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Breast Cancer in Mauritania: Mutations in Exon 4 of the *TP53* Gene

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Abstract: Cancer is one of the leading causes of death worldwide, with approximately 10 million cancer-related deaths reported in 2020. Approximately 70% of these deaths occurred in developing countries. In 2020, 2.3 million women were diagnosed with breast cancer and 685,000 deaths due to breast cancer were reported worldwide. Cancer is characterized by the presence of most triggers in the genome. Mutations in genes, either passed from one generation to the next or acquired throughout life, can cause breast cancer. Tumor suppressor genes encode proteins that negatively regulate cell proliferation and repress certain oncogenes. One of these genes, the *TP53* gene, has multiple biological functions. Understanding the mechanism of action of p53 in breast carcinogenesis has been an important challenge in cancer research. This study aimed to investigate the involvement of alterations in exon 4 of the *TP53* gene in Mauritanian patients with breast cancer. The study was conducted using 45 tumor tissue sequences and 35 control sequences. The nature and position of the mutations were determined using Mutation Surveyor V5.1.2 The pathogenicity of the mutations was determined using Polyphen2, SIFT, and Mutation Tester, and their three-dimensional structure was determined using the I-Tasser server. DnaSP version 5.10, MEGA version 7.014, and Arlequin version 3.1 were used to highlight the variability of exon 4 of the *TP53* gene. Our results revealed the presence of a single-nucleotide variant at position (c.139 C>T), which causes an amino acid change from proline to serine at codon 47 in the coding region of exon 4 of the *TP53* gene. Of note, this variant has already been listed in the database (rs1800371). In addition, five novel mutations were found in the cancer tissue sequences alone, with statistically significant scores. Analyses of genetic variability indicated a relatively high polymorphism in tumor tissue sequences compared with control sequences. This variability may contribute to the involvement of exon 4 mutations in the occurrence of breast cancer in our population, and serves as the first data on *TP53* exon 4 alterations in Mauritania.

Keywords: Breast Cancer, Mauritania, *TP53*, Exon 4

1. Introduction

The breast undergoes important changes throughout life, particularly during gestation, lactation, and involution. These physiological stages are characterized by three important cellular mechanisms: proliferation, differentiation, and cell death. However, disturbances at the molecular level during this process may lead to the development of cancer cells [1]. Breast cancer is the abnormal proliferation of cells in the mammary glands, most often a carcinoma arising in the epithelium of the glands or their ducts [2]. Breast cancer is the most common tumor affecting women worldwide [3].

Multiple genetic changes occur during the evolution of normal cells into cancerous cells. This evolution is facilitated by the loss of fidelity in processes that replicate, repair, and segregate the genome. Dysfunction is caused by one or more mutations that alter the expression of genes involved in the regulation and control of cells in the body [4, 13].

Breast cancer is associated with different types of somatic genetic alterations, such as mutations in oncogenes and tumor suppressor genes. *TP53* mutations are considered as the most common genetic alterations in human cancers and are found in 20-40% of breast cancers [4]. Carriers of the germline *TP53* mutation are at a very high risk of developing breast cancer [5], and more than 20-40% of sporadic breast cancers exhibit a somatic mutation in the *TP53* gene [6], indicating the involvement of the loss of function of the p53 protein in the pathogenesis of breast cancer.

The *TP53* gene encodes a tumor suppressor protein (393 amino acids) that contains transcriptional activation, DNA-binding, and oligomerization domains. The encoded proteins respond to various cellular stressors by regulating the expression of target genes, thereby inducing cell cycle arrest, apoptosis, senescence, DNA repair, or metabolic changes. Mutations in this gene are associated with various human cancers, including breast cancer [7]. Based on prior studies, patients with TP53 mutant tumors have poorer survival rates than those with *TP53* wild-type tumors [8, 9].

Mutations in the *TP53* gene have been identified in European, American, and Asian populations, but rarely in African populations [7]. According to studies by Baba and Mohamed [11, 12], breast cancer is the most frequent cancer among Mauritanian women, with percentages differing according to ethnicity. Moreover, diagnosis is often late and management is difficult and costly [12]. The general objective of this study was to evaluate the involvement of alterations in exon 4 of the *TP53* gene in breast cancer in Mauritanian patients and identify its genetic variability and genetic diversity.

2. Methodology

2.1. Study Population

This study comprised 46 Mauritanian patients with breast cancer and 38 healthy women (controls). Patients were recruited from the National Hospital Centre (CHN) in Nouakchott and the Military Hospital Centre (CHM) in Nouakchott, the capital of Mauritania, in accordance with the ethical principles of the World Medical Association's HELSINKI Declaration.

2.1.1. Samples

For each patient, a fresh surgical specimen was obtained from the tumor, collected in a dry tube, and preserved in 96% alcohol. These patients were of different ages and ethnicities and had histologically confirmed breast cancer. In addition, some patients had undergone neoadjuvant chemotherapy.

