LETTER TO JMG

Complete allelic analysis of *BRCA1* and *BRCA2* variants in young Nigerian breast cancer patients

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J Med Genet 2005;**42**:276–281. doi: 10.1136/jmg.2004.020446

Breast cancer is a leading cause of cancer deaths among women, and is expected to claim the lives of nearly 40 000 individuals in the USA each year (American Cancer Society *Breast Cancer Facts and Figures 2003–2004*). Only 5–10% of breast cancers are associated with mutations in the susceptibility genes *BRCA1* and *BRCA2*. However, in cases associated with strong family history, mutation rates are higher, ranging from 16% to 26% for *BRCA1* [1–3] and from 7% to 13% for *BRCA2*.[2 3] However, many breast cancer patients with strong family histories have no obvious mutations in *BRCA1*/2. While there is an active search for other breast cancer susceptibility genes, it is possible that the true contributions of *BRCA1* and *BRCA2* to early onset breast cancer have been underestimated. Indeed, one study has shown that only 63% of breast cancer families linked to *BRCA1* are associated with detectable mutations in *BRCA1*.[4] Several reasons for this discrepancy are possible. For example, mutations in *BRCA1* promoter sequences might be undetectable by current detection techniques. Additionally, inherited genomic rearrangements that inactivate *BRCA1* and *BRCA2* but cannot be detected by conventional polymerase chain reaction (PCR) based assays have been reported.[5–10] Finally, it is possible that some genetic variants previously dismissed as "unclassified variants" or "polymorphisms" may have hitherto underappreciated effects on protein synthesis or function.

Most studies of *BRCA1* and *BRCA2* associated breast cancers have focused on white populations, yet several observations suggest that there might be a genetic component to breast cancer susceptibility in families of African ancestry.[11] Breast cancer is less common in African populations than in other populations but, when it does occur, it is characterised by an early age of onset and a higher mortality.[12–14] Additionally, histopathological studies have revealed striking similarities between breast tumours that occur in *BRCA1* mutation carriers and patients of African descent, including a higher likelihood of being high grade, hormone receptor negative, and showing increased S-phase and nuclear atypia.[15–21]

Thus it is important to know whether *BRCA1* and/or *BRCA2* mutations play any role in the early onset breast cancers that disproportionately affect patients of African ancestry. We previously described the first analysis of truncating *BRCA1* and *BRCA2* alleles in a population of Nigerian breast cancer patients aged 40 years or younger.[22] This study showed that, while protein truncating alleles are surprisingly less frequent in the Nigerian cohort than other populations studied, the total level of genetic variability in these genes was very high. In the present study, we examine whether non-truncating alleles of *BRCA1* or *BRCA2* are associated with breast cancers in a similar cohort. Specifically, we addressed whether *BRCA1/2* sequence variations are more frequent in African breast cancer populations than in other populations; whether there is a distinctive spectrum of *BRCA1*/2 sequence variations in African patients that may identify functionally critical protein domains; and whether there are founder mutations that occur frequently in breast cancer populations of African descent.

Key points

- Breast cancer in African women is less common than in other groups, but it strikes at an earlier age and has a higher mortality. Breast cancers that result from inherited *BRCA1* and *BRCA2* mutations are also associated with early age of onset and poor prognosis.
- The frequency and mutational spectra of *BRCA1* and *BRCA2* germline mutations were analysed in 39 Nigerian breast cancer patients aged $\leqslant$ 40 years unselected for family history. In this cohort, 29/39 (74%) carried at least one *BRCA1/2* genetic variation, with 69% having sequence variations in *BRCA2*. This represents a higher frequency of *BRCA2* variants than previously seen in a clinic based high risk white cohort.
- Despite this extensive amount of genetic variation, only one truncating allele was detected (*BRCA2* 3034del4). The remaining alleles included 24 exonic single base substitutions, of which four (E425E, L1357L, E1806E, V2171V) have been reported only in Africans or African Americans.
- While no candidate founder mutations emerged from this analysis, five of the 13 different variants detected (38%) were rare non-protein-truncating *BRCA2* alleles and undetected in a population of 74 unaffected Nigerian control subjects. As this frequency difference of rare alleles in cases and controls cannot be statistically significant, modelling software was used to predict whether these alleles represent functionally disruptive amino acid substitutions or potential splicing defects.
- 11 different *BRCA1/2* alleles were shown to be potentially deleterious by one of these criteria, suggesting that the significant level of genetic variation in *BRCA1* and *BRCA2* may contribute to breast cancer risk in populations of African ancestry.

