Breast Cancer Res Treat (2007) 102:189–199
DOI 10.1007/s10549-006-9303-z

EPIDEMIOLOGY

BRCA1 and *BRCA2* status in a Central Sudanese series of breast cancer patients: interactions with genetic, ethnic and reproductive factors

Khalid Dafaallah Awadelkarim · Gitana Aceto · Serena Veschi · Ahmed Elhaj · Annalisa Morgano · Ahmed Abdalla Mohamedani · Elgaylani Abdalla Eltayeb · Dafaallah Abuidris · Mario Di Gioacchino · Pasquale Battista · Fabio Verginelli · Alessandro Cama · Nasr Eldin Elwali · Renato Mariani-Costantini

Received: 1 June 2006 / Accepted: 3 June 2006 / Published online: 1 March 2007
© Springer Science+Business Media B.V. 2007

Abstract The etiology of breast cancer in Africa is scarcely investigated. Breast cancer was responsible for 456/2,233 cancer patients (20.4%) ascertained between 1999 and 2004 at Gezira University, Central Sudan. Male breast cancer accounted for 16/456 patients (3.5%), 275/440 female patients (62.5%) were pre-menopausal and 150/440 cases (34%) occurred in women with ≥5 childbirths. We characterized for germline *BRCA1/2* mutations a one-year series of patients (34 females, 1 male) selected by diagnosis within age 40 years or male gender. Overall 33/35 patients were found to carry 60 *BRCA1/2* variants, of which 17 (28%) were novel, 22 (37%) reported in populations from various geographic areas and 21 (35%) reported worldwide. Detected variants included 5 truncating mutations, one of which (in *BRCA2*) was in the male patient. The 55 non-truncating variants included 3 unclassified variants predicted to affect protein product and not co-occurring with a truncating mutation in the same gene. Patients were from different tribes but AMOVA showed that most *BRCA1/2* variation was within individuals (86.41%) and patients clustered independently of tribe in a phylogenetic tree. Cluster analysis based on age at cancer diagnosis and reproductive variables split female patients in two clusters that, by factor analysis, were explained by low versus high scores of the total period occupied by pregnancies and lactation. The cluster with low scores comprised all 4 patients with truncating mutations and 3 of the 4 carriers of unclassified variants predicted to affect protein product. Our findings suggest that in Central Sudan *BRCA1/2* represent an important etiological factor of breast cancer in males and young women less exposed to pregnancy and lactation. Factors other than *BRCA1/2* may contribute to breast cancer in young highly multiparous women who breast-fed for prolonged periods.

Keywords Africa · Breast cancer · *BRCA1* · *BRCA2* · Ethnicity · Germ-line mutation · Lactation · Parity

Electronic Supplementary Material The online version of this article (doi: 10.1007/s10549-006-9303-z) contains supplementary material, which is available to authorized users.

Khalid Dafaallah Awadelkarim and Gitana Aceto equally contributed to this work.

K. D. Awadelkarim · G. Aceto · S. Veschi · A. Morgano · M. Di Gioacchino · P. Battista · F. Verginelli · A. Cama · R. Mariani-Costantini (✉)
Unit of Molecular Pathology and Genomics, Center for Sciences on the Ageing (CeSI), "Gabriele d'Annunzio" University Foundation, Via Colle dell' Ara, 66013 Chieti, Italy
e-mail: rmc@unich.it

A. Elhaj · E. A. Eltayeb · D. Abuidris · N. E. Elwali · K. D. Awadelkarim
Departments of Molecular Biology, Oncology, Institute of Nuclear Medicine, Molecular Biology and Oncology (INMO), University of Gezira, Wad Medani, Sudan

A. A. Mohamedani
Faculty of Medicine, Department of Pathology, University of Gezira, Wad Medani, Sudan

Introduction

Breast cancer (BC), a classical hormone-dependent malignancy [1] that represents the commonest cancer of women in the world, is a biologically heterogeneous

disease influenced by complex interactions between multiple genetic and environmental risk factors [2–8]. This most likely contributes to the geographic and ethnic variation observed in worldwide BC incidence [9].

BC and its associated risk factors have been predominantly investigated in the industrialized Western world, whose populations share relatively similar lifestyles and socio-cultural environments and have the highest age-standardized BC incidence rates worldwide [9, 10]. Much less is known about BC in the developing world, with particular regard to populations indigenous to sub-Saharan Africa, which strongly differ from Western populations in ethnicity, lifestyle and environmental exposures [11, 12]. Age-adjusted BC incidence rates in indigenous African women are estimated to be much lower than rates in Western women [9, 13], nevertheless BC remains the first or second most common malignancy of females in African hospital-based cancer series and BC incidence in Africa is predicted to rapidly increase [9, 13–15].

BC in Africa seems to be characterized by advanced stage, poor prognosis, presentation in mainly multiparous premenopausal women and relatively high male BC (MBC) frequency [15–17]. These characteristics are consistent with demographics, especially high fertility rates, young population age and low life expectancy, and with delayed diagnosis [13]. Furthermore, comparatively low BC incidence rates in women predict relatively high proportions of males in BC series. Therefore, beyond lack of adequate health care systems, the features of BC in Africa may reflect population structure and comparatively low female BC (FBC) risk. This would imply that the role(s) of etiological factors responsible for MBC and for early onset BC, particularly in parous women, could be magnified in African cancer series.

African women are predominantly non-contracepting and their reproductive lifestyle is characterized by early childbearing, high multiparity and very extended postpartum breast-feeding [18]. Reproductive variables, main determinants of lifetime exposure to female hormones, were shown to have dual effect on BC risk [3–5]. Parity, multi-parity and early age at first full-term pregnancy (≤20 years) impart a well-documented and long-lasting protective effect, particularly in the postmenopausal period [3–5, 19]. This could be due to several not mutually exclusive reasons, including interruption of ovulatory cycles, modification of the hormonal milieu and induction of a new persistent mammary epithelial cell population refractory to mutagenic damage [1, 20]. On the other hand, BC risk is transiently increased postpartum and in the years immediately following childbirth [3, 21, 22], an apparent paradox that may reflect a cancer-promoting effect of sex hormone stimulation [23]. Parity-associated BC risk may be modulated by race and could be higher in Black women [2, 7, 24]. Lack or short lifetime duration of breast-feeding is regarded as a contributor to the high BC incidence in developed countries and a number of epidemiological studies reported a protective effect of lactation, with the strongest risk reduction in women who breast-fed for longer periods [2, 5, 19, 25–30]. However, some studies did not detect any protective effect of breast-feeding, particularly among Black women [31, 32]. Reproductive variables could interact with constitutional BC risk factors which are predicted to facilitate cancer initiation in mammary epithelia [33]. In this respect it is intriguing that the clinical-pathological features of African BC are somewhat reminiscent of those of hereditary BC associated with deleterious germ-line mutations in either of the two major BC susceptibility genes, *BRCA1* (GenBank accession no. U14680; MIM#113705) and *BRCA2* (GenBank accession no. U43746; MIM#600185) [17]. Mutations in these genes are the most important risk factors for early-onset female BC (FBC) and MBC [34]. Thus the hypothesis that BC in Africa could be enriched in *BRCA1/2*-related disease needs to be verified.

