RESEARCH ARTICLE

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# Genetic Mutations in Senegalese Women with Breast Cancer

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## Abstract

**Objective:** This study aimed to understand the effect of nucleotide mutations and microsatellite locus instability on breast cancer in Senegalese women. **Methods:** A total of 120 healthy and cancerous tissue samples were analyzed. After PCR/sequencing analysis, the nucleotide variability of two mitochondrial genes (MTCYB and D-loop) and polymorphisms of two microsatellite loci (BAT-25 and BAT-26) were determined. Additionally, structural and correlation analyses were performed between the clinicopathological features and polymorphisms of the genes studied. **Result:** Multiple variations in MTCYB and the D-loop were identified with much higher frequencies in cancerous tissues. However, MTCYB appeared more affected than the D-loop during carcinogenesis. Regarding the BAT-25 and BAT-26 loci, breast carcinogenesis was associated with instability of these microsatellite loci, with an MSI-H phenotype in 60.71% of tumors. Furthermore, the number of pears and the histological grade impacted patient survival. In addition, mutations at D-loop sites 150 and 152 and BAT-25 stability negatively impacted patient survival. In contrast, BAT-26 instability conferred the advantage of longer post-operative survival. Molecular variance analysis revealed that the clinical heterogeneity of the tumors was a function of the number of invading lymph nodes. **Conclusion:** This study highlights the high penetrance of mitochondrial mutations in breast cancer in Senegalese women, as well as the predominant role of BAT-25 marker stability and BAT-26 marker instability in breast carcinogenesis.

**Keywords:** Cancer- breast- mutations- polymorphism- microsatellites

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## Introduction

Breast cancer is a major global public health concern. According to the World Health Organization, approximately 7.8 million women live with this disease [1]. It is the most common cancer in women, with approximately 2.3 million new cases diagnosed in 2020 [2]. Incidence rates remain the highest in most developed regions, but mortality is much higher in poorer countries due to a lack of early detection and access to treatment [1]. In sub-Saharan Africa, breast cancer is the second most common cancer in women, after cervical cancer [3]. According to a study conducted at the Institut Curie of the Cheikh Anta Diop University in Dakar, breast cancer accounts for 42% of gynecological and mammary cancers [4]. The main characteristic of breast cancer in Africa, particularly in Senegal, is that it increasingly occurs in very young individuals in an aggressive form and is often diagnosed late. Similar to most cancers and many other diseases, breast cancer is a multifactorial disease with a clear genetic component that is modulated by environmental factors. Thus, the discovery of oncogenes and their counterparts, tumor suppressor genes, has established a clear pattern of cancer initiation and progression due to the spontaneous occurrence of genetic mutations. Therefore, the acquisition of the tumor phenotype is closely linked to an increase in genetic instability, whether extrinsic or intrinsic in origin. The most widespread and studied variations in the human genome are single nucleotide polymorphisms (SNPs), which are commonly used as molecular markers of genotype/phenotype associations. These genetic alterations include modifications to both the mitochondrial and nuclear DNA.

Some studies have demonstrated that mitochondrial dysfunction, such as mtDNA mutations, can cause tumor initiation and progression [5, 6]. However, the discovery of a link between the occurrence of certain cancers and the presence of anomalies in the DNA mismatch repair (MMR) system has opened new avenues for studying human carcinogenesis [7, 8]. These anomalies lead to nucleotide instability of the nuclear DNA, mainly affecting the microsatellite sequences of the genome without associated chromosomal abnormalities. Indeed, many genomic alterations in breast cancer are correlated with clinicopathological parameters and can serve as prognostic markers. This information is invaluable, as it can lead to a better understanding of many pathologies and the development of treatments for them [9]. Therefore, approaches using SNP data and/or microsatellite locus polymorphisms to study the occurrence and progression

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of breast cancer may be useful. The present study investigated two mitochondrial markers (MTCYB and D-loop) and two microsatellite markers (BAT-25 and BAT-26). This study aimed to determine the effects of nucleotide mutations and microsatellite locus instability on breast cancer carcinogenesis in Senegal.

## Materials and Methods

*Samples*

The study involved 120 surgical samples of healthy and cancerous tissue from patients with breast cancer treated at the Aristide Le Dantec Hospital Cancer Institute. Blood samples were also collected from control subjects. Clinicopathological data, such as patient identification, family history, clinical presentation, and histology, were collected for correlation analysis. This study was approved by the Ethics and Research Committee of the Cheikh Anta Diop University, Dakar (protocol 0271/2018/CER/UCAD).

*DNA extraction, polymerase chain reaction (PCR), and sequencing*

Total DNA from tissue and blood was extracted using the Qiagen Standard method (Qiagen DNeasy Tissue Kit, Qiagen, Germany) and the Qiagen DNeasy Blood method, respectively. PCR was performed in a reaction volume of 50 μL. The compositions of the reaction mixtures for each target gene are listed in Table 1. Amplification was performed using an Eppendorf thermal cycler under the conditions listed in Table 2. After UV visualization, the PCR products in which the primers were hooked were sequenced.

*Genetic analysis*

*Mutation research*

To test the association between breast cancer and the studied mitochondrial markers, the MTCYB and D-loop sequences of healthy and cancerous tissues were compared with the revised Cambridge sequence [10] (NC_012920) in the MITOMAP database. Differences between the reference sequence and MTCYB and D-loop sequences were recorded as variations. Thus, any variation not previously described in the MITOMAP database was considered novel. Odds ratios (ORs) were used to characterize the associations.

*Analysis of MTCYB encoded amino acid variability*

The frequency distributions of the 20 amino acids in healthy and cancerous tissues were also studied. Chi-square test was used to identify amino acids with significant differences between healthy and cancerous tissues. A level of 5% was considered statistically significant.

