*British Journal of Medicine & Medical Research*
*4(14): 2627-2638, 2014*

**SCIENCEDOMAIN** *international*
www.sciencedomain.org

# Correlation of Clinical and Pathological Parameters with the Diversity and Genetic Evolution of Breast Cancer in Senegalese Women

**F. Mbaye[1,5*], A. Dem[2], M. Fall[1], E. S. Mbaye[2], G. Diop[1], R. Ndiaye[3], M. S. Niang[4], A. Dieye[4] and M. Sembene[1,5]**

*[1]Department of Animal Biology, Faculty of Science and Technology, University CA Diop, BP 5005 Dakar, Senegal.*
*[2]Institute of Cancer, Faculty of Medicine, Pharmacy and Odonto-Stomatology, University Cheikh Anta Diop, Dakar, Senegal.*
*[3]Laboratoire of Pharmaceutical Biochemistry, Faculty of Medicine, Pharmacy and Dentistry, University Cheikh Anta Diop, Dakar, Senegal.*
*[4]Institut of Avenue Pasteur Pasteur-BP220-Dakar, Senegal.*
*[5]Centre for Biology and Management of Populations, Institute of Research for Development, IRD / Bel-Air, Senegal.*

***Authors' contributions***

*This work was carried out in collaboration between all authors. Author FM designed the general study, carried out the molecular genetic studies, participated in the sequence alignment and drafted the manuscript. Author AD contributed to administrative, technical or material support (such as clinical samples collection). Author MF participated in its design and coordination and helped to draft the manuscript. Authors ESM and GD participated in the sequence alignment, performed the statistical analysis. Authors RN and MSN drafted the manuscript. Author AD revised the manuscript and provided the funding. Author MS conceived of the study and participated in its design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.*

***Original Research Article***

***Received 13th September 2013***
***Accepted 28th November 2013***
***Published 22nd February 2014***

## ABSTRACT

**Aims:** The purpose of this study was to assess the hypothesis that genetic diversity of breast cancer is related to clinical and pathological tumors characteristics.

**Corresponding author: Email: fatimata.mbaye@ucad.edu.sn*

**Study Design:** Representative sequences of mitochondrial Cytochrome b gene of cancerous tissues were analyzed to determinate diversity and genetic evolution of breast tumors in Senegalese women.
**Place and Duration of Study:** Department of biology Animal (University Cheikh Anta Diop), laboratory of molecular biology (IRD-CBGP), 2011-2013.
**Methodology:** Genetic diversity of breast tumors in 57 Senegalese patients was estimated by analyzing sequences of the Cytochrome b mitochondrial coding gene. The Cytochrome b sequences from cancerous tissues were aligned using BioEdit version 7.0.8. Number of polymorphic sites, parsimony informative sites, the rate of transitions/transversions and the nucleotide frequency were calculated using MEGA 5.05. Genetic distance (d) was calculated with MEGA 5 using the Kimura 2-parameter (K2P) model. The index of genetic differentiation (Fst) used to describe the distribution of the genetic diversity between sub-groups was calculated with the software DnaSP version 10.5.01. The significance level (*P*-value) was 0.05.
**Results:** Analyses have revealed a high score of haplotype (Hd=*0.991+/-0.008*) and nucleotide diversity (Pi=*0.15650+/-0.01326*). Statistical correlations have shown a positive association between the risk factors (age of the patients, appearance of the first menstruation beyond 12 years, number of gestation) and nucleotide diversity of tumors (*P*<0.0001). Statistical analyses based on t-test showed a positive association between age, date of onset of the first menstruation and nucleotide diversity (Pi), between healthy and cancerous tissues for each patient (*P*<0.0001).
**Conclusion:** Our results have shown an important genetic diversity in breast tumors. A strong genetic differentiation was noted depending on the number of gestations.

