referring young women to professionals experienced in navigating "...through the psychosocial trauma of a breast cancer diagnosis". In a small group of (six) patients Crump[29] discerned that "genetic testing, screening and prophylaxis have not provided peace of mind"

What is clear from the present study is that BRCA mutations are relatively common in Sudan, with the expected increased incidence in cancer families, especially where there is a male affected relative, but perhaps with BRCA I mutations being more common in the latter group. In view of the sociological, cultural and educational considerations outlined above, protocols for screening and surveillance might be appropriate but need to be constructed with great care and sensitivity.

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