*Analysis of genetic variability in MTCYB and D-loop*

For each gene, the number of polymorphic sites, parsimony-informative sites, number of synonymous and nonsynonymous substitutions, and transition/transversion rate (R) were calculated using MEGA 6 software [11]. The average number of nucleotide differences (k), haplotypic diversity (Hd), and nucleotide diversity (π) indices were estimated using DnaSP version 5.10.01 [12].

*Analysis of genetic differentiation and evolution*

Intra- and inter-tissue genetic distances were calculated using the MEGA 6 [11]. Population-pair genetic differentiation values between healthy and cancerous tissues [13] (FST) and associated probabilities were estimated using ARLEQUIN version 3.0 software [14]. The evolution of cancerous tissues was investigated by extracting mismatch distribution curves generated using the DnaSP software version 5.10.01 [12]. The demographic indices SSD (sum of squared deviation) and RI (Harpending's raggedness index), which allowed us to test the validity of the expansion model [15, 16], were calculated using ARLEQUIN version 3.0 [14]. The significance level was set at 5% for all tests.

*Genetic analysis of BAT-25 and BAT-26 loci*

The two microsatellite loci sequences were also cleaned, corrected, and aligned using the BioEdit software version 7.1.9 [17]. Differences in the size and pattern of the loci between cancer and control tissues were considered unstable. In addition, tumors with simultaneous BAT-25 and BAT-26 instability were defined as MSI-H (a high level of microsatellite instability in tumors).

*Statistical analyses*

*Association analyses and risk assessment*

Classical multifactorial risks and prognostic factors were assessed using the Cox model. The genetic structuring of tumors according to clinicopathological parameters, such as age, number of pairs, size, number of invaded lymph nodes, and histological grade, was investigated using analysis of molecular variance [18]. Survival tests were performed using the Kaplan- Meier method to test whether mutations at certain sites are naturally polymorphic [19] and whether BAT-25 and BAT-26 instability could be risk factors [20]. Survival times were compared using a log-rank test [21].

*Survival and correlation analyses*

We tested the association between breast cancer in Senegalese women and the mitochondrial markers studied. The association study is based on the principle of linkage disequilibrium (LD). An association between a marker and a disease suggests the presence of an LD between the two. The existence of an association with a particular allele is then verified. MTCYB and D-Loop are markers with k alleles, denoted a1,...., ak. To test allelic association, the distribution of alleles in cancerous and healthy tissues for each marker studied is compared. To test the null hypothesis H0: P (ai / TC) = P (ai / TS) with i= 1,....., k, we use the chi2 test. The chi2 test was analyzed using Statview (Version 5, 1992-1998, SAS). If the distribution between healthy and cancerous tissue is significantly different, the allele is said to be associated with disease. Odds ratios are then used to characterize the association. The odds' ratio (probability ratio between the probability of occurrence of the ai allele in CT and the probability of occurrence of the ai allele in SC) is

calculated from the following equations (P=probability): P (ai/TC)/1-P (ai/TC) and P (ai/TS)/1-P (ai/TS). The odds' ratio is interpreted similarly to the relative risk. An odds' ratio of 1 mean no association. In the case of a positive association, the odds' ratio is > 1, and the association is inverse when the odds' ratio is < 1. The further the odds' ratio is from 1, the stronger the association.

The study of classic risk and prognostic factors in mono and multifactorial analysis was carried out using the Cox model, which is useful for determining the impact of explanatory variables on a patient's survival time. It expresses the instantaneous risk (hazard rate) of experiencing the event studied after a given exposure time as a function of a linear combination of explanatory factors. It can also be used to quantify and test the effects of individual characteristics. The Cox model includes age (<50y and ≥50y), date of first menstrual period (≤12 and >12), and number of parity (nulliparous women and those who are multiparous) as risk factors. Stage including tumor size, number of invaded lymph nodes and presence of metastasis, histological grade (SBRI, SBRII, and SBRIII), and phenotype (HER2, TN, LA, and LB) were considered prognostic factors. Multivariate analysis should be emphasized for its greater accuracy; in fact, it considers the interaction of criteria on each other. The statistical significance threshold is set at 5%, with a confidence interval of 95%.

Survival tests were carried out using the Kaplan-Meir method to ascertain whether the mutations could affect patient survival and constitute a risk factor [20]. This calculation method enables survival curves to be established. Survival times were compared using the log-rank test [21].

The genetic structuring of tumors according to clinico-pathological parameters such as age, number of parities, tumor location, quadrant, size, number of invaded lymph nodes and histological grade has been investigated by an Analysis of Molecular Variance (AMOVA) [18]. AMOVA is a variance/covariance of haplotype (or allele) frequencies, which, in addition to frequency information, uses molecular data, considering the number of substitutions between haplotypes (or alleles).

We analyzed the impact of BAT-25 and BAT-26 instability to see which of the two markers could serve as prognostic markers. Correlation analysis of BAT-25 and BAT-26 instability according to clinico-pathological parameters [Age, stage, grade and response to chemotherapy [<25%; 25-75% and >75%] was performed with Fisher's exact test. The significance level was 5% with a 95% confidence interval. According to the two markers studied, survival analyses were performed using the Kaplan Meir test [20]. Survival times were compared using the log-rank test [21].