The Sudan, the largest African country, is an ethnic mosaic of about 700 tribes, many of which traditionally endogamic [35, 36]. Diet is based on vegetable foods, with meat and animal fat accounting for less than 1/4th of the dietary energy supply [37], a pattern considered protective against BC [38] and that could be relevant to the prevention of *BRCA1/2*-related disease [39]. The fertility rate is high (from 5 to 6 between 1993 and 2004) and infants are extensively breast-fed [37, 40, 41].

Little is known about BC in the Sudan [13]. We report here the salient features of a Central Sudanese BC series ascertained between 1999 and 2004 at the newly established Institute of Nuclear Medicine, Molecular Biology and Oncology (INMO) of Gezira University in Wad Medani. Revision of this BC series prompted us to explore in a selected series of patients the relationships between *BRCA1/2* status, ethnic and reproductive variables.

Patients and methods

Patients

The study has been approved by the Review Boards at the participating Sudanese and Italian Institutions. The medical records of the BC patients ascertained at

INMO from May 1999 through December 2004 were reviewed with regard to age at diagnosis and reproductive data. Patients with pathologically confirmed BC ascertained between September 20, 2001 and October 23, 2002, were consecutively selected using the following criteria: age at diagnosis ≤ 40 years (34 FBC patients); MBC at any age (1 patient). Individual data, reported in a standardized questionnaire after interview in person and review of medical records are summarized in online Table S1. Hormone receptor data were not available. Acid citrate–dextrose anticoagulated blood (10 ml) was collected from each study subject and preserved frozen at –20°C in two 5 ml aliquots for replicate genetic testing. All patients gave informed consent to the study.

Genetic testing

Genomic DNA was isolated from frozen blood using the QIAamp DNA Blood Mini Kit (QIAGEN Inc, Chatsworth, CA). Protein truncation test (PTT), denaturing high performance liquid chromatography (DHPLC) and direct sequencing were used for mutation detection. *BRCA1* exon 11 and *BRCA2* exons 10–11 were screened by PTT as previously described [42]. The entire *BRCA1/2* coding sequences, including intron-exon boundaries, were analyzed by DHPLC using the Wave®Nucleic Acid Fragment Analysis System (Transgenomic Inc., San Jose, CA). Primers for exons 2–24 of *BRCA1* and 2–27 of *BRCA2* were as reported in the literature [43, 44]. DHPLC conditions were adapted based on the manufacturer's software and published literature [43, 44]. Samples that showed PTT shifts or altered DHPLC profiles were directly sequenced using an ABI PRISM™310 genetic analyzer (Applied Biosystems, Foster City, CA). Variants nomenclature follows the guidelines of the Human Genome Variation Society (HGVS). Variants were confirmed on replicate 5 ml blood aliquots. Unavailability of fresh blood precluded allele expression studies of non-truncating mutations.

Genetic and statistical analyses

Tolerability prediction of amino acid changes was tested by SIFT version 2 (available at http://blocks.fhcrc.org/sift/SIFT.html) [45]. The *BRCA1/2* genetic structure of study patients was tested through analysis of molecular variance (AMOVA) implemented in Arlequin version 3.01 using phased genotypes estimated by Bayesian approach (ELB algorithm) [46]. Genetic diversity among tribes, among individuals within tribes and among all individuals regardless of tribes was examined by the *F* statistics of Wright [47]. Tribes represented by only one patient in the series were not considered. We calculated the matrix of pairwise *Fst* values between tribes and tested for statistical significance after 1,000 permutations. Moreover, population differentiation was analyzed by exact test of non-random genotype distribution, also implemented in Arlequin version 3.01. A phylogenetic tree based on all *BRCA1/2* sequence data retrieved from study patients was constructed through neighbor joining (NJ) algorithm by MEGA3 version 3.0 [48]. The genetic distance matrix was based on Kimura 2-parameter with uniform rate of heterogeneity [49]. Significance was determined from bootstrap percentages obtained after replication of 1,000 trees.

Unsupervised cluster analysis (CA) was used to investigate the natural clustering of the 33 FBC patients for whom reproductive data were available. CA was performed by STATISTICA 5.0 (Statsoft, inc., Tulsa, OK) using Euclidean distance measurements to obtain a dissimilarity matrix based on age at BC diagnosis and reproductive variables known to determine lifetime exposure to sex hormones, including age at menarche (or, when not available, median age at menarche of patients in the series), number of children and total lactation years. All parous patients admitted extended breast-feeding, but duration of each lactation period could not be defined and was uniformly set at 1.6 years (19 months) according to the most recent FAO estimate for the Sudan [37]. The period of fertile life occupied by pregnancies and lactation should be regarded as a maximum estimate, because it does not take into account overlaps. Abortions (reported by 3 patients) were not considered. Ward's method was applied to the dissimilarity matrix to build a tree [50]. This method uses analysis of variance to evaluate distances between clusters, minimizing the sum of squares of any two hypothetical clusters that can be formed at each step.

Factor analysis using principal components analysis (PCA) was applied to age at BC diagnosis and to the above-described reproductive variables. This was done to determine the minimum number of factors among those considered that retained most of the dataset variance and to quantify the significance of the explained variance for each variable in dataset grouping(s). A scoring algorithm that loaded each individual variable most strongly onto the factor with which it was most correlated created summary factors. The adopted extraction method was a sum of squared factor loadings (eigenvalue) >1. PCA was developed by SPSS version 11.5 (SPSS Inc, Chicago, IL). A default setting

of 25 maximum iterations of algorithm steps to obtain convergence was used to extract factors. Factor scores were shown graphically, with a high factor score indicating a high influence of the factor on the clustering of the patient. *BRCA1/2* status was then correlated with the observed clustering.

Results

Cancer cases treated at INMO from May 1999 through December 2004 were reviewed. BC was the leading cancer and accounted for 20.4% (456/2,233) of all cancer patients admitted in the 5-year period. The MBC frequency was 3.5% (16/456), with mean age at MBC diagnosis of 51.9 years (±17.9, age range: 22–85 years). Mean age at diagnosis of the 440 FBC patients was 46.1 years (±13.7, age range: 20–90 years) and 275/440 patients (62.5%) were premenopausal (age 50 years or less). Overall 335/440 FBC cases (76%) occurred in parous women: 150/440 women (34%) reported ≥5 childbirths, 185/440 (42%) <5 childbirths and 105/440 (24%) were nulliparous.