Blood samples from healthy women (controls) of different ages and ethnicities were collected by nurses in the maternity ward at the Nouakchott National Hospital. Informed consent was obtained before sample collection. The samples were transported to the genomic laboratory of the Department of Animal Biology of the Faculty of Science and Technology of the University of Cheikh Anta Diop in Dakar (Senegal). The tissues were stored at -20°C before the performance of any molecular analysis.

2.1.2. Extraction, Amplification, and Sequencing of Exon 4 of the TP53 Gene

Total DNA was extracted from each sample using the standard Zymo Research Kit protocol. Exon 4 of the *TP53* gene was amplified in the DNA extracts in a 25-µl reaction volume. The following primers were employed:

F- (5'-CCTGGTCCTCTGACTGCTCT-3') and R- (5'-GCCAGGCATTGAAGTCTCAT-3').

PCR was performed using an Eppendorf thermocycler with the following cycling conditions:

preliminary denaturation at 94°C for 7 min, 35 cycles of denaturation at 94°C for 1 min, primer hybridization at 64°C for 1 min, complementary DNA strand elongation at 72°C for 1 min, and final elongation at 72°C for 10 min. To ensure that exon 4 of TP53 was amplified, electrophoretic migration was tested using 2% agarose gel. The sequencing method described by Sanger (1977) was used to identify the nucleotide sequence of exon 4 of the *TP53* gene.

2.2. Search for Mutations in Exon 4 of the TP53 Gene

The presence of any mutation and its position at the chromosomal level in exon 4 of the *TP53* gene were determined by inputting raw sequencing data into Mutation Surveyor version 5.0.1, an anti-correlation technology that compares sample sequences to a reference sequence from chromatograms. This software has excellent accuracy and sensitivity and low false-positive and false-negative rates in DNA variant analyses. The mutation score indicates the level of confidence of a "true mutation." This score is based on the concept of Phred scores, where quality scores are logarithmically related to error probabilities. The lower the score, the greater the number of mutation calls. A mutation is considered if the Phred score is ≥20, with an accuracy of 99%. The detected mutations were inputted into the *TP53* database, a software that helps interpret genetic mutations identified in the human genome.

2.2.1. Prediction of the Pathogenicity of the Mutations Found

The resulting non-synonymous variants (which induce amino acid changes) were inputted into three software programs (Polyphen-2, http://genetics.bwh.harvard.edu/pph2/); (Mutation Taster, https://www.genecascade.org/MutationTaster2021/#chrpos); and (SIFT, https://sift.bii.a-star.edu.sg/www/Extended_SIFT_chr_coords_submit.html) to obtain relevant annotations on pathogenicity. This information was then combined with data from the literature to determine whether the oncogenic (oncogenic or tumor suppressor) effects of the gene were correlated with somatic alterations.

2.2.2. Prediction of the 3D Structure of Protein p53

To determine the functional impact of the identified mutations at the three-dimensional level, we simulated the structure of the protein encoded by the *TP53* gene using the I-Tasser web server. The iterative thread assembly refinement server (I-TASSER, https://zhanggroup.org/I-TASSER/) is an integrated platform for automated prediction of protein structure and function based on the sequence-structure-function paradigm. Given an amino acid sequence, I-TASSER first generates three-dimensional (3D) atomic models from multiple threading alignments and iterative structural assembly simulations. Thereafter, the function of the protein is inferred via structural matching of the 3D models with other known proteins.

2.2.3. Analyses of Variability, Diversity, and Genetic Structuring

Polymorphisms in exon 4 of the *TP53* gene were assessed in both healthy tissue (TH) and cancerous tissue (TC) using different parameters, including the total number of sites, variable sites, singleton variable sites, total number of synonymous and non-synonymous sites (Eta), percentage of transitions (s), transversions (v), and mutation rate (R). The parameters, nucleotide frequency and genetic distance [14], were determined using MEGA software version 7.0.14 [15]. The average number of nucleotide differences (k) and the number of haplotypes (h), haplotypic diversity (Hd), and nucleotide diversity (π) indices, which enable inference of the genetic diversity within TH and TC, were obtained using DnaSP software version 5.10 [16]. The genetic differentiation index (F_{ST}) was determined using the ARLEQUIN V3.1 software [17].

2.3. Analysis of Amino Acid Variability in Exon 4 of the TP53 Gene

The frequency distribution of the 20 amino acids in TH and TC tissues was determined using MEGA version 7.0.14 [15] by selecting the universal genetic code and best reading frame. To determine whether a difference in the frequency distribution of each amino acid exists between control and cancer tissues, the database was submitted to the R software version 4.0.0 [26], the Shapiro Wilk normality test was performed to determine whether the data follow a normal distribution. The Student's t-test was performed for cases of normal distribution while the Wilcoxon test was performed for cases of non-normal distribution. A significance threshold of 5% was retained.