## Results

*MTCYB and D-loop mutations*

Compared to the Cambridge Reference Sequence (rCRS), 237 variations were observed in MTCYB, 67 of which have already been reported in the MITOMAP database. In 58.28% of the cases, these substitutions resulted in a change in amino acids. A total of 40 samples showed significant differences (P<0.05) between healthy and cancerous tissues. Variants T15530d, C15556G, C15604T, T15622G, A15628C, C15637G, C15637T, C15643T, A15653G, T15654C, A15655C, C15667T, and C15754A were more frequent in cancerous tissues. In contrast, variants C15664A, C15664CA, A15689T, C15700T, C15704A, C15704T, C15732T, C15749T, C15767A, T15787G, and T15792C were absent in healthy tissues. Of the 230 variations detected in the D-loop, 38.69% were reported in the MITOMAP database, and 61.30% were novel. The distributions of T146C, T152TC, and T152d showed significant differences (P<0.05) between healthy and cancerous tissues.

Mutation frequencies for both mitochondrial markers were higher in cancerous tissues than in healthy tissues (MTCYB: TC=90.29% and TS=41.77%; D-loop: TC=75.21% and TS=48.69%).

*Genetic variability of MTCYB and D-loop in cancer tissues*

For both MTCYB and the D-loop, the nucleotide compositions of A and T (56.9% and 52.5%, respectively) were predominated over those of C and G (43.1% and 47.5%, respectively). The percentages of transitions (57.12% and 79.77%) were higher than those of transversions (42.88% and 20.23%). Diversity analysis revealed high values for haplotypic diversity (Hd = 0.998+/-0.004 and Hd = 1+/-0.009) and low values for nucleotide diversity ($\pi$ = 0.164+/-0.018 and 0.039+/-0.015). The average number of nucleotide differences (k) was 53.543 and 22.867 for MTCYB and D-loop, respectively. The results are summarized in Table 3.

*Variability of MTCYB encoded amino acids*

Amino acids, such as Glu, Gly, Arg, Gln, Ile, Leu, Met, Trp, and Val, showed marked differences between healthy and cancerous tissues. However, the frequencies of Glu, Gly, Arg, Met, and Trp were lower in cancer tissues than those of Gln, Ile, and Val, which were considerably higher in cancer tissues (Table 4).

*Genetic differentiation between cancerous and healthy tissue*

Genetic dissimilarity was higher in cancerous tissues than in healthy tissues for both mitochondrial markers (Table 5). Genetic differentiation (Fst) between healthy and cancerous tissues was not significant [MTCYB: Fst=0.001 (P=0.51); D-loop: Fst=0.003 (P=0.56)].

*Demo-genetic evolution*

Analysis of MTCYB and D-loop mismatch distribution curves revealed a multimodal appearance (Figure 1). However, the demographic indices SSD and RI were not significant.

*BAT-25 and BAT-26 microsatellites loci polymorphism*

Differences in size and pattern were observed between the two markers studied in patients with cancer. Of the 38 tumors analyzed, 34 (89.47%) were unstable for BAT-25 and 24 (70.58%) for BAT-26 (Table 6). Control samples

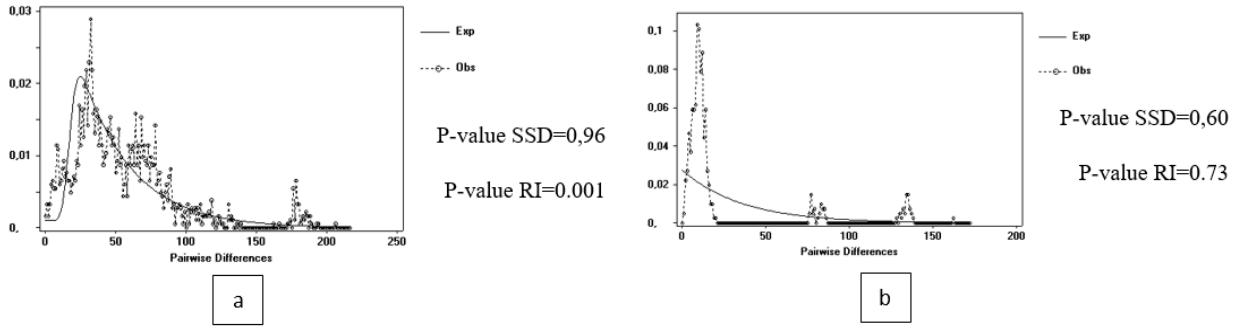


Figure 1. Mismatch Distribution Curves for MTCYB (a) and D-loop (b).

consisted of 25T and 25A for BAT-25 and BAT-26, respectively.

*MSI status*

Of the patients for whom both markers could be sequenced, one (1) was stable for both markers, 8 (28.57%) were unstable for the BAT-25 marker, 2 (7.14%) were unstable for BAT-26, and 17 (60.71%) were MSI-H (unstable for both markers). The results are summarized in Table 7.

*Multivariate analysis of clinicopathological factors*

The number of pairs (HR=1.369; CI=0-0.602; P=0.019) and histological grade (HR=4.357; CI=0.002-0.727; P=0.017) significantly affected patient prognosis (Table 8). 3.9 Genetic structuring according to clinicopathological parameters

For the mitochondrial matrix, moderate genetic structuring was noted only between N0-N1 tumors (Fst=0.119; P=0.035) and between N0-N2 tumors (Fst=0.148; P=0.002). In other words, as the number of lymph nodes increased, genetic differentiation was observed in node-less tumors.

*Correlation between survival time and genetic markers*

Polymorphisms in D-loop sites 150 and 152 and BAT-26 instability were considerably associated with longer post-operative survival. Furthermore, the survival of patients with stable BAT-25 decreased drastically, but the difference was not statistically significant (P=0.473). The results are shown in Figure 2.

## Discussion

This study aimed to elucidate the role of genetic mutations in breast cancer in Senegalese women.

