CLINICAL GENETICS An International Journal of Genetics, Molecular and Personalized Medicine

Clin Genet 2013
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Published by Blackwell Publishing Ltd

CLINICAL GENETICS
doi: 10.1111/cge.12132

Short Report

The spectrum of *BRCA1* and *BRCA2* mutations in breast cancer patients in the Bahamas

Akbari MR, Donenberg T, Lunn J, Curling D, Turnquest T, Krill-Jackson E, Zhang S, Narod SA, Hurley J. The spectrum of *BRCA1* and *BRCA2* mutations in breast cancer patients in the Bahamas.
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We sought to identify the full range of founder mutations in *BRCA1* and *BRCA2* in the Bahamas and to estimate the proportion of all *BRCA1* and *BRCA2* mutations that are accounted for by founder mutations. We studied 214 Bahamian women with invasive breast cancer, unselected for age or family history. A founder mutation had previously been identified in 49 patients. We conducted full sequencing of the *BRCA1* and *BRCA2* genes and multiplex ligation-dependent probe amplification (MLPA) for 156 patients. A novel founder mutation in *BRCA2* (exon 17 818delA) was seen in four different patients and five other unique mutations in *BRCA1* and *BRCA2*, including a large deletion (exons 8–9) in *BRCA1*. In total, a mutation was seen in 58 of the 214 patients (27%); 92% of carriers carried one of the seven founder mutations. Approximately 27% of unselected cases of breast cancer in the Bahamian population are attributable to a mutation in *BRCA1* or *BRCA2*, a prevalence which far exceeds that of any other country. The majority of women who carry a mutation in the Bahamas, carry one of the seven founder mutations, making it possible to offer genetic testing to all women at risk for breast cancer in the Bahamas.

Conflict of interest

The authors declare that they have no conflict of interest.

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Key words: Bahamas – *BRCA1* – breast cancer – hereditary

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Received 2 January 2013, revised and accepted for publication 19 February 2013

The Bahamas is a Carribean island country with a population of approximately 300,000. There are approximately 100 new cases of breast cancer per year in the Bahamas. The average age of onset of breast cancer in the Bahamas is 42 years and one half are diagnosed in pre-menopausal women. In 2011, we reported six founder mutations in *BRCA1*, one of which was present in 49 of 214 unselected breast cancer patients (23%) (1). The mutation prevalence was particularly high (45%) for women diagnosed with breast cancer before age 40. In the context of developing a national cancer genetics program, we asked if there were founder mutations beyond these six (particularly in *BRCA2*) and we sought to estimate what proportion of all *BRCA* mutations in the Bahamian population are accounted for by founder mutations. To do so, we performed full gene sequencing of both *BRCA1* and *BRCA2* and multiplex ligation-dependent probe amplification (MLPA) analysis on 156 unselected breast cancer patients for whom a *BRCA* mutation had not been found.

Materials and methods

Breast cancer patients were recruited from public and private clinics in Grand Bahama, Nassau, Eleuthera, Harbor Island, Abaco and Spanish Wells between September 2008 and January 2010. Women were

eligible if they had been diagnosed with invasive primary breast cancer at any age, in any year and if at least one parent was born in the Bahamas (1). Accrual centers were set up at the Princess Margaret Hospital Oncology Centre, at the Cancer Society in Nassau, at the Cancer Association in Freeport, at Cancer Societies in Eleuthera and Abaco, at the Government Clinic of St George's Cay and at a private home in Harbor Island. In addition, breast cancer patients who were born in the Bahamas were eligible for the study in Miami, at the University of Miami, Jackson Memorial Hospital and the Mount Sinai Cancer Center. All women provided written consent. The study protocol was approved by the ethics review board of the University of Miami, Jackson Memorial Hospital, Mount Sinai Cancer Center, Princess Margaret Hospital, Doctors Hospital and the Bahamian Ministry of Health. In total, 214 women from 204 different families agreed to participate. A saliva sample was taken from each woman and a medical and family history was taken. The family history asked about cancer diagnoses in first- and second-degree relatives. In the event that additional cases in more distant relatives were reported these were recorded on the pedigree. Of 165 women who previously tested negative for mutations in our first study, 156 underwent full testing.

Mutation analysis

Saliva was collected using the Oragene® DNA sample collection kit, (OG-250 format, DNA Genotek, Kanata, ON, Canada) and extracted following the manufacturer's instructions. DNA was then quantified using the NanoDrop ND-1000 Spectrophotometer (ThermoScientific Inc., Wilmington, DE).

Sequencing

All 22 coding exons (exons 2–24) of *BRCA1* and 26 coding exons (exons 2–27) of *BRCA2* were amplified in 34 and 47 amplicons respectively. The primers have been designed to cover all coding exons and their adjacent consensus splicing sites. The amplified DNA fragments were sequenced using the BigDye Terminator Cycle Sequencing kit on an ABI 3500xl DNA Analyzer (Applied Biosystems Co., Foster City, CA). Generated sequencing chromatograms by the sequencer instrument were analyzed for variant detection using Mutation Surveyor software (SoftGenetics LLC, State College, PA). All sequences were compared to the *BRCA1* (U14680.1) and *BRCA2* (U43746.1) reference sequences for variant detection.

