

## BRCA1 c.68\_69delAG (exon2), c.181T>G (exon5), c.798\_799delTT and 943ins10 (exon11) mutations in Burkina Faso

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Burkina Faso, with HBC were collected at the University Hospital Yalgado OUE-DRAOGO(CHU-YO) of Ouagadougou in Burkina Faso. c.68\_69delAG (exon2), c.181T>G (exon5), c.798\_799delTT and 943ins10 (exon11) mutations were not detected in any of the 15 women diagnosed with family breast cancer history. Genetic analysis in this study, we show that targeting relevant exons in BRCA1 genes did not allow detection of mutations in the population of Burkina Faso. Therefore, such an approach may be of interest to perform a complete sequencing of BRCA1 and BRCA2 genes in families at a high risk of developing breast cancer in Burkina Faso.

### Introduction

The incidence of breast cancer is increasing in sub-Saharan Africa, including Burkina Faso.<sup>1,2</sup> The actual cause of breast cancer is unclear but studies globally have implicated a wide variety of factors like germline mutation/heredity, age, gender, reproductive status, diet, anthropometric characteristics, psychological factors and environmental factors as possible etiological factors.<sup>3</sup> About 5-10% of all breast cancer cases and 25-40% of cases under the age of 35 years are due to hereditary factors. An accounting for 15-40% of the breast cancer (BC) cases with familial are due to germline mutations of the two major cancer susceptibility genes, BRCA1 and BRCA2. The high risks genes *BRCA1*, *BRCA2*, *P53*, *PTEN*, and *ATM*. Mutations in *BRCA1* and *BRCA2* have at least five germline mutations that predispose to breast cancer. The germline mutations of other genes such as P53, PTEN that predispose to Li-Fraumeni cancer syndrome and to Cowden disease, respectively, constitute risk factors for breast cancer.<sup>4</sup> Germline mutations in the BRCA1 and BRCA2 genes significantly increase the risks of breast and ovarian cancer. A recent meta-analysis of 10 studies estimated that the risk of developing breast cancer by the age of 70 was 57% and 49% for BRCA1 and BRCA2 mutation carriers, respectively.<sup>5</sup> One major Ashkenazi Jews mutation of BRCA1 gene, c.68\_69delAG/185AGdel (exon2), was identified in Egyptian and Tunisian women. However, the 185AGdel founder alteration was found in two independent screening tests of Jewish and non-Jewish Moroccan populations.<sup>6</sup>

The c.181T>G/300T>G (exon5) mutation has reported as founder mutation in Central European, Morocco and Algerian populations.<sup>7</sup>

Moreover, in a recent study, c.798

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799delTT (exon11) was introduced as a non-Jewish founder *BRCA1* gene identified in the *BRCA1* gene with a frequency of 5.12% in Tunisian, Moroccan and Algerian BC patients. The c.798\_799delTT alteration of BRCA1 was shown in Tunisian BC cases from Algeria.<sup>8</sup>

In all the few African population assays, which have been reviewed, Egyptian women demonstrated founder mutations in both *BRCA1* and *BRCA2* genes. However, African triple negative BC patients from the United States showed 943ins10 (exon11) mutation of *BRCA1* as founder of West Africa.<sup>6</sup>

According to our knowledge, no study has investigated these breast cancer mutations in Burkina Faso. The aim of this study was to evaluate the frequency of these four well-defined mutations in a sample of the population of Burkina Faso. These mutations have been described in previous studies of populations around the Mediterranean. Thus, targeting frequent mutations in the North African population,

### Abstract

The worldwide variation of BRCA mutations is well known. The c.68\_69delAG, c.181T>G, c.798\_799delTT mutations in BRCA1 were observed in Moroccan, Algerian and Tunisian Breast Cancer families and were described founder mutation in Northern Africa. The 943ins10 is also recognized a founder mutation in West Africa. To our knowledge no study has been published on BRCA1/2 germline mutations and hereditary breast cancer (HBC) in population of Burkina Faso. The aim of the present study (first in Burkina Faso) was to screen for these four mutations in 15 unrelated patients with HBC. Mutation analysis was performed by Sanger sequencing of coding exon2, Exon5 and exon11A sequences of the BRCA1 gene. Blood specimens of 15 patients from

we can identify and confirm the hypothesis of founder mutations and study on a larger sample to provide a genetic profile of the mutation in the population of Burkina Faso.