Abbreviations: ESE, exonic splicing enhancer; HA, heteroduplex analysis; PTT, protein truncation test; SNP, single nucleotide polymorphism

Table 1 Variants detected in *BRCA1* and *BRCA2* in 39 Nigerian patients

Patient		Age (years)	*BRCA1*	*BRCA2*
Variants detected				
1	98-168-1	27		N991D
				P1088P
				T1414M
				E1806E
				D1902N
				L1904V
				H2440R
				ex21–36C→G
2	98-169-1	38		V2171V
3	98-171-1	33		ex18+109G→A
4	98-172-1	32		A248T
				ex18+109G→A
5	98-173-1	31	K1183R	I2944F
6	98-174-1	35		ex2–11C→T
7	98-176-1	37		ex7–19C→T
8	98-181-1	37	I379M	
9	98-185-1	38	P938P	ex2–11C→T
10	98-187-1	40		ex7–19C→T
11	98-194-1	40		I2944F
				ex17–40A→G
12	98-198-1	40		ex17–40A→G
13	98-203-1	40		L1904V
14	98-215-1	40		N991D
15	98-219-1	37		L1521L
				V2171V
				ex17–40A→G
16	98-220-1	29		ex26+106delT
17	98-221-1	38		E425E
				G3212R
				Q713L
				ex26+106delT
18	98-228-1	38		N987I
				L929S
19	98-231-1	38		958X
20	98-244-1	35		E425E
				G3212R
				Q713L
				ex2–11C→T
				ex11–43T→C
				ex11–43T→C
21	98-245-1	33		ex12–200insC
22	98-247-1	33		P1088P
				E1806E
				H2440R
23	98-254-1	40		N991D
				I2944F
24	98-255-1	39	K820E	
25	98-260-1	38		N991D
				ex2–11C→T
				N289H
26	98-263-1	38		V2171V
27	98-267-1	38		V2171V
28	98-270-1	40		L1357L
				Q2384K
				S2414S
29	98-274-1	30		ex22–70C→T
No variants detected				
30	98-182-1	34		
31	98-188-1	37		
32	98-225-1	35		
33	98-227-1	30		
34	98-229-1	30		
35	98-232-1	31		
36	98-234-1	35		
37	98-236-1	33		
38	98-253-1	40		
39	98-261-1	39		

METHODS

Human subjects

Genomic DNA was prepared from peripheral blood from breast cancer patients using DNA isolation kits from Puregene (Gentra Systems, Minneapolis, Minnesota, USA) according to the manufacturer's instructions. Patients were all Nigerian women diagnosed with breast cancer at the age of 40 years or younger (median age 37 years; range 27 to 40), and samples were selected from a consecutive series of newly diagnosed breast cancer cases seen at the University College Hospital, Ibadan, Nigeria. Thirty three of 39 patients (85%) reported no family history of cancer. Of the remaining six (15%), five reported one case of breast cancer in a first degree relative, and one reported cervical cancer in her sister and grandmother. The patient distribution among Nigerian ethnic groups was as follows: 25 Yoruba (64.1%); three Igbo (7.7%); three Urhobo (7.7%); two Edo (5.1%); one Delta (2.6%); one Goemai (2.6%); one Kagoro (2.6%); one Ishan (2.6%); one Owan (2.6%); and one unknown (2.6%). Thus most of the cases were Yorubas.