2.4. Codon-Based Selection Signature Detection

The codon Z-test is a method used to determine whether positive selection occurs in a gene and involves a comparison of the relative abundances of synonymous (dS) and non-synonymous (dN) substitutions that have occurred in gene sequences. *TP53* exon 4 gene sequences were analyzed in both TH and TC. For each codon, the numbers of synonymous (dS) and non-synonymous (dN) substitutions were estimated. These estimates were produced using joint maximum likelihood reconstructions of ancestral states under a Muse-Gaut model of codon substitution and a Tamura-Nei model of nucleotide substitution. The dN-dS test statistic was used to detect positively selected codons. A positive value for the test statistic indicated an overabundance of non-synonymous substitutions. In this case, the probability of rejecting the null hypothesis of neutral evolution (p-value) was calculated. Statistical significance was set at $P < 0.05$. These analyses were performed using MEGA version 7.0.14 [15].

3. Results

The DNA extracts obtained and migrated on a 2% agarose gel indicated the good quality and quantity of the DNA.

Exon 4 of the *TP53* gene was amplified from the DNA extracts of 84 samples, 46 of which were cancer tissues (TC) and 38 of which were control tissues (TH). Clear sample bands were observed on the electrophoretic migration profile, except for one TC sample and three TH samples that had no bands. A total of 45 TC and 35 TH amplicons were identified in the gene of interest.

3.1. Nature and Position of the Mutations Found

Analysis of the chromatograms using Mutation Surveyor software indicated the presence of a single-nucleotide variant at position (c.139 C>T), which caused an amino acid change from proline to serine in codon 47 of the coding region of exon 4 of the *TP53* gene. This variant, which is already listed in the database (rs1800371), was identified in one sequence from THs and three sequences from tumor tissues. Five novel mutations were found in the cancerous tissue sequences, two of which did not result in an amino acid change (p.Q80Q and p.G85G) and the other two causing amino acid frameshifts (p.D57H and p.P72A), with statistically significant scores compared with the cytosine deletions found in the two sequences. The results are presented in Table 1 and Figure 1.

Table 1. *Nature and position of the mutations.*

Chromosomal position	Variant nucleotide	Variant protein	Nature of variants	Affected tissues	SNP	Score
17: 7579518	c.169 G>C	p.D57H	Non-Syn	TC	New	37.73
17: 7579548	c.139 C>T	p.P47S	Non-Syn	3TC /1TH	rs1800371	39.21/105.9
17: 7579413	c.214 C>G	p.P72A	Non-Syn	TC	New	30.09
17: 7579372	c.255 C>G	p.G85G	syn	TC	New	106.73
17: 7579574	c.112 del C	-	-	2TC	-	14.56
17: 7579387	c.240 G>A	p.Q80Q	syn	TC	New	76.62

TH= Healthy Tissue; TC= Cancerous Tissue

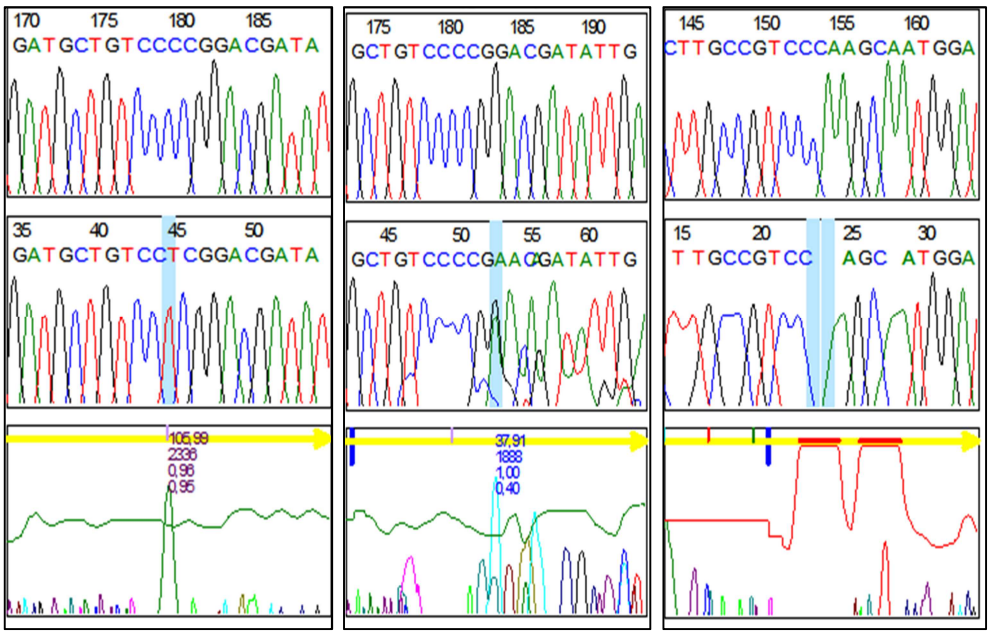

Figure 1. *Some SNPs in exon 4 of the TP53 gene.*

3.2. Pathogenicity of the Mutations

Pathogenicity analysis of missense mutations revealed that all exon 4 variants identified in both the control and tumor tissues were predicted to be single polymorphisms using all three software packages (Table 2). Except for the guanine-to-cytosine variant at position 169, which is related to the coding region of exon 4, the amino acid change from asparagine to histidine at codon 57 was predicted to be potentially damaging to Polyphen-2, with a significant score (0.607).