## Materials and Methods

### Patients

The present study included 15 Burkinabe familial breast cancer cases selected from patients treated at the University Hospital Yalgado OUEDRAOGO of Ouagadougou in Burkina Faso between 2015 and 2016. Patients were selected according to specific family history criteria: Three or more first or second-degree relatives with breast cancer diagnosed at any age in the same familial branch. Patients who gave informed consent were selected from patients diagnosed with breast cancer. All patients were asked to provide detailed information regarding per-

sonal and family history of cancer by interview. Clinical and pathological characteristics including age at diagnosis, mono- or bilateral tumor location, marital status, histology, stage of disease, tumour grading were collected from medical records and pathology reports (Table 1). The study was approved by the National Ethics Committee of Health Ministry and written informed consent was obtained from each subject. Blood samples were taken from at least one affected woman of each patient and stored in EDTA tubes.

### Molecular analysis

DNA was extracted using the salting out method,<sup>9</sup> confirmed by agarose gel electrophoresis and then quantified using the NANODROP 2000 spectrophotometer (Thermo Scientific). The exon2, exon5 and exon11A of BRCA1 genes were amplified in a final volume of 25 µl containing: 1x reaction buffer, 10 µM primers (forward and reverse), 1.25 U MyTaq™ DNA

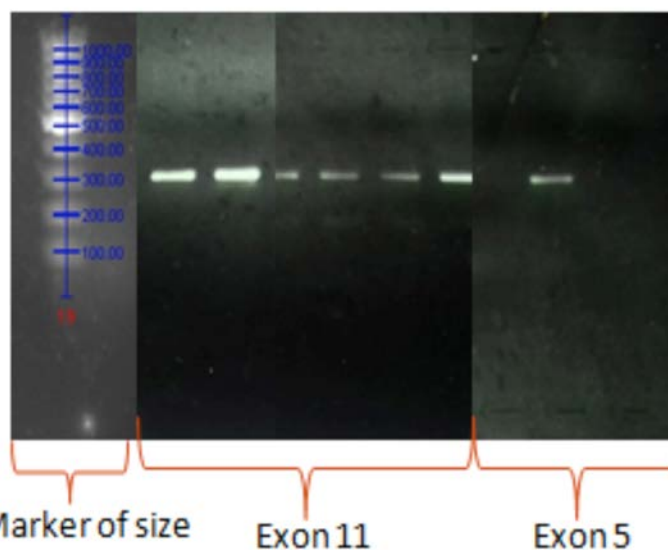
Polymerase and 50 ng genomic DNA.

Amplification cycles were: 92 °C for 3 min followed by 40 cycles of 92 °C for 30 sec, 53 °C for 30 sec (exon2), 57 °C for 30 sec (exon5), 55 °C for 30 sec (exon11A), 72 °C for 1 min 30sec and ended with 7 min incubation at 72 °C. PCR products were verified by electrophoresis agarose 1, 5% and visualized by exposure to ultraviolet light (Figure 1). Information's on the primers used are shown in Table 2. The amplified products were purified by Exo-Sap enzymatic digestion (GE Healthcare, USA), according to the manufacturer's instructions. Sequence reactions were performed in forward strands on ExoSap-purified PCR products using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster city, CA, USA), and then runned on a ABIPRISM 3130 XL Genetic analyzer (Applied Biosystems, Foster city, CA, USA). Sequence analyses were performed using Sequence Scanner v1.0 (Applied Biosystems, Foster city, CA, USA) software

**Table 1. Characteristics of the breast cancer cases.**

Parameters	Breast cancer cases (n=15)
Patients' age (year±SD)	47.4±1.11
Matrimonial status	
Single	3
Married	11
Divorced	0
Widow	1
Tumor localisation	
Right breast	7
Left breast	8
Histology	
IDC	15
ILC	0
Other	0
Stage	
I	1
IIA&IIB	3
IIIA&IIIB	9
IV	2

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma.