All BC patients diagnosed within 40 years of age or of male gender ascertained between September 20, 2001 and October 23, 2002 were consecutively recruited and screened for *BRCA1/2* mutations using PTT, DHPLC and direct sequencing. This series included 34 FBC patients (age range 23–40 years, mean 34.3 ± 4.7 years) selected out of a total of 74 women with pathologically diagnosed BC (age range 23–80 years, mean 44 ± 11.7 years), and one 65-year-old MBC patient. Selected patients derived from 18 Central Sudanese tribes, including Ja-alia (9 patients), Kawahla (6 patients), Arakeia (4 patients), Danagla (2 patients) and other 14 tribes with one patient each. Twenty-two patients were from rural and 13 from urban settings. Most cases were diagnosed as advanced stage ductal carcinomas. The distribution of the selected FBC patients with regard to age at disease diagnosis and reproductive and clinical-pathological features is detailed in online Table S1.

A total of 60 sequence variants (32 in *BRCA1*, 28 in *BRCA2*) were detected in 33 of the 35 cases. Mutations, predicted effects, carrier frequencies, tribal origins of carriers, reported occurrence(s) of variants in major ethnic groups worldwide and relevant references are listed in online Table S2.

Truncating mutations (2 in *BRCA1*, 3 in *BRCA2*, Table 1) were detected in 5/35 patients (14%). These comprised the MBC patient and 4/34 FBC patients (12%), including 1/3 in the series with bilateral BC and 1/2 with clinically ascertained BC in a first-degree relative. With regard to tribal background 3 carriers were Arakeia; the other 2 belonged respectively to the Ja-alia and Hawsa tribes. Ages at BC diagnosis of the 4 FBC patients with truncating mutations ranged from 28 to 36 years, two patients were nulliparous and two reported two children each.

The majority of the studied cases (33/35, 94%) carried at least one of the 55 non-truncating variants. Five of these were predicted to affect protein product and were previously reported as unclassified (UV, 4 variants) or were novel (Table 1). These included 3 missense UVs (i.e., *BRCA1* p.Ser186Tyr, *BRCA1* p.Lys820Glu, *BRCA2* p.Arg2034His), a novel *BRCA2* missense variant (p.Pro41Leu) and a controversial *BRCA2* UV in the splice acceptor site of intron 2 (c.68-7delT), predicted to delete the amino-terminal transactivation domain encoded in exon 3 [51, 52]. *BRCA1* p.Ser186Tyr and *BRCA2* p.Pro41Leu were found in more than one patient and co-occurred with a trun-

Table 1 *BRCA1/2* truncating mutations and unclassified or novel non-truncating variants predicted to affect the *BRCA1/2* gene products identified in 11/35 BC patients

Mutation	Effect	Carrier(s) (%)
***BRCA1* c.3999delT**	**Stop (codon 1335)**	**S10 (3%)**
BRCA1 c.4065_4068delTCAA	Stop (codon 1364)	S15 (3%)
BRCA2 c.3195_3198delTAAT	Stop (codon 1075)	S8 (3%)
***BRCA2* c.6406_6407delTT**[a]	**Stop (codon 2139)**	**S20 (3%)**
***BRCA2* c.8642_8643insTTTT**	**Stop (codon 2907)**	**S35 (3%)**
BRCA1 c.557C>A[b]	p.Ser186Tyr (SIFT-intolerated)	S8, S10, S11, S33 (11%)
BRCA1 c.2458A>G	p.Lys820Glu (SIFT-tolerated)	S9 (3%)
***BRCA2* c.122C>T**[b]	**p.Pro41Leu** (SIFT-tolerated)	**S26, S35 (6%)**
BRCA2 c.6101G>A	p.Arg2034His (SIFT-tolerated)	S16 (3%)
BRCA2 c.68-7delT	In-frame deletion, exon 3	S26, S27 (6%)

Novel mutations in bold characters

[a]Detected in the MBC patient

[b]Co-occurring with a truncating mutation in the same gene

cating mutation in the same gene. Therefore these two mutations were regarded as most probably neutral [34, 53, 54]. *BRCA1* p.Lys820Glu and *BRCA2* p.Arg2034His were both SIFT-tolerated. The remaining 50 non-truncating variants, without reported or predicted effects, included polymorphisms, synonymous variants and sequence changes in introns and un-translated regions (detailed in online Table S2).

The *BRCA1/2* mutations detected in the BC series were highly diverse. Of 60 detected variants 17 (28%) were novel and are thus far unique to the Sudan, 21 (35%) were reported worldwide (i.e., in ethnic groups from at least 3 continents), 2 (3.3%) were reported only in Africans and the remaining variants comprised mutations previously detected in ethnic groups from Europe, Asia and the Americas (details in online Figure S1 and online Table S2).

It could be argued that the diverse mutational spectrum could reflect the tribal heterogeneity of the patients. To address this issue the *BRCA1/2* genotype structure of the study series was investigated using genetic and phylogenetic analyses [48, 49, 55]. Twenty-nine (29) *BRCA1/2* variants (48%) recurred in patients from different tribes (details in online Table S2).

AMOVA showed that 2.27% of the observed *BRCA1/2* variation was among tribes, with a low co-ancestry coefficient *Fst* of 0.022 ($P < 0.0001$); 11.32% was among individuals within tribes, with a relatively low inbreeding coefficient *Fis* of 0.115 ($P = 0.0254$), and 86.41% among individuals regardless of tribe with a high *Fit* coefficient of 0.135 ($P = 0.0273$). Altogether this indicates no association of *BRCA1/2* phased genotypes with tribes. Comparisons between pairs of tribes computed by pairwise of *Fst* values and exact test of population differentiation were both not significant, indicating lack of tribe-related differentiation.

The NJ tree, built to investigate relationships between individual sequence variation and tribal origin in a phylogenetic context, split patients in two clusters, identified with Roman numerals in Fig. 1, with cluster II showing significant bootstrap support (over 50%), which indicates phylogenetic relevance. Tribes represented by more than one patient (i.e., Ja-alia, Kawahla, Arakeia and Danagla) always split to both clusters. Four of the five carriers of truncating mutations (i.e., S8, S10, S35, Arakeias, and S15, Ja-alia) joined cluster II, one (S20, Hawsa) clustered in I. The NJ tree also highlighted that the truncating mutations detected in Arakeia patients S8 and S10 arose in individuals that were strictly related (as supported by bootstrap value) but that did not diverge significantly from the other patients, being embedded in the same cluster II genetic background. Overall genetic and phylogenetic