3.3. Prediction of the Structure of the p53 Protein

Prediction of the 3D structure of the p53 protein based on the three protein variants, namely p.P47S, p.D57H, and p.P72A, revealed no conformational change in the protein compared to the reference obtained after alignment. The results are shown in Figure 2. I-TASSER generates ligand-binding site predictions by matching the target model with the proteins. The results revealed no changes in the protein ligand receptors, despite changes in the P53 protein sequence (Figure 2).

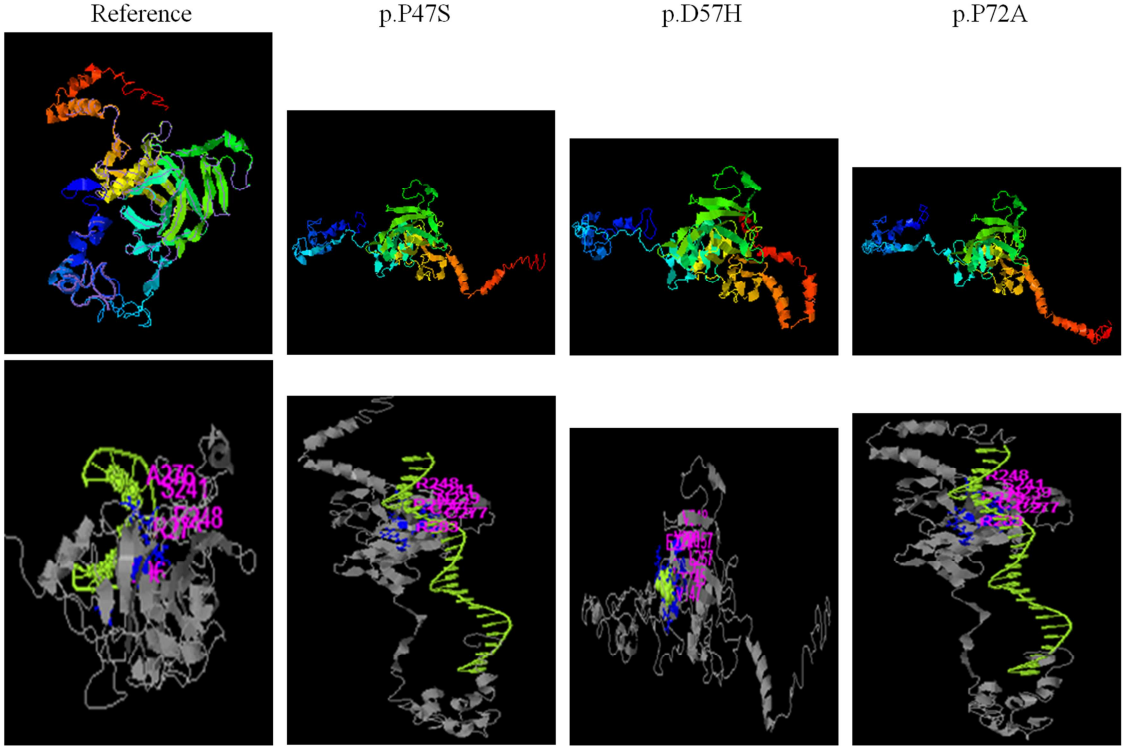

Figure 2. *3D structure of the p53 protein according to the identified mutations.*

Table 2. *Effect of non-synonymous mutations on the function of the protein and their pathogenicity.*

Variants	Effect on protein	Pathogenicity		
	TP53 Database	Polyphen-2	Mutation Taster	SIFT
c.139C>T p.P47S	Supertrans	Benign (0.017)	Benign (0.91)	Tolerated (0.98)
c.169G>C p.D57H	Functional	Probably Damaging (0.607)	Benign (0.91)	Tolerated (0.12)
c.214C>G p.P72A	_	Benign (0.00)	Benign (0.91)	Tolerated (0.12)

3.4. Study of the Genetic Polymorphism of Exon 4 of the TP53 Gene

3.4.1. Alignment and Cleaning of the Nucleotide Sequences of Exon 4 of the TP53 Gene

Sequences of the two tissue groups (TC and TH) obtained after sequencing were aligned and corrected. Eighty TH and TC sequences were obtained after processing. The size of the TH and TC sequences was 256 bp (Figure 3).