MLPA

We screened *BRCA1* and *BRCA2* genes for large insertions/deletions using MLPA method. The *BRCA1* (Cat#: P002) and *BRCA2* (Cat#: P090) MLPA kits (MRC-Holland Inc., Amsterdam, the Netherlands) contain 35- and 43 pairs of probes respectively that bind to different exons of the genes. Probes were hybridized to the genomic DNA and then adjacent probe pairs were ligated and amplified. The fragment analysis of the amplified probes was implemented on an ABI 3500XL DNA analyzer. Generated fragment analysis data were analyzed for detecting large insertions/deletions using GeneMarker software (SoftGenetics LLC). The identified insertions/deletions were confirmed by a second MLPA test using different probe sets (P087 for *BRCA1* and P077 for *BRCA2*) from the initial probe sets used for screening.

Results

We completed full genetic sequencing for 156 women with breast cancer for whom a founder mutation in *BRCA1* had not been found previously. Among these 156 women, a mutation was identified in 9. Four women carried the same nucleotide deletion in *BRCA2* (8128delA) and there were four unique mutations in *BRCA1* and *BRCA2* (1127insGT, 3477delGT, 1538elAAGA and 2190delA). In addition, we found a large deletion encompassing exons 8 and 9 of *BRCA1* in one patient through MLPA analysis.

There were 214 patients studied from 204 different families. The patients represent prevalent cases of cancer and were not selected on the basis of family history, and five families were represented by more than one case. In total, including the 49 mutations previously identified, we have now extended the number of mutations in the panel of 214 women to 58, which represents 27% of all the cases (Table 1). There was one family with five cases included in the study, one family with four cases and three families with two cases each. The other 199 families included one case each. If we restrict the testing to the 204 unrelated women (one per family) there were 49 mutations detected among 204 patients (24.0%).

The mutation prevalence was 52% in 63 women diagnosed at age 39 and under, was 26% in 74 women diagnosed from age 40 to 49 and was 10% in 77 women

Table 1. Distribution of 58 mutations found in 214 breast cancer patients

Gene	Exon	Mutation	Number of patients	Percentage of patients with mutation	Number of families with mutation	Previously reported
BRCA1		IVS13+1G>A	30	52	24	Yes
BRCA1	15	4730insG	6	10	3	Yes
BRCA1	21	T5443G	5	9	5	Yes
BRCA1		IVS16+6T>C	3	5	3	Yes
BRCA1	2	185delAG	2	3	2	Yes
BRCA1	11	943ins10	3	5	3	Yes
BRCA1	11	3477delGT	1	2	1	Yes
BRCA1	11	2190delA	1	2	1	Yes
BRCA1	8 and 9	deletion	1	2	1	Yes
BRCA2	17	8128delA	4	7	4	No
BRCA2	10	1127insGT	1	2	1	No
BRCA2	10	1538delAAGA	1	2	1	Yes

Table 2. Prevalence of mutations by age of diagnosis

Age group	Number of subjects	Number of mutations			Mutation prevalence (%)
		All	BRCA1	BRCA2	
20–29	13	9	8	1	69.2
30–39	50	24	23	1	48.0
40–49	74	19	16	3	25.7
50–59	59	5	4	1	8.4
60+	18	1	1	0	5.6
All ages	214	58	52	6	27

Table 3. Mutation prevalence, by family history of cancer

Family history	Number of probands	Number of mutations	Percentage with mutation
Breast cancer only	108	37	34
Ovarian cancer only	5	0	0
Breast and ovarian cancer	20	9	45
Neither cancer	72	11	15
Unknown	9	1	11
Total	214	58	27

diagnosed at age 50 and above (Table 2). The mean age of diagnosis of the women with a mutation was 38.2 years (range 22–70 years) and the mean age of diagnosis of the women without a mutation was 47.6 years (range 25–80 years). A mutation was present in 33% of 142 patients with a family history of breast and ovarian cancer and in 15% of 72 patients without a family history (Table 3).

Of the 58 mutations, 53 were founder mutations (91%). A single mutation (*BRCA1* IVS13+1G>A) represented 52% of all the mutations. Five other mutations were seen in three or more women (*BRCA1*: IVS13+1G>A, 943ins10, IVS16+6T>C, 5443T>G, 4730insG and *BRCA2*: 8128delA). There were five patients with a unique (non-founder) mutation (*BRCA1*: 3477delGT, 2190delA, del exons 8–9 and *BRCA2*: 1538elAAGA, 1127insGT). Of these, three had a strong family history of breast cancer.