Table 1. Sequences of Primers Used and Conditions for PCR Reactions

| Regions | Sequences | | Programs |
|---|---|---|---|
| MTCYB | H15915 | TCTCCATTTCTGGTTTACAAGAC | 94 °c 3 min; 40 cycles (92 °c 45 s, 50 °c 1 min, 72 °c 1 min 30 s) 72 °c 10 min |
| | L14723 | ACCAATGACATGAAAAATCATGGTT | |
| D-loop | H408 | TGTTAAAAGTGCATACCGCCA | 95 °C 15 mins; 35 cycles (95 °c 30 s, 62 °c 30 s, 72 °c 2 min) 72 °c 10 min |
| | L16340 | AGCCATTTACCGTACATAGCACA | |
| BAT-25 | BAT 25 (F) | TACCAGGTGGCAAAGGGCA | 95 °C 10 min; 30 cycles (94 °C 45 s; 57 °C 45 s; 74 °C 45 s); 74 °C 7 min |
| | BAT 25 (R) | TCTGCATTTTAACTATGGCTC | |
| BAT-26 | BAT 26 (F) | CTGCGGTAATCAAGTTTT | 95 °C 5 min; 35 cycles (95 °C 30 s; 47 °C 1 min; 70 °C 1 min); 70 °C 10 min |
| | BAT 26 (R) | AACCATTCAACATTTTTAACCC | |

Table 2. PCR Reaction Mixture Compositions

| Components | Volume: concentration or quantity | | | |
|---|---|---|---|---|
| | Amplified genes | | | |
| | MTCYB | D-loop | BAT-25 | BAT-26 |
| Water MilliQ | 23.8 µL | 32.9 µL | 34.9 µL | 34.9 µL |
| Tampon 10X | 5 µL | 5 µL | 5 µL | 5 µL |
| dNTP | 2 µL | 2 µL | 2 µL | 2 µL |
| $MgCl_2$ | 1 µL | 3 µL | 1 µL | 1 µL |
| Primers (forward and reverse) | 5 µL | 5 µL | 5 µL | 5 µL |
| Taq polymerase | 0.2 µL | 0.1µL | 0.1 µL | 0.1 µL |
| DNA extract | 8 µL diluted 1/10 | 2 µL | 2 µL | 2 µL |

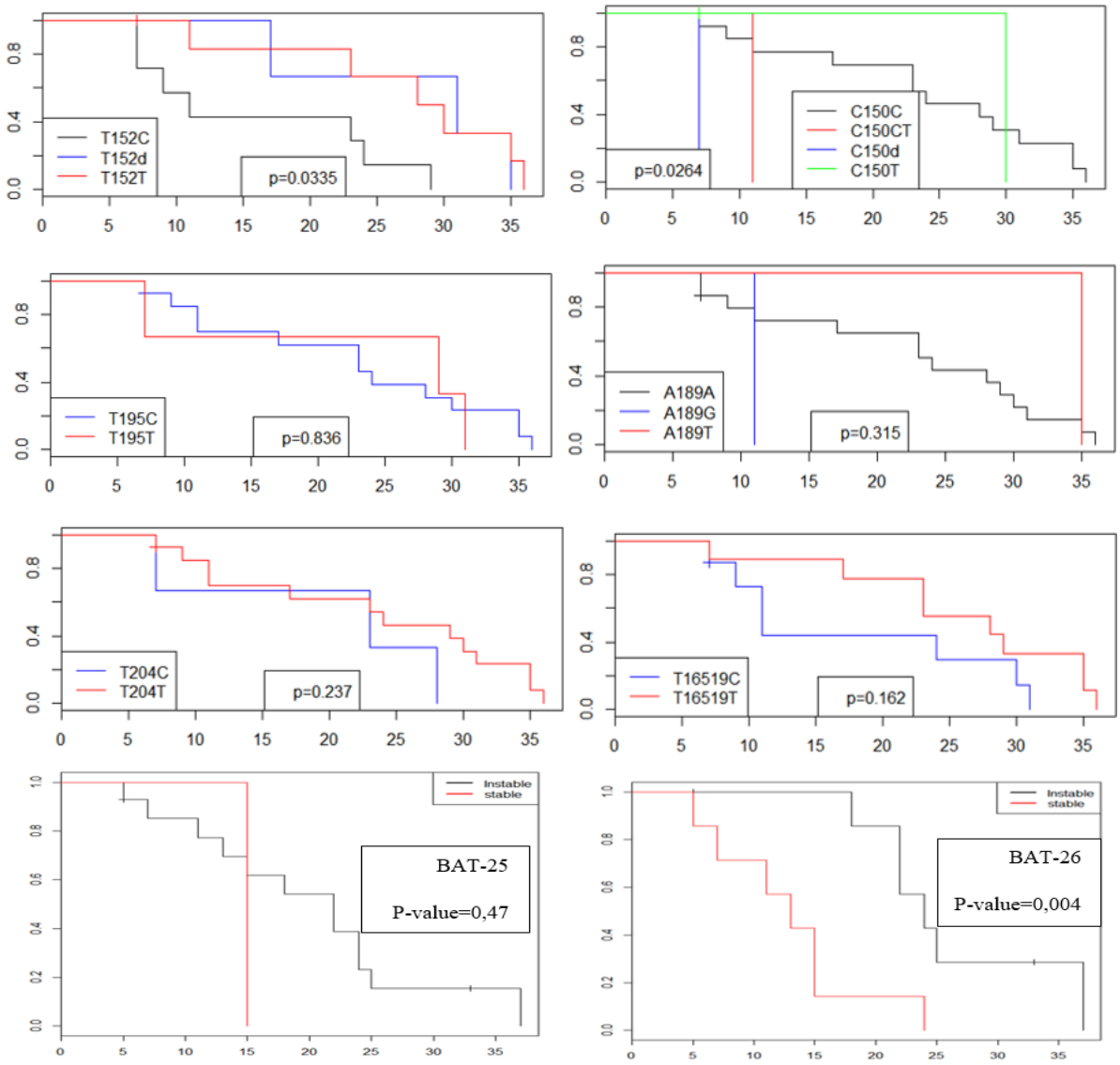


Figure 2. Survival Curves as a Function of Nucleotide Mutations in D-loop and Instability and Stability of BAT-25 and BAT-26.