*Keywords: Cancer; breast; genetic diversity; evolution; DNAmt; cytochrome b; Senegal.*

## 1. INTRODUCTION

The factors that influence variability of the human genome are fundamental in population genetics and in evolutionary biology. Understanding how these factors operate allows better understanding of the history of populations and their associated diseases, the distribution of genetic and phenotypic variability of populations and facilitating the identification of genes involved in complex pathologies such as breast cancer. Breast cancer is the most frequent neoplasia of women worldwide causing ~ 30% of cancers and ~ 16% of deaths due to cancer in the female population [1]. It is the second most prevalent cancer in Sub-Saharan Africa after cervix cancer [2]. The standardized incidence is estimated at 27.8 per 100000 women in West Africa [3]. A recent study at the Curie Institute, Cheikh Anta Diop University of Dakar (UCAD) reported that breast cancer represented 42% of gynecologic and mammary cancers. In this study, the average age of the patients was 47.6 years and more than half of the subjects were pre-menopausal women [4]. The most important characteristic of breast cancer in Africa, particularly in Senegal, is the increasing frequence of the disease, in an aggressive form in very young subjects. This cancer can occur from age 20 and the frequency increases from 30 years to reach a peak in the age group of 40-44 years [4]. Multiple pregnancies are a major risk factor. More than 50% of affected women have at least five children. According to data from the Senegalese Institute of cancer, breast cancer patients are diagnosed at an advanced stage. Only 1.2 % and 7.21 % of the tumors are diagnosed at stage I and II respectively, while 63% are discovered at stage III and 20.12 % at stage IV. Infiltrating ductal carcinoma is the most frequent histological type and the majority of the tumors are undifferentiated. Like the majority of cancers and many other

diseases, breast cancer has a multifactoral component with genetic predisposition being a major risk factor.

The discoveries of oncogenes and of tumor suppressive genes have led to a better understanding of the biology of cancers and have shown that the formation and progression of cancer are the results of somatic mutations. It is now known that the genomes of tumors undergo many changes that alter their structure and profoundly affect tumor functions [5]. Additionally, mutations in the mitochondrial genome have been associated with the mechanisms of carcinogenesis. Indeed, given the important role played by mitochondria in cell energy metabolism, production of free radicals and apoptosis during mitochondrial function has long been suspected to contribute to the development and progression of cancer [6]. Given their high frequency in cancer and their presence in early stages of the disease, mitochondrial mutations could be used as new molecular markers for early detection of breast cancer.

In a previous study the variable penetrance genetics of Cytochrome b, encoded by a mitochondrial gene has been reported in Senegalese patients with breast cancer [7]. In this study, we evaluated the hypothesis that the genetic differences in breast cancer are related to the clinical and pathological characteristics of the tumors. We also aimed to determine whether risk factors including age of patients, date of onset of the first menstruation and multiple pregnancies are correlated to the genetic evolution of the tumors.

## 2. MATERIALS AND METHODS

### 2.1 Patients and Samples

57 Patients with breast cancer were followed at the Senegalese Institute of cancer in Aristide Le Dantec hospital, Dakar. Clinical and pathological data were obtained from the patient's records. Other parameters were recorded including age, date of onset of the first menstruation, number of pregnancies, location of the tumor (right or left breast), quadrant super-external (QSE), quadrant super-internal (QSI), quadrant inferno-external (QIE), quadrant inferno-internal (QII) of the tumor, size of the tumor ranging from $T_2$ to $T_4$, invasion of the lymph nodes (ranging from $N_0$ to $N_2$), histology and grade of the tumors determined according to the classification of Scarff Bloom and Richardson [8]. Most of the patients had an infiltrating ductal breast cancer (77.19%), one (1) subject had an infiltrating lobular carcinoma, two (2) had a mixed carcinoma and four (4) an atypical hyperplasia. No histological data were available from the six (6) remaining patients. The patients were aged between 19 and 78 years with an average age of 47.02. The average age of onset of the first menstruation was 14.23 and 94.1% of the patients had their first period after 12 years. Five (9.09%) patients were less than 30 years old and 50 (90.9%) were over 30 years. The numbers of pregnancies were known for 49 (85.96%) patients. Among these subjects, 8 (16.35%) were nulliparous; 7 (14.29%) patients had 2;5 (10.20%) had 3, 3 (6.12%) had 4 and 26 (53.06%) had more than 4 gestations. 30 patients had a tumor at the right and 24 at the left breast. 20 patients had a tumor located at the QSE, 3 at the QIE, 6 at the QSI and 7 at the QII. The size of the tumors was determined for 47 (82.45%) patients, 35 (74.47%) had a tumor sized T4, 3 (6.38%) of size T3, 8 (17.02%) of size T2 and 1 (2.13%) of size T1. In 48 patients (84.21%) for whom this information was available, 36 (75%) had a tumor that invaded the lymph nodes while no invasion was observed in 12 (25%) subjects. 12 patients had tumors of grade SBR III, 18 of grade SBR II and 3 of grade SBR I. The grade was not

determined for the 24 remaining cases. Clinical and pathological characteristics of patients are shown in Table 1.