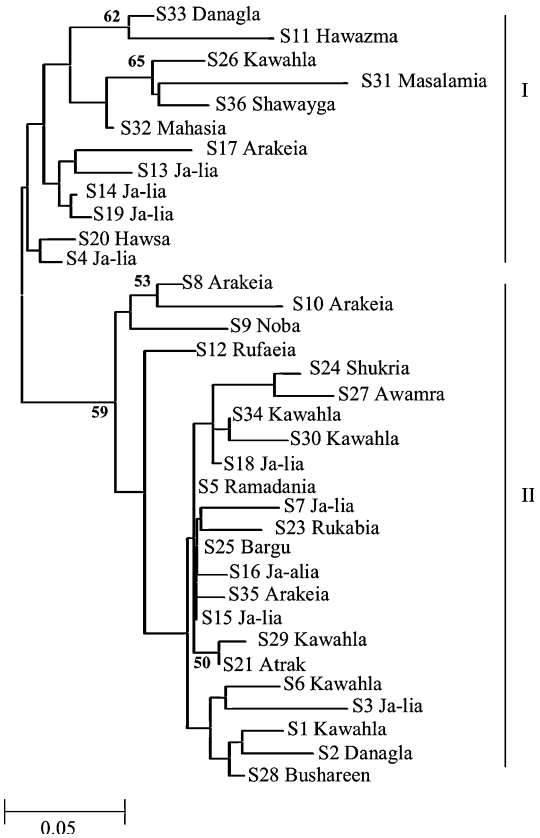

Fig. 1 Phylogenetic tree of the studied individuals based on the *BRCA1/2* genes. *BRCA1* and *BRCA2* sequences were aligned and analyzed for phylogenetic relationships using NJ algorithm, with the Kimura 2-parameter (uniform rate of heterogeneity applied) as distance measure. Support values are indicated at nodes when found in at least 50% of 1,000 bootstrap trees. The NJ tree split the individuals in two clusters (I and II) independently of tribal origins (distance bar is shown below the tree)

evidences show that the detected *BRCA1/2* sequence diversity was independent of tribal origins.

CA based on age at BC diagnosis and reproductive factors was used to verify the natural clustering of the 33 FBC patients for whom reproductive data were available. The obtained NJ tree split patients in two major clusters, respectively comprising 19 and 14 patients, designated A and B in Fig. 2 (data of patients detailed in online Table S3). Mean ages at BC diagnosis were 33.4 ± 5 years (range: 23–40 years) in A versus 35.7 ± 3.6 (range 30–40) in B, mean ages at menarche 13.7 ± 1.3 years (range: 12–18 years) in A versus 13.8 ± 1.2 (range 11–16) in B, patients in A reported 0–3 children and 0–5 individual lactation years versus 4–10 children and 6–16 lactation years for patients in B. Factor analysis using PCA was performed to better understand the features of the two clusters highlighted by the NJ tree. The goal was to determine the minimum number of factors among the

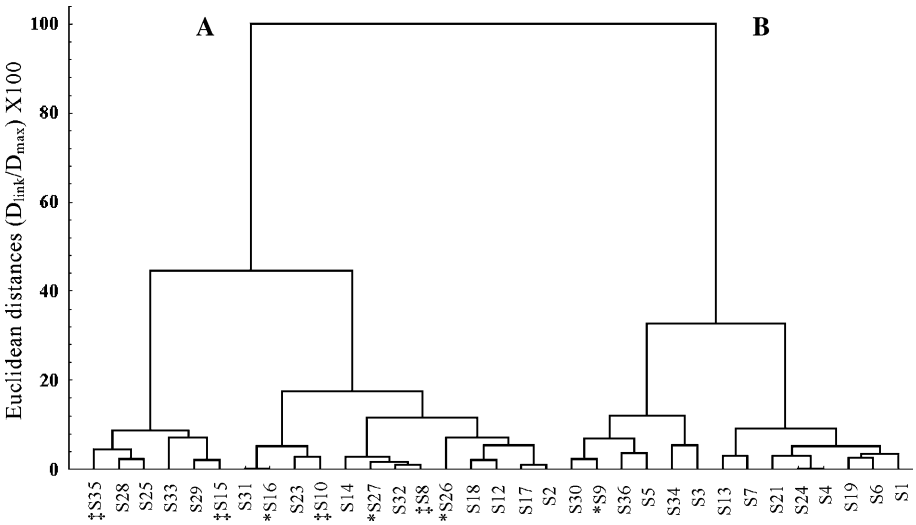

Fig. 2 Hierarchical clustering tree (Ward's method, Euclidean distances) based on four variables (age at cancer diagnosis, age at menarche, number of children and estimated total lactation period). The linkage distance (D_{link}) is presented as a percentage of the maximum linkage distance (D_{max}). The tree split patients in two clusters. Cluster A comprised 19 patients that included the 4 carriers of truncating mutations and 3 carriers of unclassified variants predicted to affect the *BRCA1/2* protein products. Cluster B comprised 14 patients, including 1 carrier of an unclassified missense variant. Carriers of truncating mutations and of unclassified variants predicted to affect the *BRCA1/2* protein products are designated by ‡ and * respectively

selected variables that retained most of the observed dataset variance. As expected, complete correlation ($r^2 = 1$) was observed between number of children and lactation years ($P < 0.05$). Although not significant, a slight positive correlation ($r^2 = 0.339$) was also observed between age and, respectively, number of children and lactation years. Factor analysis showed that two factors explained 80.093% of the dataset variance. The first factor extracted (eigenvalue = 2.192) accounted for the largest proportion of variance (54.812%) and corresponded to the total time period occupied by pregnancy and lactation, with a load of 0.972 each. The second factor (eigenvalue = 1.011) explained 25.281% of variance and corresponded to age at menarche, with a load of 0.979. A third factor (eigenvalue = 0.796) explained 19.907% of variance and corresponded to age at BC diagnosis, with a load of 0.806. The total variance of the three extracted factors explained 100% of the dataset variance. Factor scores of the total individual time periods occupied by pregnancy and lactation ranged from –1.303 to 2.734, with a median of 1.121. Factor scores ranged from –2.472 to 3.625 for age at menarche (median –1.324) and from –2.253 to 2.134 (median –1.100) for age at BC diagnosis. As indicated by factor scores the total individual time periods occupied by pregnancy and lactation was crucial for the NJ clustering of the patients (Fig. 3). Patients that joined cluster A had factor scores ≤–0.2 (Fig. 3), while factor scores ≥0.16 determined inclusion in cluster B (except for case S13, detailed below, that had a low factor score of –0.173). The more limited proportions of variance explained by age at BC diagnosis and age at menarche indicate that these factors had a low influence on the observed grouping pattern, with the exception of case S13, that joined cluster B because of the low value of the age at menarche factor (–1.551).

As shown in Figs. 2 and 3, cluster A included all 4 patients with truncating mutations and 3 out of the 4 carriers of unclassified variants predicted to affect gene product that did not co-occur with a truncating mutation in the same gene (i.e., patients S26 and S27, with *BRCA2* c.68-7delT, predicted to delete the amino-terminal transactivation domain, and patient S16, with *BRCA2* p.Arg2034His, reported as UV in BIC but SIFT-tolerated). Cluster B included patient S9, carrier of *BRCA1* p.Lys820Glu, also reported as UV in BIC but SIFT-tolerated.