**Figure 1. Photo of amplification gel.**

**Table 2. PCR primers.**

BRCA1	Systematic nomenclature	BIC nomenclature	Mutation	Sequence 5' - 3' F: forward and R: reverse	Amplion size (pb)
Exon2	<i>c.68_69delAG</i>	<i>185delAG</i>	Deletion	F: GAAGTTGTCATTTTATAAACCTTT R: TGCTTTTCTCCCTAGTATG	258
Exon5	<i>c.181T&gt;G</i>	<i>300T&gt;G</i>	Substitution	F: CTCTTAAGGGCAGTTGTGAG R: TTCCTACTGTGTTGCTTCC	235
Exon11A	<i>c.798_799delTT</i>	<i>917delTT</i>	Frameshit	F: CCAAGGTGATGAAGTATGTAT R: TGTATGTTGGCTCCTTGCT	364
Exon11A	-----	<i>943ins10</i>	Insersion	F: CCAAGGTGATGAAGTATGTAT R: TGTATGTTGGCTCCTTGCT	364

(Figures 2-4). The samples were not screened for the presence of large deletions and duplications. All mutations and variants are cited according to Human Genome Variation Sequence systematic nomenclature HGVS (<http://www.hgvs.org/mutnomen/>) using GenBank entries: U14680 for BRCA1 and following the rule in which the A of the first ATG translational initiation codon nucleotide is +1. The Breast Cancer Information Core (BIC) nomenclature, based on reference sequences stated above and where A of the ATG translation initiation codon is at positions 120 of BRCA1 indicated in Table 2 and Human Genome Variation Sequence systematic (HGVS) nomenclature.

### Ethics approval and consent to participate

The whole research was funded by personal resources of researchers. The study was approved by the National Health Ethic Committee of Burkina Faso (reference number 2014-7-085 of July, 7th 2014). All study participants gave their free written and informed consent according to the Helsinki Declarations. Any personal identifier was encoded.

## Results and Discussion

According to the literature, the BRCA1 or BRCA2 mutations may be responsible for about 10% of cases of ovarian cancer and 3-5% of cases of breast cancer. A mutation in the BRCA1 gene leads to a 70-80% lifetime risk of developing breast cancer and a 50% risk of developing ovarian cancer. On the other hand, a mutation of the BRCA2 gene constitutes a lifetime risk of 50-60% occurrence of breast cancer and a risk of 30% occurrence of ovarian cancer.<sup>10</sup>

The estimated carrier frequency is 1 over 300 for BRCA1 and 1 over 800 for BRCA2 in the general population, with the exception of Ashkenazi-Jewish women, who have a 2% to 5% carrier frequency for 3 founder mutations in BRCA1 and BRCA2.<sup>11</sup>

A total of 15 Burkinabe subjects were selected for our study. General characteristic of the subjects including distribution of tumor characteristic such as histological grade and location of cancer were obtained from the patients' medical records and listed in Table 1. In total, 15 cases with family history breast cancer were successfully screened for the c.68\_69delAG, c.181T>G, c.798\_799delTT and 943ins10 mutation of the BRCA1 gene using the sequencing technique. None of the 15 Burkinabe breast cancer patients carried the mutations. This find-

ing suggests that these mutations are probably not present or are present at an extremely low frequency in the population of Burkina Faso.

### c.68\_69delAG/185delAG mutation (exon2)

The c.68\_69delAG/185delAG mutation is one of three founder mutations as muta-

tion 5382insC in BRCA1 gene and 6174delT in the BRCA2 genes, which have been identified in Ashkenazi Jews.<sup>12</sup> In Morocco, for the first time, the 185delAG mutation reported in females from two Moroccan families. Thus, further investigations with larger cohort of patients into this mutation are going to confirm its founding aspect or not. This may simplify genetic

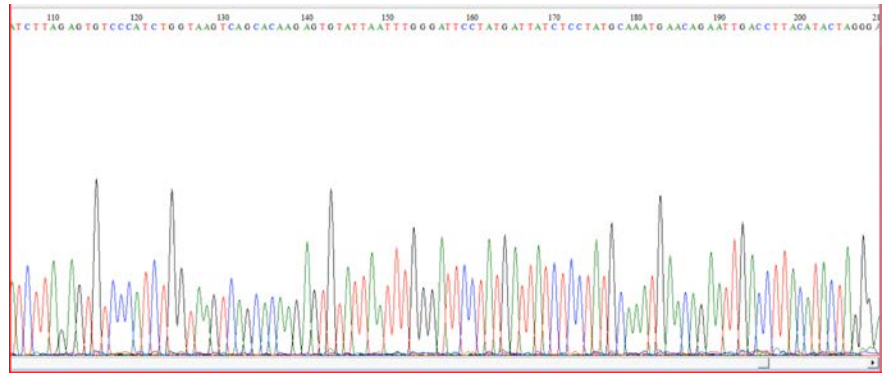


Figure 2. Electrophoregram of Exon2.

