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Identification of *BRCA1/2* p.Ser1613Gly, p.Pro871Leu, p.Lys1183Arg, p.Glu1038Gly, p.Ser1140Gly, p.Ala2466Val, p.His2440Arg variants in women under 45 years old with breast nodules suspected of having breast cancer in Burkina Faso

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Abstract: Breast cancer is the top cause of cancer mortality among women in the world and the second in Africa. The aims of this study were to: i) identify women with breast nodules suspected of having breast cancer ii) sequence the *BRCA1* and *BRCA2* genes and iii) screen mutations. From

2015 to 2016, 112 women aged from 35 to 44 years, who had come for consultation in the gynecology/obstetrics and the oncology department of the University Hospital Yalgado Ouedraogo, voluntarily agreed to participate to this study. Whole blood was collected from those with mammary nodules. The genomic DNA was extracted using Qiagen kit. FAST KAPA was used for genomic DNA amplification and the purified PCR products were analyzed by direct sequencing using Big Dye v1.1 and ABI 3730 automated sequencer. Nucleotides substitutions were determined. We identified *BRCA1* SNPs rs1799966, rs799917, rs16942, rs16941, rs2227945, and *BRCA2* SNPs rs169547, rs4986860. These identified variants are found mostly in cases of benign tumors of breast or ovarian cancer with familial history of breast cancer. This study in Burkina-Faso, is the basis for improved and more specific genetic testing, and suggests that additional genes contributing to an increased risk of breast cancer should be analyzed.

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Introduction

Breast cancer is the most common cancer in women, especially in those over 50 years old for whom it is the leading cause of death worldwide. The global trend in the epidemiology of breast cancer in African countries is not far from that observed in the western countries of the world. Breast cancer is the second most frequent cancer both in the totality of African countries and in the

specific area of Western Africa (which comprises Burkina Faso) accounting for the 25 to 30% of cases of cancers [1]. Nevertheless, its incidence is higher in developed than in developing countries (64.2/100,00 vs 23.1/100,000 women) [2].

Breast cancer is a malignant tumor of the mammary gland. This cancer is most common in women, with 89 cases per 100,000. 5 to 10% of these cancers have a clear genetic basis, whereas 85-90% of cases (called sporadic or non-hereditary) have a complex etiology still poorly understood [3-6]. In Burkina Faso (one of the west African countries) breast cancer is being responsible for 17.7% of women's deaths [7]. A recent study provide preliminary information about breast cancer risk decreasing with multiparity among women in Burkina Faso [8, 9]. Genetic and non-genetic factors are involved in the etiology of breast cancer.

The genetics of breast cancer in African countries is generally undetermined. A genetic test may reveal the risk to women from a family where there is a history of mutations in a specific gene [10, 11]. The lack of data on the genetics of breast cancer in African countries might be due to lack of diagnostic tools in molecular biology, preventing women from performing this test [2, 12-15]. Two predisposing genes for breast cancer have been identified [16, 17] so far: *BRCA1* on chromosome 17 (more than 500 mutations or sequence variations have been identified) and *BRCA2* on chromosome 13 (over 100 different mutations have been reported). Women who have inherited mutations in *BRCA1* (17q21, chromosome 17: base pairs 43,044,294 to 43,125,482) or *BRCA2* (13q12.3, chromosome 13: base pairs 32,315,479 to 32,399,671) have an increased risk of developing breast and ovarian cancers [11, 17]. The probability of developing breast cancer in a carrier of a *BRCA1* mutation is about 65% before the age of 70 and 45% for carriers of mutations in the *BRCA2* [3, 18, 19]{Couch, 1997 #2200;Whittemore, 1997 #2219;Mote, 2004 #2212;Whittemore, 1997 #2219}. In a study by Rebbeck *et al*, among *BRCA1* mutation carriers, 46% of women were diagnosed with breast cancer, 12% with ovarian cancer, 5% with breast and ovarian cancer, and 37% without cancer. And among *BRCA2* mutation carriers, 52% of women were diagnosed with breast cancer, 6% with ovarian cancer, 2% with breast and ovarian cancer, and 40% without cancer [20].