Discussion

Little is known about BC in the Sudan. We report that BC was the most frequent malignancy clinically ascertained at INMO, Central Sudan, in the 5-year period 1999–2004. This agrees with the published cancer case series from the Radiation and Isotope Centre (RICK) in Khartoum (up to 1999 the only Sudanese cancer hospital), where BC is also reported as the leading malignancy [13]. Thus the available evidence suggests that BC is a major cancer of Suda-

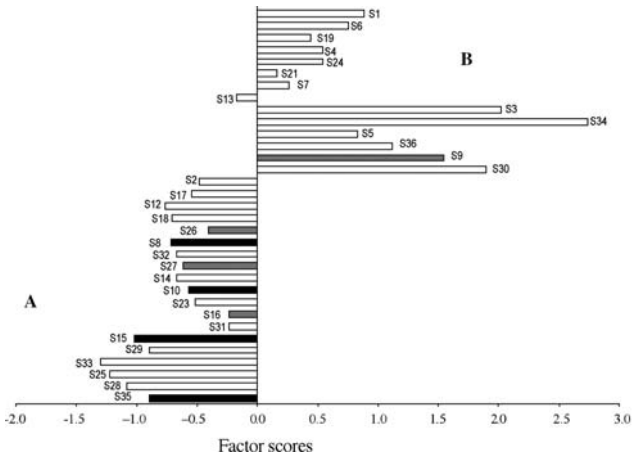

Fig. 3 Factor scores for the total time period occupied by pregnancy and lactation. This factor accounted for the largest proportion of dataset variance (54.812%), with a load of 0.972 each, and was crucial for hierarchical clustering. Patients that joined cluster A had factor scores ≤–0.2, while factor scores ≥0.16 determined inclusion in cluster B, except for S13 that joined cluster B because of the low value of the age at menarche factor (–1.551). Black bars, carriers of truncating mutations; gray bars, patients with UV variants predicted to affect the *BRCA1/2* protein products. Individual scores are in the order obtained by hierarchical clustering tree in Fig. 2

nese women, although in hospital-based series it might be over-represented relative to other malignancies, being a tumor that over time becomes readily evident.

Largely premenopausal presentation, considerable proportion of highly multiparous patients that lactated for prolonged periods and relatively high MBC frequency are relevant features of the INMO BC series. This agrees with the major characteristics of hospital-based BC series from other parts of sub-Saharan Africa [13–17] and is consistent with the young age structure and high fertility rate of the Sudanese population [37, 40, 41]. The above-mentioned characteristics also suggest an enrichment with disease due to highly penetrant etiologic factors of early onset and/or male BC, such as germ-line *BRCA1/2* mutations [3, 21, 22].

The prevalence of *BRCA1/2* carriers among clinic- or population-based series of FBC patients diagnosed within 40 years of age varies from up to about 10% in out-bred White Western populations to up to 30% in Ashkenazi Jews, an endogamous population with strong ''founder effect'' [34]. Only few studies investigated *BRCA1/2* mutation carrier status in indigenous African patients. In a Nigerian clinic-based series of 70 BC patients mostly diagnosed within 40 years of age the proportion (4%) with truncating *BRCA1/2* mutations was lower than described among young White FBC patients [56]. A study in the Sudan tested 1 MBC and 19 age-unselected FBC cases for mutations in *BRCA2* exon 11, with no identified mutations [57].

Our study focused on a series including all young (age ≤40 years) FBC patients and one MBC ascertained in a one-year period at INMO. Clearly deleterious truncating *BRCA1/2* mutations were detected in 5/35 patients (14%) (Table 1). One of these mutations (in *BRCA2*) occurred in the unique MBC patient, the other 4 (2 in *BRCA1*, 2 in *BRCA2*) were detected in 4/34 FBC patients (12%), of which 2 showed individual or family history characteristics known to be associated with *BRCA1/2* carrier status. Thus our data, although based on a small series, suggest that at INMO the proportion of early-onset FBC cases due to *BRCA1/2* could be at least comparable to the highest proportions found in series of young Western FBC patients [34]. As in most studies we probably underestimated deleterious *BRCA1/2* mutations because current screening techniques, although highly sensitive for point mutations and small insertions/deletions (the most common *BRCA1/2* mutation types), do not detect genomic rearrangements (that could contribute to the homozygous *BRCA1/2* variants observed in the series), mutations in regulatory sequences and epigenetic alterations [34, 58]. Many non-truncating *BRCA1/2* mutations considered as probably neutral in this study may need to be verified with additional epidemiological evidences from Central Sudan. Classification of rare missense variants is a major challenge [34, 53, 54]. Furthermore, the role of the controversial *BRCA2* c.68-7delT mutation in the splice acceptor site of intron 2, detected in two patients, remains highly ambiguous. This variant, predicted to delete *BRCA2* exon 3, which encodes a highly conserved transcriptional activation domain, was originally detected as a somatic mutation in sporadic endometrial and colorectal cancers with high-level microsatellite instability [51, 52, 59]. However, the role of *BRCA2* c.68-7delT is difficult to interpret, because exon 3 undergoes alternative splicing physiologically and normal tissues, including mammary gland, express a BRCA2 protein lacking the putative aminoterminal transcriptional activation domain [52, 59].

The spectrum of detected *BRCA1/2* mutations comprised a relevant fraction of unique variants combined with variants known to occur in worldwide populations. This is not unexpected in view of the well-documented genetic diversity of Africans [60] and of the *BRCA1/2* variation found in African-Americans [44, 56, 61–64]. The interpretation of the considerable sequence diversity observed in the currently studied series is complicated by the fact that the Sudanese population is organized in tribes [35, 60]. Addressing this issue we examined the relationships between *BRCA1/2* sequence variation and tribal origin in a phylogenetic context.

Although the Sudanese tribes are considered traditionally endogamous, AMOVA results showed that most of the detected *BRCA1/2* genetic variation was not associated with tribes. Furthermore, tribal pairwise differences and the exact test of population differentiation indicated lack of tribe-related differentiation. The observed *BRCA1/2* sequence diversity agrees with reported data concerning mitochondrial DNA variation in the Upper Nile River Valley [65] and with the historic/archaeological record of the region [66, 67]. These lines of evidence concordantly suggest that within the past few hundred to few thousand years Central Sudan has been a corridor for bi-directional migrations between Mediterranean and sub-Saharan Africa rather than a genetically and culturally isolated region [65]. Based on *BRCA1/2* sequence variation the patients split in two discrete clusters independent of tribes, one of which had bootstrap support indicating phylogenetic relevance. These clusters may reflect distinct ethnic components that contributed to the population of Central Sudan prior to the definition of the present tribes [65].