**Table 1. Clinical and pathological characteristics of 57 cases analyzed**

| Variables | Number of patients (%) |
|---|---|
| **Age (n= 55)** | |
| ≤30 | 5 (9.09) |
| >30 | 50(90.90) |
| **Gestation (n= 49)** | |
| 0 | 8(16.32) |
| 1 | 0 |
| 2 | 7(14.29) |
| 3 | 5(16.20) |
| 4 | 3(6.12) |
| >4 | 26(51.02) |
| **Tumor localization (n= 54)** | |
| Right Breast | 30(55.56) |
| Left Breast | 24(44.44) |
| **Quadrant (n= 36)** | |
| QSE | 20 (55.56) |
| QIE | 3(8.33) |
| QSI | 6(16.67) |
| QII | 7(19.44) |
| Aerole | 0 |
| **Size of the tumor (n= 47)** | |
| T1 | 1(2.13) |
| T2 | 8(17.02) |
| T3 | 3(6.38) |
| T4 | 35(74.47) |
| **Clinical adenopathy (n= 47)** | |
| N0 | 12(25.53) |
| N1 | 13 (27.66) |
| N2 | 22(46.80) |
| **Grade (n= 33)** | |
| SBRI | 3(9.09) |
| SBRII | 18(54.55) |
| SBRIII | 12(36.36) |

## 2.2 DNA Extraction, Amplification and Sequencing

Whole genomic DNA was extracted from tissues after digestion with proteinase K and purified on a column (QIAGEN) as previously described [7]. The Cytochrome b gene was amplified by PCR using two primers, H15915 (TCT-CCA-TTT-CTG-GTT-TAC-AAG-AC) and L14723 (ACC-AAT-GAC-ATG-AAA-AAT-CAT-GGT-T). The PCR reactions were carried out in an Eppendhorf thermal cycler with an initial denaturation step at 94ºC for 3 min. followed by 40 cycles corresponding to 45 sec. of denaturation at 92ºC, 1min. annealing at 50ºC and 1 min. 30 sec. elongation at 72ºC. A 10 min. elongation step was performed after the final cycle. The obtained amplicons were shipped to Macrogen, South Korea for purification and sequencing using primer H15915.

## 2.3 Genetic Analyses

The sequences of the Cytochrome b alleles from healthy and cancerous tissues were aligned using BioEdit version 7.0.8 view [9]. The number of polymorphic sites, the number of informative sites in parsimony, the rate of transitions/transversions and the frequency of nucleotide involved were calculated using MEGA 5.05 [10]. Genetic distance (d) at intra sub-groups between healthy and cancerous tissue was calculated with MEGA 5 [10] using the model Kimura 2-parameter (K2P). The index of genetic differentiation (Fst) used to describe the distribution of the genetic diversity between sub-groups was calculated with the software DnaSP version 10.5.01 [11]. The significance level (*P*-value) was 0.05. The demographic expansion of cancerous tissues was studied by calculating the haplotype (Hd) and nucleotide (Pi) diversities, the Tajima D [12] and Fs-Fu [13] indexes, and by analyzing the disparate distribution [14] with the editor DnaSP version 10.5.01 [11]. The statistical parameters were calculated using the editor DnaSP version 10.5.01 [11] and the significance level was assessed after 10000 coalescing simulations. As for the D of Tajima and the Fs of Fu, the expected value of these two tests under neutrality is zero. The significance level (*P*-value) was 0.05.

## 2.4 Statistical Analyses

Associations between the genetic development of tumors and the risk factors (age of the patients, date of onset of the first menstruation, and number of gestations) were determined by statistical analyses based on t-test, ANOVA and PLSD test of Fisher using the Statview software version 5.0 [15]. The significance level was 0.05.