Two mitochondrial (MTCYB and D-loop) and two microsatellite markers (BAT-25 and BAT-26) were analyzed. The results revealed that most of the analyzed cancerous tissues had variations in MTCYB. Overall, of the variations detected in MTCYB, 40 showed marked differences between healthy and cancerous tissues, some of which were much more frequent in cancerous tissues with deleterious effects. Overall, 58.28% of substitutions resulted in amino acid changes. The values found in this study generally appear to be higher than those described

Table 3. MTCYB and D-loop Genetic Variability Parameters

| Parameters | MTCYB | | | | D-loop | | | |
|---|---|---|---|---|---|---|---|---|
| Number of polymorphic sites | 306 (94.15%) | | | | 194 (33.85%) | | | |
| Number of informative sites | 230 (70.76%) | | | | 44 (20.68%) | | | |
| Transitions (%) | 57.12 | | | | 79.77 | | | |
| Transversions (%) | 42.88 | | | | 20.23 | | | |
| Transitions/Transversion rate=R | 1.278 | | | | 4.205 | | | |
| | T | C | A | G | T | C | A | G |
| Nucleotide frequency | 30.5 | 12.4 | 26.4 | 30.7 | 26.8 | 18.1 | 25.7 | 29.4 |
| Haplotypic diversity (Hd±SD) | 0.998±0.004 | | | | 1±0009 | | | |
| Nucleotide diversity ($\pi \pm$ SD) | 0.164±0.018 | | | | 0.039±0.015 | | | |
| k | 53.543 | | | | 22.867 | | | |

Table 4. Amino Acid Frequencies Encoded by MTCYB between Healthy and Cancerous Tissues

| Non-essential amino acids | Healthy tissue | Cancerous tissue | P-value |
|---|---|---|---|
| Ala | 0.694 | 0.922 | 0.089 |
| Cys | 2.894 | 1.978 | 0.118 |
| Asp | 0 | 0.301 | 0.5 |
| Glu | 3.646 | 3.47 | 0.015 |
| Gly | 18.697 | 16.549 | 0.038 |
| His | 0.347 | 0.804 | 0.24 |
| Asn | 1.678 | 1.408 | 0.055 |
| Pro | 1.041 | 1.877 | 0.177 |
| Gln | 2.112 | 2.364 | 0.035 |
| Arg | 6.396 | 5.499 | 0.047 |
| Ser | 3.039 | 4.04 | 0.089 |
| Tyr | 1.302 | 1.643 | 0.073 |
| Essential amino acids | Healthy tissue | Cancerous tissue | P-value |
| Ile | 3.299 | 3.336 | 0.003 |
| Leu | 21.389 | 20.808 | 0.008 |
| Lys | 2.518 | 3.185 | 0.074 |
| Met | 6.454 | 6.254 | 0.01 |
| Phe | 2.431 | 3.018 | 0.068 |
| Thr | 0.984 | 1.877 | 0.192 |
| Trp | 8.162 | 7.662 | 0.02 |
| Val | 12.908 | 12.994 | 0.002 |

Ala, alanine; Cys, cysteine; Asp, aspartic acid; Glu, glutamic acid; Gly, glycine; His, histidine; Asn, asparagine; Pro, proline; Gln, glutamine; Arg, arginine; Ser, serine; Tyr, tyrosine; Essential amino acids, amino acids that cannot be synthesized by the body and must be obtained from the diet; Ile, isoleucine; Leu, leucine; Lys, lysine; Met, methionine; Phe, phenylalanine; Thr, threonine; Trp, tryptophan; Val, valine

in the literature. Parella et al. found that 61% of primary breast tumors had mtDNA mutations, with 25% of the mutations inducing an amino acid change in the protein sequence [22]. This is supported by the fact that all the analyzed cancer tissues contained variations at the D-loop level. The number of tissues with variations was higher than that reported by Tseng et al. [23], who found that 30% of breast tumors had D-loop mutations in Caucasian populations. A mutation rate of 75.21% in the D-loop was found in cancerous tissues. This result is consistent with previous reports indicating that D-loop mutations are in the range of 20–78% in human cancers [24, 25]. In the present study, variations were found in healthy tissues;

Table 5. Intra- and Inter-Tissue Genetic Distance (d) and Degree of Differentiation (Fst)

| Amplified genes | | Genetic distance | | Fst (P-value) |
|---|---|---|---|---|
| | | Intra | Inter | |
| MTCYB | TS | 0.185 | 0.196 | 0.001 (0.51) |
| | TC | 0.208 | | |
| D-loop | TS | 0.033 | 0.038 | 0.003 (0.56) |
| | TC | 0.044 | | |

TS, healthy tissue; TC, cancer tissue

Table 6. Instability of BAT-25 and BAT-26 markers in cancerous tissue

| BAT-25 | | BAT-26 | |
|---|---|---|---|
| Motifs | Number of Cancerous tissues (%) | Motifs | Number of Cancerous tissues (%) |
| Stable | 4 (10.53%) | Stable | 10 (29.42%) |
| Unstable | 34 (89.47%) | Unstable | 24 (70.58%) |

Table 7. MSI Status of Breast Tumors

| Number of patients | Loci | | Status MSI |
|---|---|---|---|
| | BAT-25 | BAT-26 | |
| 1 | - | - | ? |
| 8 | + | - | ? |
| 2 | - | + | ? |
| 17 | + | + | MSIH |

Note: (+), unstable for marker; (-), stable for marker; (?): Status undetermined

however, their frequency was lower than that observed in cancerous tissues, demonstrating the involvement of MTCYB and D-loop variations in breast carcinogenesis in Senegalese women.