Literature data about the relationship between reproductive factors and *BRCA1/2*-related BC risk are rather conflicting. Narod and co-authors [68] found a higher BC rate and an earlier age at BC diagnosis in recent birth cohorts of women with *BRCA1* mutations compared to older relatives, a finding which could be partially explained by differences in reproductive history [68]. This would agree with studies suggesting an association between parity and decreased lifetime BC risk in *BRCA1* carriers, particularly with early age at first live birth and parity of 3 or more [68–70]. However, other studies suggest that in women with *BRCA1/2* mutations parity may increase BC risk, particularly before 40 years of age [71–74]. More recently a large international case–control study found that BC risk in the 2 years after childbirth slightly increased in *BRCA2* carriers, whereas lifetime risk was modestly reduced among *BRCA1* carriers with 4 or more children [75]. A slight to moderate reduction in BC risk was also reported in *BRCA1* carriers who breast-fed for at least one year, although no effect of lactation could be demonstrated for *BRCA2* carriers [76]. With respect to this debate it is of interest to explore interactions between *BRCA1/2* status and parity combined with prolonged lactation, a situation that, due to the comparatively long breast-feeding dependency of infants, reflects a physiological adaptation of the human mammary gland, but that is currently unusual among Western women [19].

After CA based on age at BC diagnosis, age at menarche, number of childbirths and estimated total lactation, we observed that the tested Sudanese FBC patients split in two clusters, being the lifetime period occupied by pregnancy and lactation crucial for clustering. The two clusters were respectively characterized by low versus high factor scores of the total individual time periods occupied by pregnancy and lactation. The cluster with low factor scores comprised all the 4 carriers of truncating mutations, together with 3 out of the 4 carriers of unclassified variants predicted to affect gene product that did not co-occur with a truncating mutation in the same gene (i.e., S26 and S27, with *BRCA2* c.68-7delT, predicted to delete exon 3; S16, with *BRCA2* p.Arg2034His, reported as UV in BIC but SIFT-tolerated). Patient S9, who carried *BRCA1* p.Lys820Glu, the other missense mutations reported as UV in BIC (also SIFT-tolerated), joined the cluster with high factor scores.

In conclusion, we explored interactions between BC and hereditary, ethnic and reproductive factors in a specific sub-Saharan African context. Overall, our findings suggest that in Central Sudan *BRCA1/2* mutations represent an important etiological factor of BC in males and young women less exposed to pregnancy and lactation, regardless of tribal origin. On the other hand other risk factors, possibly promoted by pregnancy and lactation, could contribute to the disease ascertained in young highly multiparous women, who might instead be protected from *BRCA1/2*-related BC.

This study highlights the importance of the two major BC susceptibility genes in the etiology of BC ascertained in a specific sub-Saharan African context, but could also be relevant to wider contexts. A better understanding of the causes of BC in Africa could provide new insight into the factors that influence mammary carcinogenesis and could contribute to the design of BC preventive strategies, particularly in the setting of developing nations.

Acknowledgments We thank the patients who took part in this research. Our study was supported by *Associazione Italiana per la Ricerca sul Cancro* (AIRC), the Italian Ministry of Education, University and Research (MIUR-COFIN project 2003 ''Molecular epidemiology and analysis of tumor progression in familial breast-ovarian cancer'', coordinated by S. Venuta). Dr. Awadelkarim is the recipient of a Research Contract supported by the Faculty of Medicine, G. d'Annunzio University, Chieti. We thank the personnel of the Italian Embassy in Khartoum and of the Sudanese Embassy in Rome (in particular Mr. Saif Mahmoud) for their kind assistance. Collaboration between CeSI and INMO is within the framework of activities developed by CeSI as Special Consultant of ECOSOC of the United Nations.