# 3. RESULTS AND DISCUSSION

## 3.1 Results

### 3.1.1 Genetic analyzes

Genetic analyses were performed on Cytochrome b allelic sequences from 50 patients out 57. The relative values of nucleotide composition showed a moderate predominance of A and T (55.36%) over C and G (44.63%) Table 2. Transition events (57%) were more frequent than transversions (43%) and the ratio transitions/transversions was 1.276 Table 2. The analysis of polymorphism revealed a high value of haplotype diversity (Hd= 0.991+/-0.008) and of nucleotide diversity (Pi= 0.15650+/-0.01326) Table 2.

Analysis of the genetic distances (d) between healthy and cancerous tissues according to the clinical and pathological parameters Table 3 revealed a higher genetic distance in patients (i) over 30 years of age (d= 0.20), (ii) who had 4 gestations (d=0.31), (iii) with a tumor at the right breast (d= 0.22), (iv) who had tumor invasion of the lymph nodes (N1) (d= 0.22), and (v) in tumors located on the QSI (d= 0.26). The genetic distance between healthy and cancerous tissues was comparable for the tumors of sizes T2 (d=0.23) and T3 (d= 0.24) Table 3. Similarly, the variation of genetic distance between the tumors of grade SBRI (d= 0.19), SBRII (d=0.16) and SBRIII (d=0.16) was not important Table 3. Comparison of the Fst values between cancerous tissues from different sub-groups showed an important genetic differentiation depending on the number of gestations Table 3 with Fst values of -0.02289 between zero (0) and two (II), 0.03340 between two (II) and three (III), 0.18671 between three (III) and four (IV), and 0.26826 in samples from patients with more than four

gestations. In contrast, there was a small variation of the Fst values according to the sizes or the grades of the tumors Table 3. The Fst values were -0.05569 between tumors of sizes T2 and T3, 0.00832 between tumors of sizes T3 and T4, 0.00570 between tumors of grades SBRI and SBRII and 0.00792 between tumors of grades SBRII and SBRIII.

In general, the haplotype diversity of cancerous tissues was important for all clinical and pathological parameters analyzed in this study. However, nucleotide diversity was more important in patients more than 30 years of age (0.14682+/-0.01421) and in women with 4 gestations (0.18231+/-0.08215) Table 4. Nucleotide diversity was also higher in tumors (i) in the right breast (0.16986+/-0.01601), (ii) seated on QIE (0.26939+/-0.13469), (iii) of size T3 (0.25850+/-0.07879) and (iv) of grade SBR I (0.21316+/-0.06015) Table 4. Nucleotide diversity was similar in tumors that invaded lymph nodes including N0 (0.15862+/-0.02752), N1 (0.16590+/-0.02521) and N2 (0.15837+/-0.02262) tumors Table 4.

The Tajima D values (-1.47176, *P*>0.10) was negative and note significant, while Fu Fs (-10,833, *P*=0) value was negative and significant. Analysis of the disparity distribution curve showed a multimodal Fig. 1.