The 146C and 152d variations in the D-loop were found at a higher frequency in cancerous tissues, with an OR greater than 1. Therefore, these variations could be associated with breast cancer in Senegalese women, causing the disease or increasing the risk of developing breast cancer. In contrast, individuals with a C insertion at position 152appeared to have a lower risk. The insertion was more frequently observed in healthy tissues, whereas the deletion was predominantly found in cancerous tissues. These data support the idea that these haplotypes represent consensus sequences in Homo sapiens. This study showed a marked decrease in amino acids, such as Glu, Gly, Arg, Leu, Met, and Trp, in cancer tissues. However, this decrease was associated with a considerable increase in Gln, Ile, and Val levels. Notably, Glu and Gly, markedly decreased in cancer tissues, are non-essential amino acids. Indeed, it has been reported that the expression of mitochondrial proteins in the Glu biosynthesis pathway and the consumption of Gly are strongly correlated with rapid cell proliferation in cancer [26]. Additionally, Trp, which plays an important role in T cell proliferation, decreased considerably in cancer tissues. Furthermore, T lymphocytes are key players in immune rejection reactions that can lead to the elimination of cancer cells and are the basis of various immunotherapeutic approaches currently

Table 8. Multivariate Analysis with Cox Model

| Factors | HR | 95% CI | P-value |
|---|---|---|---|
| Age | 1.081 | 0.985 – 1.185 | 0.097 |
| DFP | 1.546 | 0 - ∞ | 0.999 |
| Number of pares | 1.369 | 0 – 0.602 | 0.019 |
| Stade | 4.12 | 0 - ∞ | 0.999 |
| histological grade | 4.357 | 0.002 – 0.727 | 0.017 |

HR, Hazard ratio; DFP, Date of first period; CI, confidence interval

being tested. These methods stimulate the immune system to recognize and destroy tumor cells. Additionally, evidence suggests that amino acids are continuously used for cell proliferation, resulting in a higher concentration at the time of supply and a lower concentration after their consumption in the production of new tumor cells [27]. Therefore, quantifying the levels of 20 amino acids in the serum or plasma of patients with breast cancer is necessary.

Analysis of genetic variability showed that most transformations could be attributed to nucleotide substitutions. Notably, this variability between healthy and cancerous tissues differed from patient to patient, reflecting the heterogeneity between tumors, which is common in oncological pathology. The higher absolute values of genetic distance in cancerous tissues confirmed this heterogeneity between tumors. Both mitochondrial markers exhibited high haplotypes and low nucleotide diversity. This suggests that cells in breast cancer tissues undergo rapid growth from an initially low clone. These findings are consistent with those of the Darwinian model, in which neoplasia arises from a single cell that is the target of mutations that overcome the physiological mechanisms that limit its proliferation. Therefore, the succession of mutations conferring a selective advantage, followed by periods of clonal expansion, leads to the formation of malignant tumors. This was consistent with the mismatch distribution curves, suggesting a sudden expansion of mutations. This shows that the variation observed at polymorphic sites and singletons was not neutral and was fixed under the influence of the environmental effects of random genetic drift. Indeed, Chinnery et al. [28] showed that random genetic drift is powerful enough to explain the fixation of rare mtDNA mutations in tumor tissues.

This study investigated the two loci most predictive of MSI (BAT-25 and BAT-26), included in the National Cancer Institute group of markers [29, 30]. These two microsatellite loci were monomorphic with 25T and 25A, respectively, in control subjects. Contrary to what has been described in the literature, the BAT-26 locus consists of a 25A repeat in control subjects instead of a 26A repeat. According to Samb et al. [31], the Senegalese population has a 25A repeat. Indeed, according to Buhard et al. [32], both markers have natural polymorphisms, particularly among Africans. Therefore, their use should be cautious, depending on the ethnic group to which the patient belongs. Furthermore, in Senegalese women with breast cancer, BAT-25 is much less stable than BAT-26. However, 60.71% of the analyzed cancerous tissues were unstable for both markers, confirming their MSI-H status. According to Siah et al. [33], this variability suggests that mismatch repair defects that lead to increased MSI may play a role in the specific pathogenesis of breast cancer. Multivariate analysis using the Cox method showed that both the number of pairs and histological grade influenced patient survival. In other words, nulliparous women had a 1.369-fold increased risk of developing breast cancer. The interaction between pregnancy and breast cancer is complex and paradoxical. Epidemiological data on the effects of pregnancy on breast cancer risk are numerous and sometimes contradictory [34-36]. However, most studies indicate that the risk of breast cancer increases with nulliparity and late age at first pregnancy. In our series, grade, which reflects tumor differentiation and aggressiveness, influenced prognosis, with a consequent effect on patient survival. Thus, grade I tumors have a 4.357 times better prognosis than grade II and III tumors.