References

1. Medina D (2005) Mammary developmental fate and breast cancer risk. Endocr Relat Cancer 12:483–495

2. Ursin G, Bernstein L, Wang Y, Lord SJ, Deapen D, Liff JM, Norman SA, Weiss LK, Daling JR, Marchbanks PA, Malone KE, Folger SG, McDonald JA, Burkman RT, Simon MS, Strom BL, Spirtas R (2004) Reproductive factors and risk of breast carcinoma in a study of white and African-American women. Cancer 101:353–362
3. Veronesi U, Boyle P, Goldhirsch A, Orecchia R, Viale G (2005) Breast cancer. Lancet 365:1727–1741
4. Clavel-Chapelon F (2002) Differential effects of reproductive factors on the risk of pre- and postmenopausal breast cancer. Results from a large cohort of French women. Br J Cancer 86:723–727
5. Kelsey JL, Gammon MD, John EM (1993) Reproductive factors and breast cancer. Epidemiol Rev 15:36–47
6. Chlebowski RT, Chen Z, Anderson GL, Rohan T, Aragaki A, Lane D, Dolan NC, Paskett ED, McTiernan A, Hubbell FA, Adams-Campbell LL, Prentice R (2005) Ethnicity and breast cancer: factors influencing differences in incidence and outcome. J Natl Cancer Inst 97:439–448
7. Pathak DR, Osuch JR, He J (2000) Breast carcinoma etiology: current knowledge and new insights into the effects of reproductive and hormonal risk factors in black and white populations. Cancer 88:1230–1238
8. Hanby AM (2005) Aspects of molecular phenotype and its correlations with breast cancer behaviour and taxonomy. Br J Cancer 92:613–617
9. Parkin DM, Bray F, Ferlay J, Pisani P (2005) Global cancer statistics, 2002. CA Cancer J Clin 55:74–108
10. Mosher WD, Deang LP, Bramlett MD (2003) Community environment and women's health outcomes: contextual data. Vital Health Stat 23:1–72
11. Ramachandran S, Deshpande O, Roseman CC, Rosenberg NA, Feldman MW, Cavalli-Sforza LL (2005) Support from the relationship of genetic and geographic distance in human populations for a serial founder effect originating in Africa. Proc Natl Acad Sci USA 102:15942–15947
12. Strassmann BI (1999) Menstrual cycling and breast cancer: an evolutionary perspective. J Womens Health 8:193–202
13. Parkin DM, Ferlay J, Hamdi-Cherif M, Sistas F, Thomas J, Wabinga H, Whelan SL (2003) Cancer in Africa: epidemiology and prevention. IARC PRESS, Lyon
14. Amir H, Kwesigabo G, Aziz MR, Kitinya JN (1996) Breast cancer and conservative surgery in sub Saharan Africa. East Afr Med J 73:83–87
15. Adesunkanmi AR, Lawal OO, Adelusola KA, Durosimi MA (2005) The severity, outcome and challenges of breast cancer in Nigeria. Breast 15:399–409
16. Mbonde MP, Amir H, Mbembati NA, Holland R, Schwartz-Albiez R, Kitinya JN (1998) Characterisation of benign lesions and carcinomas of the female breast in a sub-Saharan African population. Pathol Res Pract 194:623–629
17. Fregene A, Newman LA (2005) Breast cancer in sub-Saharan Africa: how does it relate to breast cancer in African-American women? Cancer 103:1540–1550
18. Gelbard A (1997) Population 101. A primer. Popul Today 25:4–5
19. Collaborative Group on Hormonal Factors in Breast Cancer (2002) Breast cancer and breastfeeding: collaborative reanalysis of individual data from 47 epidemiological studies in 30 countries, including 50302 women with breast cancer and 96973 women without the disease. Lancet 360:187–195
20. Russo J, Moral R, Balogh GA, Mailo D, Russo IH (2005) The protective role of pregnancy in breast cancer. Breast Cancer Res 7:131–142
21. Lambe M, Hsieh C, Trichopoulos D, Ekbom A, Pavia M, Adami HO (1994) Transient increase in the risk of breast cancer after giving birth. N Engl J Med 331:5–9
22. Largent JA, Ziogas A, Anton-Culver H (2005) Effect of reproductive factors on stage, grade and hormone receptor status in early-onset breast cancer. Breast Cancer Res 7:R541–554
23. Hiraku Y, Yamashita N, Nishiguchi M, Kawanishi S (2001) Catechol estrogens induce oxidative DNA damage and estradiol enhances cell proliferation. Int J Cancer 92:333–337
24. Palmer JR, Wise LA, Horton NJ, Adams-Campbell LL, Rosenberg L (2003) Dual effect of parity on breast cancer risk in African-American women. J Natl Cancer Inst 95:478–483
25. Enger SM, Ross RK, Henderson B, Bernstein L (1997) Breastfeeding history, pregnancy experience and risk of breast cancer. Br J Cancer 76:118–123
26. Newcomb PA, Storer BE, Longnecker MP, Mittendorf R, Greenberg ER, Clapp RW, Burke KP, Willett WC, MacMahon B (1994) Lactation and a reduced risk of premenopausal breast cancer. N Engl J Med 330:81–87
27. Kelsey JL, John EM (1994) Lactation and the risk of breast cancer. N Engl J Med 330:136–137
28. Tryggvadottir L, Tulinius H, Eyfjord JE, Sigurvinsson T (2001) Breastfeeding and reduced risk of breast cancer in an Icelandic cohort study. Am J Epidemiol 154:37–42
29. Yuan JM, Yu MC, Ross RK, Gao YT, Henderson BE (1988) Risk factors for breast cancer in Chinese women in Shanghai. Cancer Res 48:1949–1953
30. Zheng T, Duan L, Liu Y, Zhang B, Wang Y, Chen Y, Zhang Y, Owens PH (2000) Lactation reduces breast cancer risk in Shandong Province, China. Am J Epidemiol 152:1129–1135
31. Coogan PF, Rosenberg L, Shapiro S, Hoffman M (1999) Lactation and breast carcinoma risk in a South African population. Cancer 86:982–989
32. Michels KB, Willett WC, Rosner BA, Manson JE, Hunter DJ, Colditz GA, Hankinson SE, Speizer FE (1996) Prospective assessment of breastfeeding and breast cancer incidence among 89,887 women. Lancet 347:431–436
33. Friedman LC, Kramer RM (2005) Reproductive issues for women with BRCA mutations. J Natl Cancer Inst Monogr 34:83–86
34. Thompson D, Easton D (2004) The genetic epidemiology of breast cancer genes. J Mammary Gland Biol Neoplasia 9:221–236
35. Tay JS, Saha N (1988) Genetic heterogeneity among the Negroid and Arab tribes of the Sudan. Am J Phys Anthropol 76:211–215
36. El Moghraby AI (2003) State of the environment in the Sudan. In: McCabe M, Sadler B (eds) UNEP studies of EIA practice in developing countries. United Nations Environment Programme (UNEP), Geneva, pp 27–36
37. FAO (2005) Sudan nutrition profile – food and nutrition division. FAO ftp://ftp.fao.org/es/esn/nutrition/ncp/sdn.pdf
38. Cho E, Spiegelman D, Hunter DJ, Chen WY, Stampfer MJ, Colditz GA, Willett WC (2003) Premenopausal fat intake and risk of breast cancer. J Natl Cancer Inst 95:1079–1085
39. Nkondjock A, Robidoux A, Paredes Y, Narod SA, Ghadirian P (2006) Diet, lifestyle and BRCA-related breast cancer risk among French-Canadians. Breast Cancer Res Treat 1–10, DOI 10.1007/s10549-006-9161-8, URL http://dx.doi.org/10.1007/s10549-006-9161-8
40. El Tahir Taha T (1993) Family planning practice in central Sudan. Soc Sci Med 37:685–689
41. Eltigani EE (2001) Childbearing in five Arab countries. Stud Fam Plann 32:17–24