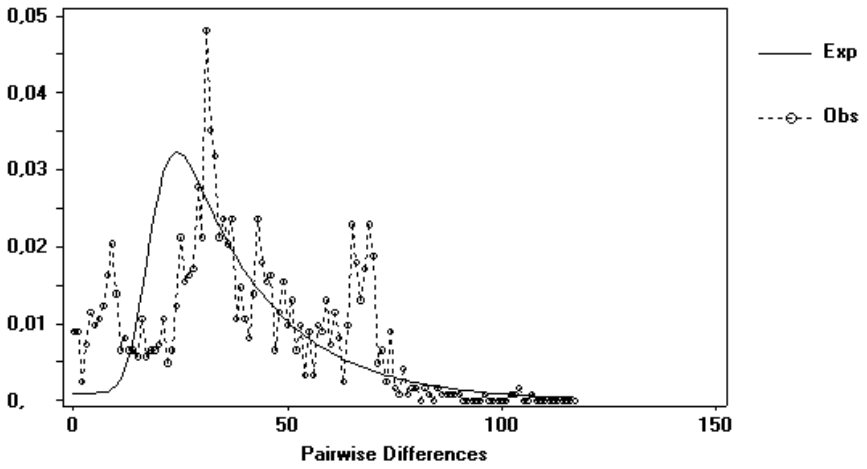


**Fig. 1. Mismatch distribution of cancerous tissues**

**Table 2. Parameters of genetic variability in cancerous tissues**

| **Number of sequences** | **50** | | | |
|---|---|---|---|---|
| **Nucleotide Frequencies (%)** | **A** | **T** | **C** | **G** |
| | 25.82 | 29.54 | 31.85 | 12.78 |
| Number of polymorphic sites | 176 (71.84 %) | | | |
| Number of informative sites in parsimony | 130 (73.86 %) | | | |
| Transitions | 57% | | | |
| Transversions | 43% | | | |
| Rate of Transitions/Transversions | 1.276 | | | |
| Haplotype Diversity (Hd) | 0,991+/- 0,008 | | | |
| Nucleotide Diversity (Pi) | 0,15650+/-0,01326 | | | |
| k | 38,342 | | | |

*k= average of the differences between the nucleotide sequences taken in pairs*

**Table 3. Distance and degree of genetic differentiation (Fst) of different sub-groups**

| **Age parameter** | | | | |
|---|---|---|---|---|
| **Genetic distances** | | **Degree of genetic differentiation** | | |
| Sub-groups | Intra sub-groups between healthy and cancerous tissues | Between sub-groups | Fst | *P*-value |
| ≤30 | 0.10 | TC_≤30 & TC_>30 | - 0.07602 | 0.6610 |
| >30 | 0.20 | | | |
| **Gestation parameter** | | | | |
| **Genetic Distances** | | **Degree of genetic differentiation** | | |
| Sub-groups | Intra sub-groups between healthy and cancerous tissues | Between sub-groups | Fst | *P*-value |
| 0_Gestation | 0.12 | TC_0_&TC_2_ Gestations | - 0.02289 | 0.3636 |
| 2_Gestations | 0.12 | TC_2_&TC_3_ Gestations | 0.03340 | 0.3575 |
| 3_Gestations | 0.13 | TC_3_&TC_4_ Gestations | 0.18671 | 0.3326 |
| 4_Gestations | 0.31 | TC_4_&TC_>4 Gestations | 0.26826 | 0.2560 |
| **Parameter of location of the tumor** | | | | |
| **Genetic Distances** | | **Degree of genetic differentiation** | | |
| Sub-groups | Intra sub-groups between healthy and cancerous tissues | Between sub-groups | Fst | *P*-value |
| Right Breast | 0.22 | TC_ Right Breast & TC_ | - 0.01300 | 0.4215 |
| Left Breast | 0.16 | Left Breast | | |
| **Quadrant parameter** | | | | |
| **Genetic Distances** | | **Degree of genetic differentiation** | | |
| Sub-groups | Intra sub-groups between healthy and cancerous tissues | Between sub-groups | Fst | *P*-value |
| QSE | 0.20 | TC_QSE & TC_QSI | - 0.03248 | 0.3995 |
| QSI | 0.26 | TC_QSI & TC_QIE | - 0.35363 | 0.3208 |
| QIE | 0.16 | TC_QIE & TC_QSE | - 0.24365 | 0.6888 |
| QII | 0.10 | TC_QII & TC_QSE | - 0.03454 | 0.4729 |
| **Size parameter of the tumor** | | | | |
| **Genetic Distances** | | **Degree of genetic differentiation** | | |
| Sub-groups | Intra sub-groups between healthy and cancerous tissues | Between sub-groups | Fst | *P*-value |
| TC_T2 | 0.23 | TC_T2 & TC_T3 | - 0.05569 | 0.3423 |
| TC_T3 | 0.24 | TC_T3 & TC_T4 | 0.00832 | 0.2426 |
| TC_T4 | 0.18 | | | |
| **Clinical adenopathy parameter** | | | | |
| **Genetic Distances** | | **Degree of genetic differentiation** | | |
| Sub-groups | Intra sub-groups between healthy and cancerous tissues | Between sub-groups | Fst | *P*-value |
| N0 | 0.19 | TC_N0 & TC_N1 | - 0.05882 | 0.3860 |
| N1 | 0.22 | TC_N1 & TC_N2 | - 0.03700 | 0.4292 |
| N2 | 0.17 | | | |
| **Grade Parameter** | | | | |
| **Genetic Distances** | | **Degree of genetic differentiation** | | |
| Sub-groups | Intra sub-groups between healthy and cancerous tissues | Between sub-groups | Fst | *P*-value |
| SBR I | 0.19 | TC_SBR I & TC_SBR II | 0.00570 | 0.3912 |
| SBR II | 0.16 | TC_SBR II & TC_SBR III | 0.00792 | 0.4705 |
| SBR III | 0.16 | | | |