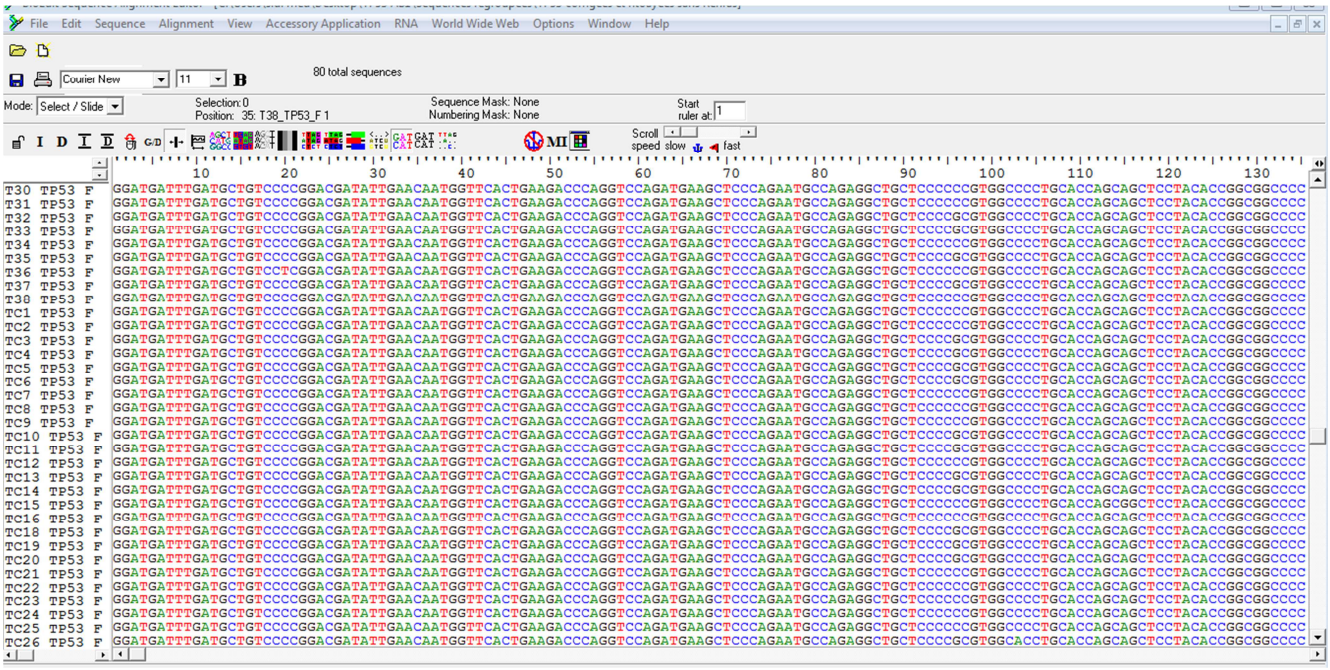

Figure 3. *Alignment of exon 4 of the TP53 gene.*

3.4.2. Diversity, Variability, and Genetic Structuring

Tables 3, 4, and 5 show the genetic variability, diversity, and structure of the two populations (TH and TC). Based on the parameters, a variability in *TP53* gene expression was found in both TH and TC. However, a higher variability was found in the sequences of cancerous tissues, with 2.73% (7/256) polymorphic sites, of which 85.71% (6/7) were variable in singletons. This higher variability in cancer tissue was also revealed by the average number of nucleotide difference (0.766) relative to the control level (0.538). Of note, 17.78% (8/45) of individuals in the cancer population shared the same nucleotide sequence while 8.57% (3/35) of individuals in the control population shared the same nucleotide sequence. A predominance of G+C over A+T nucleotide frequencies was observed in both populations. The results are summarized in Table 3.

Table 3. *Genetic Variability Parameters of the TP53 Gene.*

	Number of sites	Polymorphic sites	Non-informative sites	*K*	*h*	Nucleotide frequency	
						A+T	C+G
Total	256	7	5	0.660	8	40.2	59.8
TH	256	2	1	0.538	3	40.2	59.8
TC	256	7	6	0.766	8	40.2	59.8

The total number of mutations calculated within each group was higher in cancerous tissue (Eta=7) than in the control tissue (Eta=2). The R mutation rate was 1.34 in TC cases and 1 in the TH case. Among these mutations in exon 4 of the *TP53* gene, transitional type substitutions (change from purine base to purine base) were more important in tumor cases (59.48%) than in control cases (47.87%), in contrast to transversions (change from purine base to pyrimidine), which were more frequent in TH than in TC, with frequencies of 52.12% and 40.52%, respectively. Higher rates of synonymous substitutions (dS) than non-synonymous substitutions (dN) were found in both tumor tissues and

controls. In addition, haplotypic diversities (hd), which were higher than nucleotide diversities (π), were found in both TH and TC. As TH and TC had the same standard deviation, there was no difference in haplotypic diversity between the two groups. The results are summarized in Table 4.

Analysis of the intra-tissue genetic distances and between TH and TC revealed a genetic distance within cancerous tissues (0.003± 0.002), a low genetic distance within THs (0.02 ± 0.002), and a low inter-tissue genetic distance (0.003 ± 0.002). Based on the estimated genetic differentiation F_{ST} obtained using Arlequin v3. 1 [17], the genetic structure between the cancer and control populations had a significant F_{ST} value of 0.97190, which is close to 1.