42. Ottini L, Masala G, D'Amico C, Mancini B, Saieva C, Aceto G, Gestri D, Vezzosi V, Falchetti M, De Marco M, Paglierani M, Cama A, Bianchi S, Mariani-Costantini R, Palli D (2003) BRCA1 and BRCA2 mutation status and tumor characteristics in male breast cancer: a population-based study in Italy. Cancer Res 63:342–347
43. Gross E, Arnold N, Pfeifer K, Bandick K, Kiechle M (2000) Identification of specific BRCA1 and BRCA2 variants by DHPLC. Hum Mutat 16:345–353
44. Wagner TM, Hirtenlehner K, Shen P, Moeslinger R, Muhr D, Fleischmann E, Concin H, Doeller W, Haid A, Lang AH, Mayer P, Petru E, Ropp E, Langbauer G, Kubista E, Scheiner O, Underhill P, Mountain J, Stierer M, Zielinski C, Oefner P (1999) Global sequence diversity of BRCA2: analysis of 71 breast cancer families and 95 control individuals of worldwide populations. Hum Mol Genet 8:413–423
45. Ng PC, Henikoff S (2002) Accounting for human polymorphisms predicted to affect protein function. Genome Res 12:436–446
46. Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 1:47–50
47. Wright S (1951) The genetical structure of populations. Ann Eugen 15:323–354
48. Kumar S, Tamura K, Nei M (2004) MEGA3: integrated software for molecular evolutionary genetics analysis and sequence alignment. Brief Bioinform 5:150–163
49. Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol 16:111–120
50. Ward JH Jr (1963) Hierarchical grouping to optimize an objective function. J Am Stat Assoc 58:236–244
51. Koul A, Nilbert M, Borg A (1999) A somatic BRCA2 mutation in RER+ endometrial carcinomas that specifically deletes the amino-terminal transactivation domain. Genes Chromosomes Cancer 24:207–212
52. Santarosa M, Viel A, Boiocchi M (1999) Splice variant lacking the transactivation domain of the BRCA2 gene and mutations in the splice acceptor site of intron 2. Genes Chromosomes Cancer 26:381–382
53. Gomez-Garcia EB, Ambergen T, Blok MJ, van den Wijngaard A (2005) Patients with an unclassified genetic variant in the BRCA1 or BRCA2 genes show different clinical features from those with a mutation. J Clin Oncol 23:2185–2190
54. Chenevix-Trench G, Healey S, Lakhani S, Waring P, Cummings M, Brinkworth R, Deffenbaugh AM, Burbidge LA, Pruss D, Judkins T, Scholl T, Bekessy A, Marsh A, Lovelock P, Wong M, Tesoriero A, Renard H, Southey M, Hopper JL, Yannoukakos K, Brown M, Easton D, Tavtigian SV, Goldgar D, Spurdle AB (2006) Genetic and histopathologic evaluation of BRCA1 and BRCA2 DNA sequence variants of unknown clinical significance. Cancer Res 66:2019–2027
55. Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491
56. Gao Q, Adebamowo CA, Fackenthal J, Das S, Sveen L, Falusi AG, Olopade O (2000) Protein truncating BRCA1 and BRCA2 mutations in African women with pre-menopausal breast cancer. Hum Genet 107:192–194
57. Masri MA, Abdel Seed NM, Fahal AH, Romano M, Baralle F, El Hassam AM, Ibrahim ME (2002) Minor role for BRCA2 (exon11) and p53 (exon 5–9) among Sudanese breast cancer patients. Breast Cancer Res Treat 71:145–147
58. Unger MA, Nathanson KL, Calzone K, Antin-Ozerkis D, Shih HA, Martin AM, Lenoir GM, Mazoyer S, Weber BL (2000) Screening for genomic rearrangements in families with breast and ovarian cancer identifies BRCA1 mutations previously missed by conformation-sensitive gel electrophoresis or sequencing. Am J Hum Genet 67:841–850
59. Zou JP, Hirose Y, Siddique H, Rao VN, Reddy ES (1999) Structure and expression of variant BRCA2a lacking the transactivation domain. Oncol Rep 6:437–440
60. Saha N, Tay JS, El Sheikh FS (1989) Inbreeding effects on average heterozygosity in the Sudan. Jinrui Idengaku Zasshi 34:225–229
61. Gao Q, Tomlinson G, Das S, Cummings S, Sveen L, Fackenthal J, Schumm P, Olopade OI (2000) Prevalence of BRCA1 and BRCA2 mutations among clinic-based African American families with breast cancer. Hum Genet 107:186–191
62. Pal T, Permuth-Wey J, Holtje T, Sutphen R (2004) BRCA1 and BRCA2 mutations in a study of African American breast cancer patients. Cancer Epidemiol Biomarkers Prev 13:1794–1799
63. Freedman ML, Penney KL, Stram DO, Riley S, McKean-Cowdin R, Le Marchand L, Altshuler D, Haiman CA (2005) A haplotype-based case-control study of BRCA1 and sporadic breast cancer risk. Cancer Res 65:7516–7522
64. Freedman ML, Penney KL, Stram DO, Le Marchand L, Hirschhorn JN, Kolonel LN, Altshuler D, Henderson BE, Haiman CA (2004) Common variation in BRCA2 and breast cancer risk: a haplotype-based analysis in the Multiethnic Cohort. Hum Mol Genet 13:2431–2441
65. Krings M, Salem AE, Bauer K, Geisert H, Malek AK, Chaix L, Simon C, Welsby D, Di Rienzo A, Utermann G, Sajantila A, Paabo S, Stoneking M (1999) mtDNA analysis of Nile River Valley populations: a genetic corridor or a barrier to migration? Am J Hum Genet 64:1166–1176
66. Clark JD, Fage JD, Oliver R (1982) The Cambridge history of Africa: volume 1: from the earliest time to 500 BC. Cambridge University Press
67. Fage JD, Oliver R (1979) The Cambridge history of Africa: volume 2: from 500 BC to AD 1050. Cambridge University Press
68. Narod SA, Goldgar D, Cannon-Albright L, Weber B, Moslehi R, Ives E, Lenoir G, Lynch H (1995) Risk modifiers in carriers of BRCA1 mutations. Int J Cancer 64:394–398
69. Rebbeck TR, Wang Y, Kantoff PW, Krithivas K, Neuhausen SL, Godwin AK, Daly MB, Narod SA, Brunet JS, Vesprini D, Garber JE, Lynch HT, Weber BL, Brown M (2001) Modification of BRCA1- and BRCA2-associated breast cancer risk by AIB1 genotype and reproductive history. Cancer Res 61:5420–5424
70. King MC, Marks JH, Mandell JB (2003) Breast and ovarian cancer risks due to inherited mutations in BRCA1 and BRCA2. Science 302:643–646
71. Narod SA (2002) Modifiers of risk of hereditary breast and ovarian cancer. Nat Rev Cancer 2:113–123
72. Johannsson O, Loman N, Borg A, Olsson H (1998) Pregnancy-associated breast cancer in BRCA1 and BRCA2 germline mutation carriers. Lancet 352:1359–1360
73. Jernstrom H, Lerman C, Ghadirian P, Lynch HT, Weber B, Garber J, Daly M, Olopade OI, Foulkes WD, Warner E, Brunet JS, Narod SA (1999) Pregnancy and risk of early breast cancer in carriers of BRCA1 and BRCA2. Lancet 354:1846–1850
74. Tryggvadottir L, Olafsdottir EJ, Gudlaugsdottir S, Thorlacius S, Jonasson JG, Tulinius H, Eyfjord JE (2003) BRCA2 mutation carriers, reproductive factors and breast cancer risk. Breast Cancer Res 5:R121–128

75. Cullinane CA, Lubinski J, Neuhausen SL, Ghadirian P, Lynch HT, Isaacs C, Weber B, Moller P, Offit K, Kim-Sing C, Friedman E, Randall S, Pasini B, Ainsworth P, Gershoni-Baruch R, Foulkes WD, Klijn J, Tung N, Rennert G, Olopade O, Couch F, Wagner T, Olsson H, Sun P, Weitzel JN, Narod SA (2005) Effect of pregnancy as a risk factor for breast cancer in BRCA1/BRCA2 mutation carriers. Int J Cancer 117:988–991

76. Jernstrom H, Lubinski J, Lynch HT, Ghadirian P, Neuhausen S, Isaacs C, Weber BL, Horsman D, Rosen B, Foulkes WD, Friedman E, Gershoni-Baruch R, Ainsworth P, Daly M, Garber J, Olsson H, Sun P, Narod SA (2004) Breastfeeding and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. J Natl Cancer Inst 96:1094–1098