Seventy four control subjects, also Nigerian women seen at the University College Hospital at Ibadan, were given breast examinations to confirm that no breast cancer was present. These women had a median age of 37 years old (range 22 to 66). Seventy of them (94.6%) reported no family history of breast cancer. The remaining four (5.4%) reported cancers in first degree relatives but did not specify the type of cancer. The control distribution among Nigerian ethnic groups was as follows: 67 Yoruba (90.5%): two Igbo (2.7%); two Edo (2.7%); one Urhobo (1.4%); and one Tbibio (1.4%). Thus, as with the cases, the controls were predominantly Yorubas.

All patients and control subjects gave informed consent after appropriate counselling according to the protocols approved by the Universities of Chicago and Ibadan institutional review boards.

Heteroduplex analysis

We screened the entire coding sequence and all intron/exon junctions using heteroduplex analysis (HA) followed by DNA sequencing as previously published.[23, 24] Briefly, PCR products were heated at 95℃ for three minutes, then slowly cooled to 37℃ over 40 minutes. After mixing with 6× triple dye loading buffer at 1:5 volume ratio, the PCR products were run on 1×MDE gel (FMC BioProducts, Rockland, Massachusetts, USA) with 15% urea at 400 volts at room temperature for 14 to 20 hours. The gels were stained with in SYBR™ Green I nucleic acid (FMC BioProducts) and photographed under ultraviolet light.

Protein truncation test

In addition to heteroduplex analysis, the protein truncation test (PTT) was used to screen genomic DNAs of exon 10 from *BRCA2* and exon 11 from both *BRCA1* and *BRCA2*, as previously published.[24] The forward primers include additional 5′ sequences containing the T7 promoter and a translation start site. PTT using the TNT-T7 coupled wheat germ extract system (Promega, Madison, Wisconsin, USA) was carried out according to the manufacturer's instructions, incorporating ^{35}S-methionine or ^{35}S-cysteine. Samples were electrophoresed on 12.5% acrylamide gels and exposed to Kodak *x* ray film. Candidate positives were sequenced.

DNA sequencing

PCR products showing an electrophoretic variant pattern by HA or PTT were reamplified from the original genomic DNA and both strands were directly sequenced by the fluorometric method with automated sequencing procedures (Dyedeoxy Terminator cycle sequencing kit and ABI377 DNA sequencer; Applied Biosystems, Foster City, California, USA).

Analysis of variants

Data regarding clinical significance and ethnic occurrence of previously reported alleles were taken from the breast cancer

information core (BIC) website http://research.nhgri.nih.gov/bic/. Predictions of exonic splicing enhancer (ESE) consensus scores were made using previously described scoring matrices.[25–27] Predictions of which amino acids are not theoretically tolerated at specific positions within BRCA1 and BRCA2 were generated using the ''sorting intolerant from tolerant'' (SIFT) website http://blocks.fhcrc.org/sift/SIFT.html. This method searches public databases for protein homologs, performs alignments, and then generates a score for the theoretical tolerance of each possible amino acid substitution. Records used for single nucleotide polymorphism (SNP) frequencies are kept in the SNP500Cancer database http://snp500cancer.nci.nih.gov/.

RESULTS

In this cohort of early onset Nigerian breast cancer cases, 29 of 39 (74%) carried at least one genetic variation in *BRCA1*, *BRCA2*, or both. *BRCA2* variations were found in a surprising 27 cases (69%) (table 1). Thirty four different variants were detected, four in *BRCA1* and 30 in *BRCA2* (table 2). These included nine intronic and 25 exonic variations. Of these variations, only one (2.5%) is a known deleterious truncating mutation (*BRCA2* 3034del4). Of the 21 previously reported non-truncating exonic variants,[28] 15 are described as ''unclassified variants'' and only six are described as polymorphisms. The remaining four exonic variants are reported here for the first time. Seven of the nine *BRCA2* intronic variations have not been reported previously. It is important to note that the heteroduplex method used here identifies heterozygous genotypes, and the frequencies of the alleles reported does not include the frequencies of potential variant homozygotes.