*TC: Cancerous Tissue*

**Table 4. Haplotype (Hd) and nucleotide (Pi) Diversities of the different sub-groups depending on the parameter studied**

| **Age parameter** | | |
|---|---|---|
| **Sub-groups** | **Hd** | **Pi** |
| TC_≤30 | 1+/-0,177 | 0,11361+/-0,03382 |
| TC_>30 | 0,989+/-0,011 | 0,14682+/-0,01421 |
| **Gestation parameter** | | |
| **Sub-groups** | **Hd** | **Pi** |
| TC_0_Geste | 1+/-0,096 | 0,12925+/-0,03084 |
| TC_2_Gestes | 1+/-0,096 | 0,16463+/-0,03000 |
| TC_3_Gestes | 1+/-0,126 | 0,09918+/-0,02387 |
| TC_4_Gestes | 1+/-0,272 | 0,18231+/-0,08215 |
| TC_>4 Gestures | 0,986+/-0,018 | 0,16214+/-0,01980 |
| **Parameter of location of the tumor** | | |
| **Sub-groups** | **Hd** | **Pi** |
| TC_Sein right | 0,988+/-0,016 | 0,16986+/-0,01601 |
| Left TC_Sein | 0,995+/-0,018 | 0,14528+/-0,02396 |
| **Quadrant parameter** | | |
| **Sub-groups** | **Hd** | **Pi** |
| TC_QSE | 1+/-0,020 | 0,15402+/-0,02478 |
| TC_QSI | 1+/-0,126 | 0,20245+/-0,03395 |
| TC_QIE | 1+/-0,500 | 0,26939+/-0,13469 |
| TC_QII | 0,952+/-0,096 | 0,12595+/-0,03450 |
| **Size parameter of the tumor** | | |
| **Sub-groups** | **Hd** | **Pi** |
| TC_T2 | 1+/-0,096 | 0.18340+/-0,03548 |
| TC_T3 | 1+/-0,272 | 0.25850+/-0,07879 |
| TC_T4 | 0,980+/-0,019 | 0.15419+/-0,01664 |
| **Clinical adenopathy parameter** | | |
| **Sub-groups** | **Hd** | **Pi** |
| TC_N0 | 0,972+/-0,064 | 0,15862+/-0,02752 |
| TC_N1 | 1+/-0,045 | 0,16590+/-0,02521 |
| TC_N2 | 0,984+/-0,024 | 0,15837+/-0,02262 |
| **Grade Parameter** | | |
| **Sub-groups** | **Hd** | **Pi** |
| TC_SBRI | 1+/-0,272 | 0,21361+/-0,06015 |
| TC_SBRII | 0,989+/-0,031 | 0,12824+/-0,01838 |
| TC_SBRIII | 1+/-0,034 | 0,10866+/-0,02124 |

### 3.1.2 Statistical analyses

Statistical analyses based on t-test Table 5 showed a positive association between age, date of onset of the first menstrues and nucleotide diversity (Pi) between healthy and cancerous tissues for each patient ($P$<0.0001). The results of the ANOVA test showed that the number of gestations significantly affected nucleotide diversity ($P$= 0.0333). The PLSD test of Fisher showed important nucleotide diversities depending on the number of pregnancies Table 5 with significant differences observed between zero (0) and three (3) ($P$= 0.0190), zero (0) and four (4) ($P$=0.0006), zero (0) and eleven (XI) ($P$=0.0110), one (1) and four (4) ($P$=0.0082), two (II) and five (V) ($P$=0.0420), two (II) and six (VI) ($P$=0.0303), four (IV) and

five (V) (*P*=0.0017), four (IV) and six (0.0012), four (IV) and seven (VII) (*P*=0.0280), four (IV) and eight (VIII) (*P*=0.0053), five (5 ) and eleven (XI) (*P*=0.0219) and six (VI) and eleven (XI) (*P*=0.0162) gestations.