Table 4. *Genetic diversity parameters and genetic diversity indices for exon 4 of the TP53 gene.*

Parameters		TC	TH	Total
Eta		7	2	7
mutation rate (*R*)		1.34	1	1.34
Nature of mutation	(*S*)	59.48	47.87	59.48
	(*V*)	40.52	52.12	40.52
Type of substitution	*dS*	0.008 (0.00)	0.08 (0.00)	0.008 (0.00)
	dN	0.001 (0.00)	0.00 (0.00)	0.001 (0.00)
haplotypic diversity hd		0.633 (0.002)	0.516 (0.002)	0.58 (0.001)
Nucleotide diversity π		0.0029 (0.006)	0.0021 (0.001)	0.002 (0.005)

TH= Healthy Tissue; TC= Cancerous Tissue

Table 5. *Inter- and intra-tissue genetic distance and genetic differentiation.*

Tissues	Intra-tissue genetic distance	Inter-tissue genetic distance	F_{ST}
TH	0.02 (0.002)	0.003 (0.002)	0.97190 (0.00209)
TC	0.03 (0.002)		

3.5. Amino Acid Frequency

Amino acid frequency analysis of exon 4 of the *TP53* gene in TH and TC revealed an equitable distribution of most amino acids. No significant differences in the levels of these amino acids were found. However, the results revealed disparities, with p-values > 0.05 for six amino acids, including aspartic acid, leucine, methionine, asparagine, proline, and tyrosine, as highlighted by the bold font in Table 6. The frequency of each of these amino acids in the *TP53* exon 4 sequences differed slightly between TH and TC, without any statistical significance. Among these amino acids, aspartic acid, leucine, and proline were frequently found in the control tissues, while methionine and tyrosine were frequently found in the cancer tissues. Asparagine was only found in the tumor tissue.

Table 6. *Amino acid frequency in exon 4 of the TP53 gene.*

Amino acids	TH	TC	P-value	Amino acids	TH	TC	P-value
Ala	14.117	14.117	-	Met	2.352	2.379	0.3916
Cys	1.176	1.176	-	Asn	0.000	0.026	0.3916
Asp	7.058	7.032	0.3916	Pro	18.352	18.300	0.6960
Glu	4.705	4.705	-	Gln	3.529	3.529	-
Phe	3.529	3.529	-	Arg	2.789	2.849	-
Gly	5.882	5.882	-	Ser	10.621	10.614	-
His	1.176	1.176	-	Thr	7.058	7.058	-
Ile	1.176	1.176	-	Val	3.529	3.529	-
Lys	2. 352	2.352	-	Trp	2.352	2.359	-
Leu	5.882	5.856	0.3916	Tyr	2.352	2.359	0.3916

TH= Healthy Tissue; TC= Cancerous Tissue

3.6. Selection Test by Codon

A comparison of the relative abundance of synonymous (dS) and non-synonymous (dN) substitutions that occurred in TP53 exon 4 sequences via the codon selection test revealed that no codon was under positive selection, with P-values > 0.05 (Table 7). This selection test highlights the superiority of synonymous mutations over non-synonymous mutations in breast cancer.

Table 7. *Codon selection test for exon 4 of the TP53 gene.*

Codon	Triplet	dN-dS	*P-value*
3	TTG	0.769	0.536
7	CCG	0.506	0.664
8	GAC	0.374	0.888
32	CCC	0.5	0.666
34	GCC	-1	1
38	GCA	-1	1
60	CAG	-1.227	

4. Discussion

Breast cancer is a major cause of increased morbidity and mortality in women worldwide, particularly those residing in sub-Saharan Africa, where diagnosis is often delayed [10]. Many genetic alterations have been associated with the development of the disease, including mutations in the *TP53* tumor suppressor gene [19]. The impact of alterations in exon 4 of the *TP53* gene on the evolution of breast cancer in Mauritanian women was evaluated to decipher the mutational origin of this pathology. Alterations in this gene are very frequent in human pathologies and are found in nearly 50% of tumors, with a higher incidence in lung and gastrointestinal tumors, as well as in breast cancer, in which the pathology is aggressive at a more advanced stage [18, 19].

Alterations in the *TP53* gene are often associated with an unfavorable prognosis [21]. The prognostic value of these alterations differs between breast cancer subtypes [20] and is associated with a high rate of tumor proliferation [21]. According to Abeer et al. [22], the *TP53* gene plays an important role in breast carcinogenesis and early disease onset in Arab women. These researchers revealed a correlation between certain mutations in p53 and response to cancer treatment. In addition, p53 mutations were identified in 119 breast cancer tissue samples via direct gene sequencing (exons 4-9). Of note, 73% of patients whose tumors contained p53 mutations were younger than 50 years. For the first time, the researchers identified seven new mutations and 16 mutations that were already listed in databases in breast cancer tissues. Notably, 29% of the new mutations were found in exon 4.