The available information about breast cancer in Burkina Faso, regarding the histo-pathological characteristics of tumors and parity risk factors, is limited [8, 9], with few specific records about genetic features [8, 9, 21].

The aim of this study was to provide the first ever cancer related genetic information for a West African

country. To achieve this aim, these specific objectives were targeted: i) identify women with breast nodules suspected of being breast cancer; ii) determine genetic variants associated with breast cancer in Burkina Faso by PCR.

Materials and methods

Study population

This study took place from June 2015 to May 2016 and was approved by the Ethics Committee for Health Research of Burkina Faso (N ° 2014-8-098). The eligibility criteria were i) patients with diagnosis of breast cancer before age 45; ii) patients able to provide informed consent to complete detailed epidemiological questionnaire, which includes details of family history of cancer; and iii) patients provided a blood sample for genetic analysis. After obtaining written informed consent from each women, aged from 35 to 44 years old (with an average of 40.00 ± 3.04) with breast nodules suspected of having breast cancer, blood specimens were collected from the General Surgery and Gynecology-Obstetrics services and oncology department of the University Hospital Yalgado Ouedraogo.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Ethics Committee for Health Research of Burkina Faso (N ° 2014-8-098).

Informed consent: Informed consent has been obtained from all individuals included in this study.

BRCA1 and *BRCA2* mutation screening

The genomic DNA was extracted from blood specimens with a Qiagen kit at the Biomolecular Research Center Pietro Annigoni (CERBA) of Ouagadougou, Burkina Faso. The remaining steps for the genetic analysis of DNA were performed at the St'Orsola-Malpighi Hospital, Laboratory of Medical genetics, University of Bologna, Italy. The entire coding sequence and part of the regulatory sequence of *BRCA1* and *BRCA2* were amplified by PCR using Fast KAPA Master Mix 2x with the following cycling protocol: 95°C 1 min (1 step); 95°C 15 s, 58°C 15 s, 72°C 15 s (40 cycles); 72°C 15 s (1 step). Sequences were resuspended in Injection Buffer (10 µL, Millipore) and loaded onto the ABI 3730 automated sequencer (Thermofisher Scientific, Foster City, CA).



Figure 1: *BRCA2*, Exon 21 and 22 PCR product profile in agar gel 2%. Legend: L: Fermentas GeneRuler™ DNA Ladder #SM0313; N: Negative control; Number 1 to 9: specimens.

Sequence analysis

Data from the ABI3730 sequencing machine were analyzed using the software Chromas lite v2.5. We compared the sequences with the reference sequence reported in “Ensembl genome” browser and the variants were classified by comparison using dbSNP and ClinVar.

Results

Patient’s characteristics

From 2015 to 2016, 112 women aged from 35 to 44 (average of 40.00 ± 3.04 years), who had come for consultation in the service of General Surgery and Gynecology-Obstetrics services and the oncology department of the University Hospital Yalgado Ouedraogo, voluntarily agreed to participate in this study. Based on the eligibility criteria, 9 women were screened for genetic breast cancer. Of the 9 eligible patients, 2 had been diagnosed with cancer before the age of 36 and 2 other patients had a family history of cancer (1 breast cancer and 1 foot cancer) and all were at the level T4 of TNM.

Mutation screening

After loading the PCR products on 2% agarose gel (3 mL) they were visualized under UV light as reported in Fig 1 (examples of *BRCA1* and *BRCA2* profiles). An example of the sequencing profile of an heterozygous specimen is reported in Fig 2 in exon 10 of *BRCA1*: a non-synonymous and heterozygous change at the 6th base pair is present

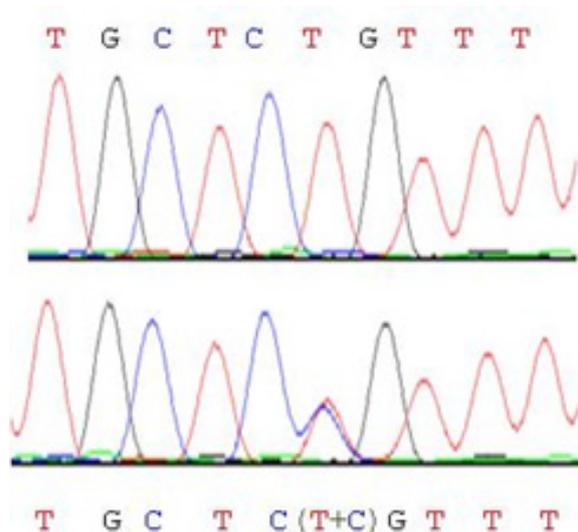


Figure 2: Sequencing profile of a DNA. Legend: variation heterozygous change at the 6th base pair (T/T+C).