**Table 5. Analysis based on the t-series test paired**

| | Average Deviation | DDL | T | *P*-value |
|---|---|---|---|---|
| Age, Pi | 46.776 | 54 | 24.810 | <0.0001 |
| DPR, Pi | 13.993 | 33 | 60.634 | <0.0001 |

*Pi= Nucleotide diversity; DPR= Date of the first menstruation*

## 3.2 Discussion

The diversity and genetic development of malignant breast tumors in Senegalese patients were analyzed in this study. A strong variability of tumors was observed. Overall, our results are in perfect correlation with the predominant view that genetic differences explain the diversity of behavior of breast tumors. This could be explained by the singularity of the mitochondrial genome characterized by heteroplasmy and mitotic segregation. This phenomenon, in addition to the threshold effect explains the high heterogeneity of the clinical expressions of diseases related to the mitochondrial genome [16].

Analysis of the genetic distances between healthy and cancerous tissues showed a more rapid evolution of the tumors in patients over 30 years of age. We observed a strong genetic differentiation that depended on the number of gestations with multiparity influencing the evolution of the disease. Several mechanisms by which multiparity influences the risk of breast cancer are known or have been proposed. It is established that multiparity confers a protection against breast cancer. However, the reproductive period seems to have a double effect with the risk of breast cancer increasing immediately after the birth and then decreasing gradually afterwards [17]. The reproductive period is accompanied by an accelerated differentiation of the breast tissue and by a rapid proliferation of the epithelium. Additionally, the changes that are initiated during the first pregnancy, in particular if it occurs early, are further increased during each subsequent pregnancy. These physiological changes explain the differential risk of breast cancer that is favored by the speed of proliferation of breast epithelial cells and, in contrast, is limited by the level of differentiation of breast tissue [18]. In our study we observed that malignancy was more frequent in the right breasts than in the lefts. The majority of the tumors were localized in the QSE. The most frequent location of breast cancer is the quadrant-external followed by the central region while the other quadrants are less often affected. This topography of breast cancer is explained by the amount of glandular tissues that are more present in the central part and in the super-external region of the breast. However, our results did not fit with these observations. The genetic distances of Cytochrome b alleles between healthy and cancerous tissues we obtained were more important in the QSI. It is possible that factors beside the large amount of glandular tissue support tumor development in the quadrant super-internal. One of the main features of breast cancer in Africa, particularly in Senegal, is the aggressiveness of the cancerous tissues with tumors of large size and of high-grade. Our results showed that the large size and the grade of the tumors did not affect their genetic development. In other words, the mutations of the Cytochrome b gene were more frequent in the early stages of the disease. The aggressiveness of certain tumors could therefore be unlinked to their genetic profile but rather depend on other factors and mechanisms involved in the tumor environment.

Our analyses of genetic diversity showed a strong value of haplotype diversity and nucleotide diversity of cancerous tissues. The results showed that the cancerous cells were stable. The tests of neutrality indicated a positive or a negative selection due to an excess of rare mutations. This observation suggests the existence of a constant size of cancerous cell population [19]. This trend was confirmed by the analysis of mismatch distribution showing a multimodal curve that characterizes cells in a state of moderate expansion.

The statistical analyses confirmed the existence of a positive association between certain parameters (age of the patients, the emergence of the first menstruation beyond 12 years, education) and the nucleotide diversity of the tumors. According to Kelsey and Bernstein [20], the age is the most important risk factor of breast cancer. Several studies showed that occurrence of the first menstruation before the age of 12 increases the risk of breast cancer [21]. We could not verify this observation since 94.1% of our patients had their first menstrues after the age of 12. The association between early menstruation and development of breast cancer relies on the early and extended exposure to hormonal supply that prevails during the period of ovarian activity. Additionally, this hormonal production is particularly important when the menstrual cycles are regular. This association is confirmed by observations showing high rates of estrogen production after menstruation in women who experienced precocious menstruation [22].

## 4. CONCLUSION

Our results have shown an important genetic diversity in breast cancer. A strong genetic differentiation was noted depending on the number of gestations. The multiparity of the patients influenced the evolution of the disease. We suggest for a future study to take into consideration the age of the first pregnancy, the reproductive period, the use of oral contraceptives and of hormone replacement therapy in order to see whether these parameters are associated or not with an increased risk of breast cancer. Identification of potential risk factors on which it is possible to act, should facilitate the implementation of effective prevention strategies.

## CONSENT

An informed consent, written according to a standardized form has been obtained for each patient.