In the present study, genetic analysis revealed the presence of a mutation in exon 4 of the *TP53* gene that is already listed in the databases (rs1800371) in the tumor tissue. Using tumor samples from African American women with breast cancer, Maureen et al. [23] demonstrated that in human cell lines and a mouse model, the P47S variant was associated with a modest decrease in apoptosis in response to most genotoxic stresses compared to wild-type p53. However, this decrease also results in significant defects in cisplatin-induced cell death [27]. The supertrans mutant was predicted to be a simple polymorphism; however, it showed an impaired ability to transactivate a subset of p53 target genes, including two genes involved in metabolism, *Gls2* (glutamine 2) and *Sco2* [23]. The same study revealed that the mutant at codon 47 was susceptible to spontaneous cancers of various histological types in homozygous and heterozygous mice. Due to the high functional significance of this variant and its specificity to people of African descent, Grochola et al. [24] suggested that it may play a role in the early onset and more aggressive nature of breast cancer in women of African descent. In our study, of the five novel mutations found in the cancerous tissue sequences, three involved amino acid changes and were predicted to be benign. Using 46 fresh tissue samples from an Egyptian woman residing in British Columbia, Auhood et al. [25] found that the *TP53* gene is mutated in approximately 30-35% of cases and loses its normal function, causing tumorigenesis. In this study, 15 somatic mutations of TP53 were found to be present in 58.7% (27/46) of patients; these mutations were all (except one) in the known hotspot regions. Further, two polymorphic variants of the *TP53* gene were detected, with p.P72R as the most frequent mutation in eight patients (17.4%), followed by p.P72A in four patients (8.7%).

Xiaona et al. [28] opted to determine the network effect of WT mutant p53 interactions and implications on p53 gene therapy. Although benign, these researchers found that codon 72 with a proline-to-alanine change was associated with hepatocellular carcinoma (HCC) and a reading frame shift. The p53 mutant may have either a dominant-negative effect or a gain-of-function that interferes with the ability of p53 to maintain genomic stability.

The arginine allele has been implicated in the predisposition to several types of cancer in different populations [29]. Segregation of the codon 72 alleles revealed that the arginine allele is associated with a predisposition to breast cancer in some families. Furthermore, in Tunisian and Turkish populations, a haplotype of p53 containing the arginine allele of codon 72 was found to be associated with a high risk of breast cancer [30, 31]. A mutation analysis performed by Khaliq et al. [32] revealed the presence of a p53 mutation in breast carcinoma in a Pakistani population; however, no significant correlation was found between the p53 mutation and tumor aggressiveness (size, nodal status, and histopathology).

In 2011, Rybarova et al. [33] found that not all p53 mutations resulted in inactivation. Some p53 mutants display only a partial loss of their DNA-binding activity. Patient survival curves revealed that patients with p53-positive tumors had significantly shorter survival times than those with p53-negative tumors.

Similar to p53 missense mutations, most deletion and frameshift mutations occur in the DNA-binding domain; however, unlike missense mutations, these mutations alter the basic integrity of the protein. Deletions are the most common nucleotide sequence abnormalities, accounting for more than 25% of abnormalities in the HGMD database. These mutations result in a free shift in the coding sequences, which leads to the appearance of a premature stop codon and the eventual presence of an incomplete protein, which is mainly nonfunctional [34]. In our study, the total number of mutations was more distinct in cancer tissues (Eta=7) than in control tissues (Eta=2). Further, we identified a deletion mutation (c.112 delC) that had no effect on the structure and function of the p53 protein.

The p53 protein is a phosphoprotein of 393 aa. The main domains of this protein include (1) an aminoterminal domain (residues 1-42), required for interaction with components of the transcriptional apparatus; (2) a region rich in proline residues (63-97), involved in apoptosis; (3) a central hydrophobic domain (102-292), whose three-dimensional structure allows specific binding to DNA, where the majority of inactivating mutations involved in various human cancers

converge; and (4) a tetramerisation domain (363-393), involved in the negative regulation of p53 [35].