To test the potential clinical significance of these variants, we examined the frequency of amino acid substitution variants in a population of 74 unaffected Nigerian samples (that is, 148 chromosomes) (table 2). *BRCA1* and *BRCA2* single base changes not expected to result in amino acid substitutions were not examined. Five of 13 predicted *BRCA2* amino acid substitution variants (38%) were not detected in the unaffected population, indicating that they may be rare high risk alleles. However, several of the alleles found in the Nigerian cohort have been described as single nucleotide polymorphisms (SNPs) by the National Cancer Institute cancer genome anatomy project ''SNP500Cancer'' database (http://snp500cancer.nci.nih.gov/). It is reported that *BRCA2* alleles N289H, L929S, N987I, N991D, S2414S, H2440R, and I2944F all exist in reference samples and are just as frequent in patients of African ancestry as in those of other ethnicities. Curiously, the *BRCA1* K1183R allele is also described as an SNP found in unaffected populations, but may be less common in individuals of African ancestry. However, the alleles found in our patient set but not in our control population (*BRCA2* A248T, Q713L, D1902N, L1904V, and Q2384K) are also not described in the SNP database. While it is possible that these rare variants found in patients may be deleterious, we did not have sufficient power to determine whether their absence from control populations was statistically significant.

To investigate whether exonic base changes found in African *BRCA1* and *BRCA2* variants might affect correct message splicing, we examined exonic base changes and the surrounding sequence for disruption of high scoring ESE consensus sequences.[25–27 30–32] ESEs are SR protein binding sequences that may lie within exons of pre-mRNAs that indicate exon identity during splicing. Mutated ESEs could therefore result in exon skipping during splicing. Eight of 24 non-truncating exonic base changes (33%) in the African population fall within high scoring predicted ESE, and the base substitutions associated with five of these alleles are predicted to cause ESE disruption (*BRCA2* A248T, Q713L, T1414M, D1902N, and V2171V). Two of these (T1414M and V2171V) are seen in our unaffected population as well as in patients. It is important to note that ESE mediated splicing patterns are complex, and not all disruptions of predicted ESE consensus motifs will necessarily result in high penetrance exon skipping phenotypes. However, such a mutation could cause shifts in relative levels of alternate splicing products, resulting in limited or conditional clinical phenotypes. Finally, we used methods on the SIFT website to predict which amino acid substitutions found in BRCA1 and BRCA2 protein variants in the African population are unlikely to be tolerated at mutant positions (see Methods). Of these variants, all the amino acid substitutions seen in *BRCA1* (I379M, K820E, and K1183R) are predicted to contain non-tolerated amino acid substitutions by scoring based on homolog alignment, and are thus potentially clinically important base changes. Interestingly, BRCA1 I379M was also identified as a potentially deleterious amino acid substitution by an ''ancestral sequence'' method.[33] Of the BRCA2 amino acid substitutions seen in the African cohort, alleles N987I, N991D, and I2944F are predicted to be non-tolerated amino acid substitutions within the limits of confidence in the alignments.

Neither ESE nor SIFT scoring mechanisms constitute definitive evidence of the clinically deleterious nature of the base changes observed. However, they suggest a rational and testable basis for anticipating that many of the functionally uncharacterised mutations described here could be deleterious alleles, perhaps some with limited penetrance. Unfortunately, we do not have additional biological materials to carry out further tests on the patients. Nonetheless, we suggest that the numerous variations in *BRCA1* and especially *BRCA2* in African breast cancer patients may contribute to disease occurrence and should be characterised functionally.