in the figure corresponding to the change c.2622C>T; p.Leu871Pro, rs799917), where a clear double peak of C/T could be identified in the lower panel. The upper panels shows a non-synonymous change but in homozygous state. All specimens had a non-synonymous change at this site.

Variants

All women in this study had at least one genetic variation, such as synonymous or non-synonymous amino acid variation. Some also had intron variations. In specimen 1, which had more genetic variation than other specimens, we identified a 3'UTR change in the last exon of *BRCA1*

Table 1: *BRCA1* and *BRCA2* genes variants by specimen.

S	S1	S2	S3	S4	S5	S6	S7	S8	S9
BRCA1									
12	-	-	-	-	-	-	-	c.4308T>C p.Ser1436=	c.4308T>C p.Ser1436=
15	rs1799966 c.4837A>G p.Ser1613Gly	-	-	-	-	-	-	-	-
23	3'UTR rs3092995 c.*36C>G	-	-	-	-	-	-	-	-
10	-	c.1236A>G p.Val 412=	c.1236A>G p.Val 412=	c.1236A>G p.Val 412=	c.1236A>G p.Val 412=	-	-	-	-
	c.2082C>T p.Ser694=	-	-	-	c.657A>G p.Gln1970=	-	-	1) c.2082C>T p.Ser694= 2) c.2311T>C p.Leu771=	-
	rs799917 c.2612C>T p.Pro871Leu	rs799917 c.2612C>T p.Pro871Leu	rs799917 c.2612C>T p.Pro871Leu	rs799917 c.2612C>T p.Pro871Leu	rs799917 c.2612C>T p.Pro871Leu	rs799917 c.2612C>T p.Pro871Leu	rs799917 c.2612C>T p.Pro871Leu	rs799917 c.2612C>T p.Pro871Leu	rs799917 c.2612C>T p.Pro- 871Leu
	rs16942 c.3548A>G p.Lys1183Arg	rs2227945 c.3418A>G p.Ser1140Gly	-	-	-	-	-	1) rs16942 c.3548A>G p.Lys1183Arg 2) rs16941 c.3113A>G p.Glu1038Gly	1) rs16942 c.3548A>G p.Lys1183Arg 2) rs16941 c.3113A>G p.Glu1038Gly
BRCA2									
11	-	-	-	-	c.3394A>G p.Lys1132=	-	-	-	-
	c.2082C>T p.Ser694=	-	-	-	-	-	-	-	-
14	1) c.7242A>G p.Ser2414= 2) rs169547 c.7397C>T p.Ala2466Val	1) c.7242A>G p.Ser2414= 2) rs169547 c.7397C>T p.Ala- 2466Val	1) rs4986860 c.7319A>G p.His2440Arg 2) rs169547 c.7397C>T p.Ala- 2466Val	rs169547 c.7397C>T p.Ala2466Val	1) c.7242A>G p.Ser2414= 2) rs169547 c.7397C>T p.Ala2466Val	rs169547 c.7397C>T p.Ala2466Val	rs169547 c.7397C>T p.Ala2466Val	c.6513G>C p.Val2171= rs169547 c.7397C>T p.Ala2466Val	rs169547 c.7397C>T p.Ala- 2466Val

S: Specimen; Ser: Serine; Gly: Glycine; Pro: Proline; Leu: Leucine; Lys: Lysine; Arg: Arginine; Val: Valine; Ala: Alanine; His: Histidine; Gln: Glutamine; Glu: Glutamic acid

Table 2: List of known *BRCA1* SNPs identified in this study and minor allele frequencies compared with other populations.