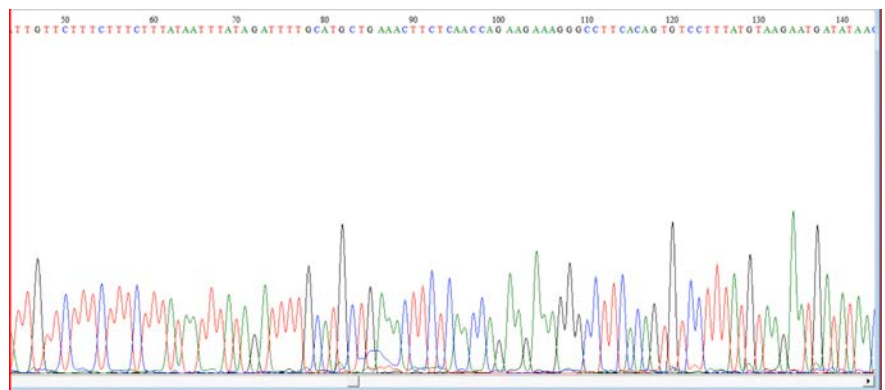


Figure 3. Electrophoregram of Exon5.

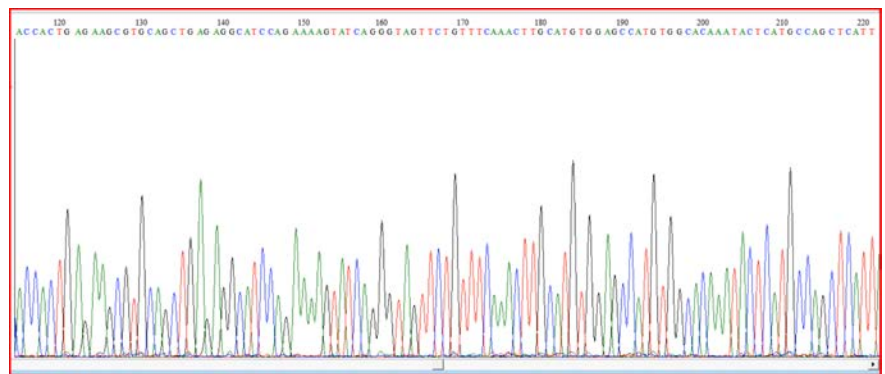


Figure 4. Electrophoregram of Exon11.

testing and have clinical and public health implications, if a significant proportion of mutations of the 185delAG mutation is confirmed in the Moroccan population.<sup>13</sup> Yawitch *et al.* evaluated 206 black South African women for 185delAG in exon 2, 943ins10 in exon 11, eleven of these patients had a family history of Breast cancer. None of these mutations were found in any of the patients studied.<sup>14</sup>

### c.181T>G/300T>G mutation (exon5)

The c.181T>G/300T>G (p.Cys61Gly) is missense mutation located in exon5 of BRCA1. It is conversion of Cysteine to Glycine which occurring in the highly conserved cysteine ligating residues in the RING finger domain.<sup>15,16</sup> The c.181T>G/300T>G mutation occurs in the amino-terminal zinc finger motif, an important functional region of BRCA1 protein. This mutation reported as founder mutation in central Europe and Algerian populations. It is also observed in Morocco, which places it among the possible changes of the Mediterranean region.<sup>7</sup>

### c.798\_799delTT/917delTT and 943ins10 mutation (exon11A)

The pathogenic mutation c.798\_799delTT (p.Ser267LysfsX19) including two bases (TT) deletion that cause a truncated protein signal at codon 285.<sup>17</sup> It is a frame-shift mutation which was reported in four Algerian families and in three unrelated Tunisian families. So, it was suggesting a North African founder mutation. This mutation was also observed in some close Mediterranean countries in particular Spain and Italy (Calabrian). The migration flow history and geographical proximity could be explained this result.<sup>7</sup>

Among the North African population, the mutational spectrum of BRCA1/2 has yet to be well characterized. Mutation c.798\_799delTT has been observed in two Algerian families, as well as in two Tunisian families with breast cancer, indicating that the first non-Jewish founder mutation to be described in Northern Africa.<sup>18</sup> Tazzite *et al.* 2012 described c.798\_799delTT/917delTT in Moroccan breast/ovarian cancer families.<sup>7</sup>

Interestingly, it has also been detected in 10 unrelated families from Italy and described as founder mutation in South and Middle Sardinia, respectively. As Mediterranean countries share a common history and migration flow history, *BRCA1* mutation c.798\_799delTT could be a Mediterranean founder mutation.<sup>8</sup>

BRCA1 mutation 943ins10 was detected in breast cancer patients from the Ivory Coast, the Bahamas, and the United States.