DISCUSSION

We previously investigated the frequency of *BRCA1* and *BRCA2* protein truncating mutations in a cohort of 70 young breast cancer patients ascertained in the surgical oncology unit at the University College Hospital Ibadan, Nigeria.[22] We reported 4% of the population carried deleterious mutations (two truncating mutations in *BRCA1* and one in *BRCA2*), well below the frequencies of deleterious mutations found in other clinic or population based cohorts.[34] However, we also reported a preliminary observation of 20 non-truncating *BRCA2* variants in 18 individuals (23%). In the present study we re-examined a subset of the original population to better define the frequency of *BRCA1* and *BRCA2* non-truncating alleles and assess whether these may also be a source of breast cancer susceptibility in young African breast cancer patients. Here we report a single truncating *BRCA2* mutation and no additional truncating mutations in the 39 patient cohort (2.6%), but 29 (74%) carried non-truncating variants in *BRCA2*. These observations are expand our previous observations and are consistent with observations by Wagner and colleagues, who found 45 different *BRCA2* sequence variants in 21 unrelated unaffected African individuals, with 33 of 45 (73.3%) being unique to African populations (reported in BIC or for the first time in 1998).[29] Other studies using different mutation ascertainment methods have shown high levels of *BRCA2* sequence variability and a distinct spectrum of variants in patients of African ancestry.[29 35] This observation is consistent with reports of an exceptionally high level of overall genetic diversity in people of African ancestry.[29 36]

Newman and colleagues reported a lower frequency of deleterious *BRCA1* mutations in black than in white women (3.3% *v* 0%) in a population of unrelated patients diagnosed

Table 2 *BRCA1* and *BRCA2* alleles detected in a Nigerian cohort

Patient	BRCA gene	Base change	Exon	Predicted amino acid effect	No of times in 39 pts	No in 74 unaffected individuals	BIC designation (occurrences in BIC)	Global heterozygosity
Single nucleotide substitutions								
1	BRCA1	1256T→G	11	I379M	1	ND	UV (15)	
2	BRCA1	2577A→G	11	K820E	1	ND	UV (28)	
3	BRCA1	2933A→G	11	P938P	1	ND	P (2)	
4	BRCA1	3667A→G	11	K1183R	1	ND	P (32)	0.403
5	BRCA2	970G→A	9	A248T	1	0	UV (1)	
6	BRCA2	1093A→C	10	N289H	1	ND	UV (2)	0.120
7	**BRCA2**	**1503A→G**	**10**	**E425E**	**2**	**ND**	**1st report (0)**	
8	BRCA2	A2366T	11	Q713L	2	0	UV* (8)	
9	BRCA2	3014T→C	11	L929S	1	5	UV (29)	
10	BRCA2	3188A→T	11	N987I	1	2	UV (29)	
11	BRCA2	3199A→G	11	N991D	4	3	UV (1)	0.15
12	BRCA2	3492T→C	11	P1088P	2	ND	P (6)	
13	**BRCA2**	**4299A→C**	**11**	**L1357L**	**1**	**ND**	**1st report (0)**	
14	BRCA2	4469C→T	11	T1414M	1	1	UV (26)	0.012
15	BRCA2	4791G→A	11	L1521L	1	ND	P* (2)	0.018
16	**BRCA2**	**5646A→G**	**11**	**E1806E**	**2**	**ND**	**1st report (0)**	
17	BRCA2	5932G→A	11	D1902N	1	0	UV (59)	0.012
18	BRCA2	5938 C→G	11	L1904V	2	0	UV (8)	
19	**BRCA2**	**6741 C→G**	**11**	**V2171V**	**4**	**16**	**P (1)**	**0.018**
20	BRCA2	7378C→A	14	Q2384K	1	0	UV (18)	
21	BRCA2	7470A→G	14	S2414S	1	ND	P (10)	0.250
22	BRCA2	7547A→G	14	H2440R	2	2	UV (79)	
23	BRCA2	9058A→T	22	I2944F	3	1	UV (115)	0.006
24	BRCA2	9862G→C	26	G3212R	2	2	UV* (8)	
25	BRCA2	3034/6 delACAA	11	958X	1	ND	trunc (63)	
Intronic variations								
26	**BRCA2**	**ex2-11C→T**		**–**	**4**	**ND**	**1st report (0)**	
27	BRCA2	ex7-19C→T		–	2	ND	UV (10)	
28	**BRCA2**	**ex11-43T→C**		**–**	**2**	**ND**	**1st report (0)**	
29	**BRCA2**	**ex12-200insC**		**–**	**1**	**ND**	**1st report (0)**	
30	**BRCA2**	**ex17-40A→G**		**–**	**3**	**ND**	**1st report (0)**	
31	**BRCA2**	**ex18+109G→A**		**–**	**2**	**0**	**1st report (0)**	
32	**BRCA2**	**ex21-36C→G**		**–**	**1**	**ND**	**1st report (0)**	
33	**BRCA2**	**ex22-70C→T**		**–**	**2**	**ND**	**1st report (0)**	
34	**BRCA2**	**ex26+106delT**		**–**	**2**	**ND**	**P (1)**	