## ETHICAL APPROVAL

This study was approved by the Ethics switched Cheikh Anta Diop University of Dakar (UCAD).

## ACKNOWLEDGEMENTS

The authors are grateful to all Senegalese women who have voluntarily agreed to participate to this study as well as those who helped with the collection of samples. We are grateful to Dr Tofféne DIOME of (CBGP) and Dr Cheikh Thiaw of (CERRAS) for their precious contributions to the genetic and statistical analyses.

## COMPETING INTERESTS

The authors declare that they have no competing interests.

## REFERENCES


1. D'hondt V, Picart M, Sotiriou C. Introduce genomic expression profiles in clinical: when will molecular signatures at the bedside. Bull cancer. 2008;95(3):336-343.
2. Ly M, Antoine M, André F, Callard P, Bernaudin JF, Diallo DA. Breast cancer among women in sub-Saharan Africa: current state of knowledge. Bull cancer. 2011;98(7):797-806.
3. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Cancer Incidence and Mortality Worldwide. IARC Cancer Base; 2008.
4. Dem A, Traoré B, Dieng MM, Ouajdi T, Lalani MT, Diop M et al. Gynecological and breast cancers Cancer Institute in Dakar. notebooks Health. 2008;18(1):25-29.
5. Theillet C. Vers un inventaire des mutations oncogéniques dans les cancers humains. Bull Cancer. 2010;97(11):1223-1229.
6. Warburg O. On the origin of cancer cells. Science. 1956;123:309-314. In Yacoubi-Loueslati B, Troudi W, Baccar A, Cherni L, Rhomdhane KB, Elgaaied ABA. Polymorphism of mitochondrial microsatellite 303-315 in breast cancer in Tunisia. Bull cancer. 2009;9(3):337-342.
7. Mbaye F, Dem A, Fall M, Sembène M. Implication of the Cytochrome B nucleotide and protein mutations in the occurrence of breast cancer in Senegal. Int Jour of Applied Biol and Phar Tech. 2012;3(2):107-114.
8. Union Internationale contre le cancer: Tumeurs du sein. In: UICC-TNM classification des tumeurs malignes: Paris: Springer-Verlag; 1988.
9. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl Acid S. 1999;44:211-232.
10. Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance and Maximum Parsimony Methods. Mol Biol Evol. 2011;28(10):2731-2739.
11. Rozas J, Librado P, Sánchez-Del Barrio JC, Messeguer X, Rozas R. DnaSP Version 5 Help Contents (Help File); 2012. Available: http://www.ub.edu/dnasp/
12. Tajima F. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics. 1989;123(3):585-95.
13. Fu YX. (Statistical Tests of Neutrality of Mutations against population growth, hitchhiking and background selection. Human Genetics Center, University of Texas, Houston, Texas. 1997;77225:915-925.
14. Rogers AR, Harpending H. Population growth makes waves in the distribution of pairwise genetic differences. Mo Bi Evol. 1992;9(3):552-569.
15. Sidel SP. Statview version 5.0. Social Science Computer Review. 1986;4:232-237.
16. DiMauro S, Schon EA. Mitochondrial DNA mutations in human disease. Am J Hum Genet. 2001;106:18-26.
17. Nkondjock A, Ghadirian P. Risk factors for breast cancer. Medecine/Sciences. 2005;21:175-180.
18. Russo J, Hu YF, Yang X, Russo IH. Developmental, cellular and molecular basis of human breast cancer. J Natl Cancer Inst Monogr. 2000;17-37.
19. Excoffier L, Laval G, Schneider S. Arlequin ver. 3.1: An integrated software package for population genetics data analysis. Evol Bioinform Online. 2005;1:47-50.

20. Kelsey JL, Bernstein L. Epidemiology and prevention of breast cancer. Annu Rev Publ Health. 1996;17:47-67.
21. World Cancer Research Fund/American Institute for Cancer Research. Expert report. Food, nutrition and the prevention of cancer: a global perspective. Washington, DC: American Institute for Cancer Research; 1997.
22. Key TJ, Verkasalo PK, Banks E. Epidemiology of breast cancer. Lancet Oncol. 2001;2:133-40.



*Peer-review history:*
*The peer review history for this paper can be accessed here:*
*http://www.sciencedomain.org/review-history.php?iid=446&id=12&aid=3790*