Cancers frequently express mutant forms of the p53 transcription factor. Based on early observations, the p53 mutant can increase the malignancy of tumor cells and immortalize primary cells [36]. In our study, pathogenicity analyses of the mutations revealed no malignancy in breast cancer pathology. This finding was confirmed by the three-dimensional structures of the mutant proteins, where no conformational changes were observed compared to those of the reference. Furthermore, p53 mutants show different degrees of dominance over the co-expressed wild-type protein, and loss of the wild-type p53 allele is frequently observed [37]. The p53 protein has an activating function on the expression of many target genes. The function of p53 as a transcription factor requires the formation of a homotetrameric structure. According to some studies, mutated p53 monomers can bind to wild-type p53 monomers to form heterotetrameric complexes [37]. The presence of mutated p53 monomers within these heterotetrameric complexes may result in the immediate inactivation of the wild-type monomers. The ability of mutated p53 to bind and inactivate wild-type p53 is termed the "dominant-negative effect." Several factors involved in this dominant-negative activity have been identified. Understanding the complex molecular functions that govern this activity constitutes one of the important aspects that would enable better discernment of the biological mechanisms involved in carcinogenesis. Among the multiple biological functions of p53, we highlight its role as a regulator of the expression of crucial genes in the cell cycle, repair of alterations in genomic DNA, angiogenesis, senescence, and programmed cell death. Under certain conditions of DNA damage or cellular stress (hypoxia and mitotic spindle depolymerization), p53 is sequestered in the nuclear compartment. This nuclear stabilization leads to the accumulation of the p53 protein and activation of its transcriptional functions. The activity of p53 is based on the importance of its transactivating power in numerous genes, such as p21, MDM2, GADD45, BAX, and XPC [37].

The transcriptional activity of p53 has diverse consequences ranging from cell growth arrest to apoptotic induction. Thus, the role of p53 in transcription is of key importance in carcinogenesis and neoplastic progression. The suppressive function of p53 involves specific binding to DNA. P53 also plays a crucial role in repairing DNA damage. In some cancer cells in which p53 is inactive, the G1/S checkpoint of the cell cycle is defective, resulting in anarchic cell growth [38]. Exon 4 is located in the activator domain of the *TP53* gene. Notably, the *TP53* gene is a transcription factor for several target genes.

A study of genetic variability and diversity parameters revealed low polymorphism in exon 4 of the *TP53* gene in cancer tissue sequences compared to controls. However, a higher variability was observed in cancer tissue sequences and polymorphic sites, which were variable in a singleton. This high variability in cancer tissues was also revealed by the average number of nucleotide differences and haplotypic diversity. Genetic diversity indices indicated a stable population signal for naturally evolving cancer tissues (hd = 0.633; Pi = 0.0029). These results are consistent with those of the codon selection test, in which no codon was under positive selection. In addition, synonymous substitutions (0.008) were greater than non-synonymous substitutions (0.001), indicating negative selection. Therefore, exon 4 of the *TP53* gene is not a polymorphic exon, and the results obtained in the present study support this observation. The frequency distribution of amino acids differed between the cancer population and controls, especially for asparagine, which is expressed only in cancerous tissues. Asparagine is not catabolized by mammalian cells and plays a central role in tumor progression [38]. In some cell types, asparagine is required for adaptation to apoptosis induced by glutamine depletion [39]. Asparagine is also involved in the exchange of extracellular amino acids, such as arginine, serine, and histidine [38]. Asparagine synthase synthesizes asparagine from aspartate using glutamine. Recently, asparagine metabolism was extensively evaluated as a new target for metabolic reprogramming in cancer [40]. In that study, asparagine synthase expression was found to be correlated with metastatic relapse in breast cancer patients and asparagine bioavailability was reported to regulate metastasis in a mouse model of breast cancer. Thus, asparagine supports cell proliferation and oncogenic gene expression in cancer cells. Analysis of the genetic structure between TH and TC revealed the beginning of genetic differentiation between the two groups of tissues, characterized by a low inter-tissue genetic distance and a significant F_{ST} value close to 1. This result indicates a low genetic differentiation of the cancerous tissues compared to the controls, which reflects the benign state of exon 4 alterations previously described in tumor cells. The small intra-Tumor genetic distance can be explained by the monoclonal origin of the tumor [41], whose development is based on successive waves of clonal expansion. A tumor is composed of different subpopulations of abnormal cells that have the common feature of early alterations and whose genesis follows the laws of a spatiotemporal evolutionary continuum [42].

5. Conclusion

The incidence of breast cancer is increasing in Mauritania, and this increase is undoubtedly due to genetic factors. In Mauritania, similar to most countries in sub-Saharan Africa, breast cancer occurs in young women (younger than 50 years) and is most often diagnosed at advanced stages (T2 and T3) with lymph node involvement (N+).

The objective of our study was to identify any mutations in exon 4 of the *TP53* gene in Mauritanian women with breast cancer. Indeed, mutations were detected in this exon. The results of our analyses of exon 4 of TP53 revealed that cancer tissue sequences have high genetic variability compared to control tissue sequences, which may contribute to the involvement of exon 4 mutations in the occurrence of breast cancer in our population. Our study provides the first data on

alterations in exon 4 of the *TP53* gene in Mauritania.

Our results lay the foundation for biochemical, proteomic, and clinical studies to determine the effects of these mutations on treatment resistance, recurrence, development of metastasis, and overall survival of these patients.

To date, cancer remains a major obstacle, challenge, and mystery worldwide, especially in developing countries where diagnosis is often delayed, and resources and capacity are limited.

Studies to elucidate the causes and identify the mutations that cause breast cancer will markedly contribute to increased understanding of this disease in our population and the discovery of effective solutions for appropriate and effective treatment and prevention.

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