Alleles reported here for the first time or found only in Africans or African Americans are in bold. Global heterozygosity frequency was known only in some cases. The heterozygosity frequency of *BRCA1* K1183R was taken from the NCBI SNP page <http://www.ncbi.nlm.nih.gov/SNP/>, and the rest were taken from Wagner *et al.*[29] The asterisk indicates alleles reported in patients of African ancestry and at least one patient of unreported ethnicity. Such alleles could be specific to populations of African ancestry, but this cannot be determined by the information available in BIC. For example, the *BRCA2* L1521L variant was shown to be specific to African individuals in the study by Wagner *et al.*[29]
ND, not determined; (P), polymorphism; pts, patients; trunc, truncating; UV, unclassified variant.

with invasive breast cancer, but not selected for early age onset or family history.[37] Other studies indicate that frequencies of deleterious *BRCA1* and *BRCA2* mutations are just as common in African American high risk patients as in other ethnic groups.[11 38–40] However, these studies used clinic based subjects who were referred for genetic testing based on family history, whereas our consecutively ascertained clinic based subjects were selected only for early onset breast cancer. Indeed, it has been shown that rigorous family history criteria predict deleterious *BRCA1* and *BRCA2* mutations just as strongly in African Americans as other populations.[11 35 38 40] However, these findings are unlikely to reflect the distribution of *BRCA1* and *BRCA2* mutations in general populations, which may explain the similarity between our results and those of Newman *et al*.[37]

Other studies of genetic contributions to breast cancer in different ethnic groups have revealed population specific patterns of *BRCA1* and *BRCA2* mutation frequencies and spectra associated with breast cancer. Patient selection criteria and mutation detection methods vary widely in these studies, so it is difficult to compare mutation frequencies among published investigations. However, several reveal that both the frequency of variants and deleterious mutations is higher in *BRCA2* than *BRCA1*. This has been seen in populations from Mexico,[41] Spain,[42] Sardinia,[43] and Japan.[44] One study that examined a population of Indian breast cancer patients showed similar frequencies of variants per se in *BRCA1* and *BRCA2*, but all the *BRCA1* variants were deleterious, while all the *BRCA2* variants were clinically unclassified.[45] Finally, several studies have described populations in which the frequency of unclassified variants and deleterious mutations in *BRCA1* are both more frequent than in *BRCA2*. These include cohorts from the USA,[46 47] Canada,[48] Germany,[49 50] and China.[51] The observation that our African cohort showed a much greater frequency of variability in *BRCA2* than *BRCA1* is surprising, as breast cancers in *BRCA1* mutation carriers have more clinical similarity to breast cancers in patients of African descent. It remains possible that some of the patients carry distant promoter mutations, splice disrupting mutations deep within intronic regions, or large scale rearrangements of *BRCA1* not detected by our methods. Additionally, it is possible that *BRCA1* mutation carriers simply have breast cancer characteristics in common with other early onset breast cancers, and such characteristics will probably occur with a high frequency in disproportionately young populations. Germline *BRCA2* mutations are associated with early onset breast cancers that are otherwise phenotypically similar to sporadic breast cancers.[52] Because of the frequency of the unclassified variants in our Nigerian population, we suggest that some of these variants may be deleterious to some extent. It is interesting to note that, in a clinic based cohort of African Americans with family histories

of breast cancer, who are thus more likely to carry *BRCA1/2* mutations, the frequency of *BRCA2* single base changes is even higher than in the age selected Nigerian cohort (Fackenthal JD and Olopade OI, unpublished observations).