Gene	SNP	Amino acid change	Protein change	Chrs:bp dbSNP	Allele Frequency	Frequency*	ASW	ESN	YRI	AFR	EUR
<i>BRCA1</i>	rs1799966	c.4837A>G	p.Ser1613Gly	17:43071077	A/G 0.356 (G)	0.111 (1/9)	0.246	0.172	0.194	0.225	0.360
	rs799917	c.2612C>T	p.Pro871Leu	17:43092919	C/T 0.544 (T)	0.889 (8/9)	0.262	0.944	0.074	0.885	0.738
	rs16942	c.3548A>G	p.Lys1183Arg	17:43091983	A/G 0.353 (G)	0.333 (3/9)	0.246	0.167	0.181	0.218	0.355
	rs16941	c.3113A>G	p.Glu1038Gly	17:43092418	A/G 0.336 (G)	0.222 (2/9)	0.148	0.126	0.122	0.157	0.357
	rs2227945	c.3418A>G	p.Ser1140Gly	17:43092113	A/G 0.010 (G)	0.111 (1/9)	0.009	0.056	0.023	0.036	0.001
<i>BRCA2</i>	rs169547	c.7397C>T	p.Ala2466Val	13:32355250	C/T 0.976 (T)	1 (9/9)	0.918	0.944	0.907	0.911	0.999
	rs4986860	c.7319A>G	p.His2440Arg	13:32355172	A/G 0.010 (G)	0.111 (1/9)	0.033	0.045	0.014	0.039	0

p-value between muted minor allele frequency in our specimens and in Yoruban population (YRI): $p = 0.409$; Chrs: chromosome; *: allele frequency in our specimens;

Population description: ASW: African ancestry in Southwest USA; ESN: Esan in Nigeria; Yoruban in Ibadan (Nigeria); AFR: African; EUR: European.

(exon 23). We identified several common polymorphisms in both *BRCA1* and *BRCA2* in our specimens (Table 1). Non-synonymous coding changes are represented with the SNP identity. The others with no SNP identity are synonymous coding.

There are more variant alleles in our population for *BRCA1* than for *BRCA2* (Table 2). For *BRCA1* we identified: A/G (c.4837A>G rs1799966; c.3548A>G rs16942; c.3113A>G rs16941; c.3418A>G rs2227945), C/T (c.2612C>T rs799917); and for *BRCA2* A/G (c.7319A>G rs4986860), C/T (c.7397C>T rs169547). All of these variants are missense mutations with benign clinical significance. Based on physical and comparative consideration these missense variants are not likely to affect protein function (ClinVar). There is no statistical significant difference in the frequency of the alleles between our population group and Yoruban population ($p > 0.05$). The SNP rs169547 (*BRCA2* non synonymous variation c.7397C>T; p.Ala2466Val) was found in all women of our study, however we found the proportion to be statistically similar to both European and most of others populations ($p > 0.05$) (Table 2).

Discussion

BRCA1 and *BRCA2* genes have been screened in young women with nodules, suspected of being breast cancer, and some variants have been found. Each specimen in this study had at least one variant. All variants identified are benign. However, others populations have high proportions of oncogenic variants. For example, in the

case of Ashkenazi families, a high frequency of causative mutations of the *BRCA1* and *BRCA2* genes, as insertions and deletions, were observed [22, 23]. A single variation 3'UTR was found in the last exon (*BRCA1* exon 23) with reported SNP rs3092995, it is possible that this variation may alter the mRNA structure in this specimen [24, 25]. All identified variants were located between c.2612C>T and c.4837A>G regions for *BRCA1* and between c.7319A>G and c.7397C>T regions for *BRCA2*. These regions are not located in the breast cancer cluster regions identified by Rebbeck and Collaborators in 2015, but some of these are in the ovarian cancer cluster region (Exon 11 of *BRCA1*) [20]. This revealed that breast cancer may have non-genetic predictor factors as well as varied genetic factors. The variants identified in this study are found mostly in cases of benign tumors. According to a study published in 2008, non-cancerous breast diseases may promote the subsequent occurrence of cancer [26]. Some of the variants we identified may be predisposing factors. In general, variants may not cause cancer. Some variants, oncogenic in other countries may not be in Africa because the genotype may be influenced by the environment [27]. Some mutations can act synergistically to cause cancer [28], especially since in this study there were patients with family history of cancer.