This study revealed that polymorphisms at sites 150 and 152 were strongly associated with patient survival. Indeed, it has been shown in the literature that replication of the mtDNA heavy strand starts at position T149 instead of position C151 if the C150T transition is present. The authors speculated that the C150T polymorphism might confer replicative and survival advantages to mtDNA. In contrast, BAT-26 instability was strongly associated with longer post-operative survival. Carvalho et al. [37] found that in colorectal cancers, the 5-year survival rate was 85% in patients with BAT-26 instability compared to that in patients with BAT-26 stability. Another crucial and classic element is the number of lymph nodes involved. In addition to tumor grade, lymph node involvement is the most important prognostic factor. For all parameters tested, genetic structuring was observed only between tumors without (N0) and those with one (N1) or two (N2) nodes involved. In other words, the genetic differentiation increased as the number of involved nodes increased. According to these results, the clinical heterogeneity of tumors was verified only at the molecular level based on lymph node invasion.

In conclusion, our study revealed a high rate of variation in cancerous tissues compared to healthy tissues, reflecting the impact of mtDNA mutations on breast carcinogenesis in Senegalese women. Consequently, any dysfunction of the mitochondria following mutations in the mitochondrial genome that affect its function could be a causal factor in carcinogenesis. Additionally, the MSI-H phenotype found in Senegalese women opens the prospect of screening for MSH2, MSH3, and MSH6 to determine the genes involved in the genetic instability of MSI-H tumors found in these patients. From a clinical standpoint, two risk factors, the number of pairs and histopathological grade, can be considered to have a significant impact on the incidence and progression of breast cancer in Senegalese women. Although the sample size was insufficient, all the results obtained from the Senegalese population are of essential interest, not only from a fundamental point of view but also from a medical perspective. This should encourage further research in this field to understand the precise role of mitochondrial genome mutations and the mismatch repair system in breast cancer.

## Author Contribution Statement

FM and MS equally contributed to the conception, design, analysis, writing, and editing of the article.

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*Scientific Approval*

This study was approved by the Ethics and Research Committee of the Cheikh Anta Diop University, Dakar (protocol 0271/2018/CER/ UCAD).

*Ethical Declaration*

The study conforms to the Declaration of Helsinki standards, and ethical approval was obtained by the Ethics and Research Committee of the Cheikh Anta Diop University, Dakar. All participants provided informed consent after being informed about the survey's objectives, relative risks, and benefits.

*Data Availability*

The data analyzed during the current study are available from the corresponding author on reasonable request

*Conflict of Interest*

The authors declare no conflicts of interest about the publication of this paper.

## References

1. World Health Organization. Breast Cancer. US: WHO, 2024. [updated 2024 March 13; cited year month day]. Available from: https://www.who.int/new-sroom/fact-sheets/detail/breast-cancer.
2. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA Cancer J Clin. 2021;71(3):209-249. https://doi.org/10.3322/caac.21660.
3. Ly M, Antoine M, André F, Callard P, Bernaudin JF, Diallo DA. Breast cancer in Sub-Saharan African women: review. Bull Cancer. 2011;98(7):797-806. https://doi.org/10.1684/bdc.2011.1392.
4. Dem A, Traoré B, Dieng MM, Diop PS, Ouajdi T, Lalami MT, et al. Gynaecological and breast cancers at the Dakar Cancer Institute. Sante. 2008;18(1):25-9. https://doi.org/10.1684/san.2008.0093.
5. Sánchez-Céspedes M. Mitochondrial DNA alterations in human tumors. Rev Oncol. 2003;5(1):15-20. https://doi.org/10.1007/BF02712836.
6. Torres JA. Mitochondrial DNA Mutation and its Relation to Breast Cancer. Revista de Especialidades Médico-Quirúrgicas. 2013;18(4):345-9.
7. Davies H, Morganella S, Purdie CA, Jang SJ, Borgen E, Russnes H, et al. Whole-Genome Sequencing Reveals Breast Cancers with Mismatch Repair Deficiency. Cancer Res. 2017;77(18):4755-62. https://doi.org/10.1158/0008-5472.CAN-17-1083.
8. Fusco N, Lopez G, Corti C, Pesenti C, Colapietro P, Ercoli G, et al. Mismatch Repair Protein Loss as a Prognostic and Predictive Biomarker in Breast Cancers Regardless of Microsatellite Instability. JNCI Cancer Spectr. 2018;2(4):pky056. https://doi.org/10.1093/jncics/pky056.
9. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, et al. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. Nature. 2010;467(7313):318-22. https://doi.org/10.1038/nature09328.
10. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet. 1999;23(2):147. https://doi.org/10.1038/13779.
11. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30(12):2725-9. https://doi.org/10.1093/molbev/mst197.
12. Rozas J, Librado P, Sánchez-Del Barrio JC, Messeguer X, Rozas R. DnaSP Version 5 Help Contents [Help File]. 2012. [updated 29 Februaru 2019; cited year month day] Available with the program at http://www.ub.edu/dnasp/.
13. Weir BS, Hill WG. Estimating F-statistics. Annu Rev Genet. 2002;36:721-50. https://doi.org/10.1146/annurev.genet.36.050802.093940.
14. Excoffier L, Laval G, Arlequin SS (version 3.0): an integrated software package for population genetics data analysis. Evol Bioinform Online. 2007 Feb 23;1:47-50.
15. Rogers AR, Harpending H. Population growth makes waves in the distribution of pairwise genetic differences. Mol Biol Evol. 1992;9(3):552-69. https://doi.org/10.1093/oxfordjournals.molbev.a040727.
16. Schneider S, Excoffier L. Estimation of past demographic parameters from the distribution of pairwise differences when the mutation rates vary among sites: application to human mitochondrial DNA. Genetics. 1999;152(3):1079-89. https://doi.org/10.1093/genetics/152.3.1079.
17. Hall TA. BioEdit: A User-Friendly Biological Sequence Alignment Editor and Analysis Program for Windows 95/98/NT. Nucleic Acids Symp Ser. 1999;41:95-98.
18. Excoffier L, Smouse PE, Quattro JM. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics. 1992;131(2):479-91. https://doi.org/10.1093/genetics/131.2.479.
19. Brandon M, Baldi P, Wallace DC. Mitochondrial mutations in cancer. Oncogene. 2006;25(34):4647-62. https://doi.org/10.1038/sj.onc.1209607.
20. Kaplan EL, Meier P. Nonparametric Estimation from Incomplete Observations. J Stat Am Assoc. 1958;53(282):457-81. https://doi.org/10.2307/2281868.
21. Peto R, Pike MC, Armitage P, Breslow NE Cox Dr, Haward SV. Organization and analysis of comparative therapeutic trials involving long-term patient monitoring. J Epidemiol Public Health. 1979;27:167-255.
22. Parrella P, Xiao Y, Fliss M, Sanchez-Cespedes M, Mazzarelli P, Rinaldi M, et al. Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle aspirates. Cancer Res. 2001;61(20):7623-6.
23. Tseng LM, Yin PH, Chi CW, Hsu CY, Wu CW, Lee LM, et al. Mitochondrial DNA mutations and mitochondrial DNA depletion in breast cancer. Genes Chromosomes Cancer. 2006;45(7):629-38. https://doi.org/10.1002/gcc.20326.
24. Wong LJ, Tan DJ, Bai RK, Yeh KT, Chang J. Molecular alterations in mitochondrial DNA of hepatocellular carcinomas: is there a correlation with clinicopathological profile? J Med Genet. 2004;41(5):e65. https://doi.org/10.1136/jmg.2003.013532
25. Czarnecka AM, Gammazza AM, Di Felice V, Zummo G, Cappello F. Cancer as a "Mitochondriopathy". J Cancer Mol. 2007;3(3):71-9.
26. Jain M, Nilsson R, Sharma S, Madhusudhan N, Kitami T, Souza AL, et al. Metabolite profiling identifies a key role for glycine in rapid cancer cell proliferation. Science. 2012;336(6084):1040-4. https://doi.org/10.1126/