Among the most clinically useful observations from *BRCA1* and *BRCA2* genetic analyses in individual ethnic populations is the existence of population specific founder mutations. For example, among Ashkenazi Jews, 20% of patients diagnosed before the age of 40 carry the *BRCA1* 185delAG allele, whereas only 10% of a general white population with early onset breast cancer may carry *BRCA1* mutations of any kind.[53 54] Additionally, the *BRCA2* 999del5 allele has been identified as a founder mutation in the Icelandic population and has been detected in 0.6% of the total population, 7.7% of all female breast cancers, and 40% of all male breast cancers.[55–57] In populations of African descent, such founder mutations have been difficult to identify, possibly because of the diversity in the African diaspora. Several reports have shown that a single allele, *BRCA1* 943ins10, is common to patients of African ancestry detected in Washington DC, Florida, South Carolina, Bahamas, and the Ivory Coast.[58–61] This allele does not occur often enough to provide expediency in clinical diagnostics, but it does suggest that the mutational spectrum of *BRCA1/2* may be distinctive in Africans and African Americans.

The mutations identified in *BRCA2* in our Nigerian cohort were distributed throughout the coding region and did not fall predominantly into known functional domains or cluster into potential novel domains. Several amino acid substitutions occurred more than once, and some of these have been reported only in African or African American individuals, or are reported here for the first time (table 2). While these variants may contribute to a unique spectrum of *BRCA2* mutations in subSaharan African populations, we cannot conclude from currently available data that these alleles are unique to individuals of African ancestry.

However, the unique spectrum of *BRCA2* alleles seen in these African patients may reflect *BRCA2* involvement in early onset breast cancer in African populations. Several observations suggest that some of these mutations may represent deleterious or incompletely penetrant alleles. First, five of 13 amino acid substitutions (38%) that were detected in the breast cancer population were not detected in the control population. While our sample size is too small for definitive statistical analysis, this observation suggests these alleles could be rare high risk alleles. Additionally, eight of 14 *BRCA2* exonic single base substitutions (57%) fell within high scoring ESE motifs, and the majority of these are motif disrupting alleles that could result in exon skipping. ESE disruption alleles that result in exon skipping have been reported in numerous cancer related genes, including *BRCA1* and *BRCA2*.[25 31 62 63] Finally, all three BRCA1 amino acid substitutions and three of 13 BRCA2 amino acid substitutions (23%) are predicted to be disruptive to protein structure based on scoring from alignment of homologs. Many DNA sequence variants that result in an alteration of protein structure, protein binding ability, or aberrant splicing patterns are likely to have limited penetrance or be dependent upon genetic or environmental backgrounds for expressivity. To address the potentially subtle nature of such hypothetical alleles, it will be necessary to construct haplotypes containing variants of interest in linkage disequilibrium with other markers. Such haplotypes are potentially more likely to cosegregate with disease than any single marker component. Taken together, these observations suggest that the high variability of *BRCA1* and *BRCA2* sequences could contribute to early onset breast cancers seen in patients of African descent. We suggest that functional characterisation of non-truncating alleles like the ones reported here will be necessary in the future in order to understand the structural requirements for *BRCA1/2* tumour suppressor functions and provide informed genetic counselling for patients from ethnically diverse backgrounds.

ACKNOWLEDGEMENTS

This work was supported by grants from the Falk Medical Research Trust, US Army Department of Defense, and NC1-CA 89085 (support to OIO). OIO is a Doris Duke Distinguished Clinical Scientist.

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Competing interests: none declared

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