Among our study population, we identified the same variation (regarding the amino acid change c.2622C>T corresponding to the protein change p.Leu871Pro) that like other variations found in our specimens, had been reported previously in dbSNP database. This common variation is on the *BRCA1* gene (SNP rs799917). Some

studies have found that *BRCA1* rs799917 polymorphism may contribute to the risk of cervical cancer in Chinese populations, and further validation in other populations would be warranted [29]. Recently in Burkina Faso, Zoure et al. targeted exon2, exon5 and exon11A for *BRCA1* sequencing [21]. These exons were described as likely to contain causative mutations of cancer in northern Africa and some west African countries' [30, 31]. But they did not detect mutations in any of the 15 women diagnosed with family breast cancer history in Burkina Faso [21]. In our study, we sequenced the whole *BRCA1* and *BRCA2* gene. This is the first reported study which screened these genes in their entirety to detect mutations in a Burkina Faso population. It is worth noting that our specimen size was very small and selected mostly based on the age of presentation. Also, in the familial cases of breast cancer, the proportion of *BRCA1* and *BRCA2* carrier is relatively low (5%) [3, 4, 32] and this could explain why known oncogenic variants have not yet been determined that confer an increased risk for breast cancer in this study.

Indeed few studies concerning *BRCA1* and *BRCA2* in the West African population have been published. We can consider the study in Yoruban populations where the *BRCA1* Y101X mutation (1.1%) has been identified [33]. A study carried out in Algeria observed that 9% of sporadic cases of early-onset breast cancer and 36% of family cases have been associated with *BRCA1* mutations [34]. In Ghana another high proportion (61%) of mutation was found among patients with breast cancer [35]. Zeng and collaborators performed a study involving Africans, in which no association between identified SNPs and breast cancer risk was identified [36]. The limited data may indicate that the west African families do not have the same access to screening for mutations in target genes compared to other countries [22, 23]. The detection of *BRCA* mutations within a family member would have predicted hereditary breast cancer [3, 18, 19]. For those at risk, also for other family members, active surveillance through early detection would be necessary. In addition, mutations may vary among individual racial and ethnic groups [22, 37] and early studies suggested that *BRCA* mutations were relatively rare among Africans [14, 33].

The variant c.4837A>G (corresponding to the protein substitution p.Ser1613Gly) has been found to be most frequent in South Asian (50%), East Asian (37%), European (36%), American (37%) and Africans (23%) populations'. About 77% of the alleles found in the African population studies are ancestral allele T (dbSNP). This proportion is similar to those found in the African sub-populations with variant c.2612C>T rs799917 SNP (88% of ancestral allele A), c.3113A>G rs16941 SNP (84% of ancestral allele

T), c.3548A>G rs2227945 SNP (96% of ancestral allele T), c.7397C>T rs169547 SNP (91% of ancestral allele C), c.7319A>G rs4986860 SNP (96% of ancestral allele A), c.4837A>G rs1799966 SNP (77% of ancestral allele T). However, the rs16942 SNP, 22% of the alleles found in the African population studies, are ancestral alleles (C).

Mutations in several other genes remain to be identified, even if *AKT1*, *TP53* and *PTEN*, *ATM* variants have been associated with hereditary breast cancer [38]. Further work is required and include additional analyses of these genes known to be involved in breast cancer. Also, some studies reported the whole spectrum of hereditary breast cancer is not summarized by only *BRCA1* and *BRCA2* mutations [39-43]. A study in a Japanese population free of *BRCA* mutation revealed some large deletions in *RAD51C* [43]. So *RAD51C*, *RAD51D* or even *PALB2* could be explored. Esteller et al., have found a high rate of loss of heterozygosity at the *BRCA1* locus that accounts for the vast majority of *BRCA1* biallelic inactivation, in most tumors from *BRCA1* families [41]. Therefore, another perspective could focus on epigenetics in tumors.

Conclusions

This study describe some variants found in *BRCA1* and *BRCA2* genes, in young women with nodules and suspected breast cancer in Burkina Faso. Breast cancer in Burkina Faso can have a genetic basis because some of variants that we identified may be predisposing, but there is need to have a large number of specimen and more specific genetic testing to confirm if this can be considered as conferring an increased risk for breast cancer in this West African population.

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