The families inheriting BRCA1 943ins10 were from widespread locales of Africa and the African diaspora: the Ivory Coast, the Bahamas, the southeastern United States, and Washington, DC. On the other hand, the 943ins10 allele has not been observed in any patients with breast cancer who identify their ancestry as solely European.<sup>19</sup>

The determination of the proportion of inherited breast cancer attributable to BRCA1 943ins10 among Burkinabe women was necessary. Given the increasing incidence of and higher mortality from breast cancer among Burkinabe women,<sup>1</sup> it would be useful to obtain as much information as possible about the roles of BRCA1 943ins10 in this population, but no mutation was detected in our study.

The BRCA1 mutations c.68\_69delAG (exon2), c.181T>G (exon5), c.798\_799delTT (exon11A) mutation which we studied, are more or less founder mutations in Caucasians<sup>20</sup> and many described in Morocco.<sup>21</sup> Thus the people of West Africa in particular Burkina Faso may have a low frequency of BRCA1 c.68\_69delAG, c.181T>G, c.798\_799delTT mutation. However, despite that studies have shown that the 943ins10 (exon11) mutation is founder in West Africa.<sup>19</sup> We have not detected mutations, in view of the previous studies, our results and the size of our sample, a large study is necessary to clarify the actual state of these mutations in Burkina Faso.

### Epigenetic regulation (BRCA promoter methylation)

This means that the lack of mutation as they obtained with 15 patients does not exclude BRCA implication in the etiology of the disease in Burkina Faso. It is important to realize that a mutation in one tumor suppressor allele is often insufficient for cancer initiation but it predisposes someone to cancer as mutation in the remaining wild-type allele can lead to epigenetic regulation and loss of heterozygosity (LOH) in a population of cells driving tumor progression. BRCA expression silencing by epigenetic regulation (BRCA promoter methylation) or activation of BRCA repressors lead to the reduction of BRCA protein with consequences similar to those of BRCA-mutations.

Epigenetic alterations are fundamentally different from mutational events in that they are more frequent, are reversible. Cell transformation is associated with epigenetic events that predominantly involve DNA methylation. Hypomethylation of CpG islands may lead to aberrant expression of genes that are normally silenced by methylation in a particular tissue or developmen-

tal stage. Changes in DNA methylation of BRCA can affect gene expression either directly through activation (or silencing) of a gene by hypomethylation (or hypermethylation) of its promoter, or indirectly by activation or silencing of other genes that in turn regulate the expression of a gene of interest. In breast cancer, genes directly affected by hypermethylation include tumor suppressor genes (BRCA1), metastasis-inhibitory genes (CDH1, TIMP-3), hormone receptor genes (ER- $\alpha$ , PR) and cell cycle control genes [p16INK4a (CDKN2A)].<sup>22,23</sup> Methylation of the BRCA1 promoter has been shown to occur in approximately 20% of breast cancer patients. Sporadic tumours with BRCA1 promoter methylation have been reported to be ER and PR negative.<sup>24,25</sup> All carcinomas that showed methylation of the promoter of BRCA1 had a high-grade serous histology.<sup>26</sup> The prevalence of the promoter methylation status of ubiquitin carboxyl-terminal hydrolase 1 (UCHL1) was 67% and 82% for patients with sporadic and hereditary breast cancer, respectively.<sup>27</sup> Methylation-specific multiplex ligation-dependent probe amplification (MS-MLPA) is used to detect methylation of the BRCA1 promoter.<sup>26</sup>

## Conclusions

The risk factors of breast cancer are not yet known scientifically. Thus, our study of the genetic factor could help us elucidate this question. No mutation was detected in the samples analyzed. However, in the near future, it is very important to screen for *BRCA1/2* germline mutations in large series of breast and/or ovarian cancer patients/families in order to know about the frequency, the spectrum, the contribution and the prevalence of *BRCA* genes mutations in our populations. The implications of these new findings in regard to genetic testing and counseling are substantial for Burkinabe patients and families at risk.

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