Discussion

In the Bahamas, we estimate that from 24% to 27% of all breast cancer patients carry a mutation in *BRCA1* or *BRCA2*. We have previously described six recurrent *BRCA1* mutations in breast cancer patients from the Bahamas (IVS13+1G>A, 943ins10, IVS16+6T>C, 5443T>G, 4730insG, 185delAG) (1). The majority of these (IVS13+1G>A, 943ins10, IVS16+6T>C and 5443T>G) are African founder mutations. (2) We used DNA sequencing and MLPA to show the full spectrum of *BRCA* mutations in the Bahamas. Six additional mutations were identified (8128delA, 1127insGT, 1538delAAGA, 3477delGT, 2190delA and a large deletion of exons 8 and 9) and are reported here. Three mutations were found in the *BRCA2* gene (8128delA, 1127insGT, 1538delAAGA). A novel *BRCA2* mutation (8128delA) was seen in four patients and thus is the seventh recurrent mutation increasing the prevalence of founder mutations in the Bahamian population to 25% of unselected breast cancer patients. The two additional *BRCA2* mutations were seen in one study participant each. *BRCA2* mutation1127insGT is a novel mutation, whereas 1538delAAGA has been reported in three patients in Nigeria (3) and one patient in Barbados and both Latin American and Western European patients [BIC (Breast Cancer Information Core) database]. Three additional mutations were found in *BRCA1* (3477delGT, 2190delA and a large deletion of exons 8 and 9). The large deletion of exons 8 and 9 has been reported previously in one African-American patient (4). The 3477delGT *BRCA1* mutation has been reported twice in families from Central and Western Europe and the 2190delA *BRCA1* mutation was seen in several families from Western Europe. There were 87 women with a positive family history of breast or ovarian cancer who did not carry one of the seven founder mutations. Of these, a unique mutation was found in three women (3.4%). In the event of a positive family history and a negative founder result, additional testing may be warranted.

The prevalence of mutations among breast cancer cases in the Bahamas is higher than that observed in any other country. We have previously estimated the mutation frequency among unselected breast cancer patients to be 12% in Ashkenazi Jews, 6% in French-Canadians and 6% in Poles (5–8). Recently, a study in Nigeria yielded 11% mutation rate in 434 unselected breast cancer patients, although this included a diverse group of mutations (3). In Canada and the United States, approximately 3% of all breast cancer cases are due to a mutation in one of two cancer susceptibility genes, *BRCA1* and *BRCA2* (9). The unique heritage of Bahamian women makes this small country an ideal location to conduct studies of genetic testing and personalized medicine.

On the basis of studies of other populations, we estimate that between 2% and 4% of all Bahamian women will carry a founder mutation in *BRCA1* or *BRCA2* (for example, mutations are present in 12% of unselected Ashkenazi Jewish breast cancer cases and in 1.5% of the unselected Jewish women) (6, 10). It is important that the mutation prevalence be measured prior to offering genetic testing outside of a personal or family history of breast cancer. Two population studies of unselected individuals from high founder rate ethnic groups have shown success with respect to interest in testing and uptake of cancer risk reduction strategies (10–12).

All women with a history of breast cancer were eligible for the study, but the patients who enrolled may have done so in response to a family history of breast cancer and may not be representative of all cases in the country. Of note, 128 of the 214 patients had a family history of breast cancer (60%). The 27% estimate is based on 58 mutations identified in 214 different patients, but five families contributed more

than one case. If we consider only unrelated individuals (one case per family) the mutation frequency was 24%

The genetic composition of the Bahamas may be distinct from that of neighboring populations because of historical patterns of migration and relative reproductive isolation. These factors may be particularly relevant for other island nations with a small number of founders. Our group is currently investigating whether other islands in the Caribbean have these founder mutations in common or those unique to their island.

In conclusion, we identified one of seven founder mutations in *BRCA1* and *BRCA2* in 27% of breast cancer patients in the Bahamas. It is possible that mutations in other genes are present in one or more of the 156 women in our study without a mutation in *BRCA1* or *BRCA2*. If so, then the hereditary proportion of Bahamian breast cancer patients might be even higher.

On the basis of our results, a seven mutation testing panel is predicted to be 91% sensitive for *BRCA* mutations and should be offered to all breast and ovarian cancer patients in the Bahamas. Patients who have a strong family history of breast and ovarian cancer, who test negative for the founder mutations in this panel should be offered more extensive testing.

We have now initiated a program in the Bahamas which offers genetic testing to all women with breast cancer. Those who test positive will be offered options of increased surveillance and preventive surgery. It is also reasonable to offer predictive testing to unaffected patients with a family history of breast or ovarian cancer if there is no affected family member available for testing. Finally, given the public health consequences of the high founder mutation rate reported here, consideration should be given to population-wide genetic screening and development of a national breast cancer prevention program in the Bahamas. It is important to estimate the prevalence of these mutations among Bahamian women at large and to estimate the penetrance of these mutations in the population.

Acknowledgements

This work was supported by Komen for the Cure, the Ride for Hope and the Bahamas Breast Cancer Initiative.

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