science.1218595.
27. Musharraf SG, Shahid N, Naqvi SMA, Saleem M, Siddiqui AJ, Ali A. Metabolite Profiling of Preneoplastic and Neoplastic Lesions of Oral Cavity Tissue Samples Revealed a Biomarker Pattern. Sci Rep. 2016;6:38985. https://doi.org/10.1038/srep38985.
28. Chinnery PF, Samuels DC, Elson J, Turnbull DM. Accumulation of mitochondrial DNA mutations in ageing, cancer, and mitochondrial disease: is there a common mechanism? Lancet. 2002;360(9342):1323-5. https://doi.org/10.1016/S0140-6736(02)11310-9.
29. Suraweera N, Duval A, Reperant M, Vaury C, Furlan D, Leroy K, et al. Evaluation of tumor microsatellite instability using five quasimonomorphic mononucleotide repeats and pentaplex PCR. Gastroenterology. 2002;123(6):1804-11. https://doi.org/10.1053/gast.2002.37070.
30. Bacher JW, Flanagan LA, Smalley RL, Nassif NA, Burgart LJ, Halberg RB, et al. Development of a fluorescent multiplex assay for detection of MSI-High tumors. Dis Markers. 2004;20(4-5):237-50. https://doi.org/10.1155/2004/136734.
31. Samb MD, Mbaye F, Ndiaye MM, Toure S, Sembene M. Bat 26 Microsatellite Instability in Oral Cavity Cancers in Senegal. J Cancer Ther. 2023;14(1):25-37. https://doi.org/10.4236/jct.2023.141003.
32. Buhard O, Cattaneo F, Wong YF, Yim SF, Friedman E, Flejou JF, et al. Multipopulation analysis of polymorphisms in five mononucleotide repeats used to determine the microsatellite instability status of human tumors. J Clin Oncol. 2006;24(2):241-51. https://doi.org/10.1200/JCO.2005.02.7227.
33. Siah SP, Quinn DM, Bennett GD, Casey G, Flower RL, Suthers G, et al. Microsatellite instability markers in breast cancer: a review and study showing MSI was not detected at 'BAT 25' and 'BAT 26' microsatellite markers in early-onset breast cancer. Breast Cancer Res Treat. 2000;60(2):135-42. https://doi.org/10.1023/a:1006315315060.
34. Hinkula M, Pukkala E, Kyyrönen P, Kauppila A. Grand multiparity and the risk of breast cancer: population-based study in Finland. Cancer Causes Control. 2001;12(6):491-500. https://doi.org/10.1023/a:1011253527605.
35. Albrektsen G, Heuch I, Thoresen S, Kvåle G. Clinical stage of breast cancer by parity, age at birth, and time since birth: a progressive effect of pregnancy hormones? Cancer Epidemiol Biomarkers Prev. 2006;15(1):65-9. https://doi.org/10.1158/1055-9965.EPI-05-0634.
36. Antoniou AC, Shenton A, Maher ER, Watson E, Woodward E, Lalloo F, et al. Parity and breast cancer risk among *BRCA1* and *BRCA2* mutation carriers. Breast Cancer Res. 2006;8(6):R72. https://doi.org/10.1186/bcr1630.
37. Carvalho F, Santos EMM, Pena SD, Vettore A, Lopes A, Ferreira FO, et al. Clinicopathological significance of BAT-26 instability in 184 patients with colorectal cancer. Appl Cancer Res. 2005;25(1):36-40.