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RESEARCH ARTICLE

Mutations in the *MT-CYB* Mitochondrial Gene in Prostate Tumors in Senegal: Molecular Analysis and Computational Prediction of Functional and Structural Effects

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Abstract

The prostate is affected by two types of tumors: benign prostatic hyperplasia and prostate cancer. These are the most commonly diagnosed tumors worldwide. Given the significance of mitochondrial genome alterations in cancerous lesions, this study aims to determine the characteristics of *MT-CYB* gene mutations in prostate tumors in Senegalese men. Twenty-seven biopsies were selected for the study. Mitochondrial DNA (*MT-CYB* gene) was extracted, amplified by PCR, and sequenced. Mutations were detected using the Mutation Surveyor software. The pathogenicity of the variants was assessed using Polyphen-2, Provean, and SNPs & GO. The impact on protein stability and molecular mechanisms was evaluated using I Mutants 2.0, MuPro, and MutPred2. Mutation3D was used to map the identified high-risk non-synonymous mutations. The results revealed 125 mutations, predominantly found in malignant tissues. Thirteen mutations are shared by both tumours, suggesting their potential role as early biomarkers of prostate cancer risk. The prediction tools identified 75 potentially pathogenic mutations. All of the non-synonymous mutations affect protein stability. Among them, 14 alter molecular mechanisms, and 16 are classified as high-risk. Although the sample size is small, these results confirm that mutations in the *MT-CYB* gene contribute to prostate tumorigenesis.

Keywords: Prostate, Cancer, *MT-CYB,* Benign Hypertrophy, Mutations, Senegal.

1. Introduction

The prostate, being a glandular structure, is affected by two types of tumors: benign prostatic hyperplasia (BPH) and prostate cancer (PCa). Prostate cancer is the second most common malignant tumor and the fifth leading cause of cancer death among men worldwide, with 1,414,259 new cases and 375,304 deaths reported in 2020 [1]. Racial/ethnic differences have long been observed, and men of African descent have higher incidence and mortality rates as well as more aggressive tumors compared to other racial groups. In Africa, prostate cancer is the most common cancer among men [2]. This cancer is one of the most heritable malignancies, and genome-wide association studies have identified more than 100 risk variants, which together account for ~30 % of familial risk [3 ; 4 ; 5]. Benign prostatic hyperplasia is a noncancerous enlargement of the prostate characterized by rapid proliferation of the gland's stromal and epithelial cells, which increases with age [6]. It is common and affects approximately 70 % of men over the age of 70 [7]. According to Kang and colleagues (2004), this condition, diagnosed histologically, has a prevalence ranging from 8 % in men aged 31 to 40, to 40–50 % in men aged 51 to 60, and to over 80 % in men over 80 years of age [8]. This condition remains largely

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underrecognized in sub-Saharan Africa where a lack of data has been noted, even though its morbidity affects patients' quality of life and the resulting financial burdens [9].

These diseases thus pose significant challenges for health care systems in most regions of the world [10]. Given the expected demographic changes that will lead to an increase in the elderly population worldwide, these problems are likely to worsen in the coming years [11 ; 12]. Consequently, improvements in risk prediction methods and prognostic models could help address these challenges.

It is well established that cancer is a group of diseases characterized by abnormal and uncontrolled cell growth. In 90 % of cases, it is caused by genetic mutations associated with environmental and lifestyle factors, while 5 to 10 % of cases are linked to hereditary genetics [13]. Carcinogenesis leads to changes in cellular energy metabolism [14]. Growing tumor cells produce high levels of lactate during aerobic glycolysis. Tumor mitochondria are characterized by elevated levels of ROS (reactive oxygen species), hypoxia, and signals that inhibit apoptosis [15]. Most somatic mtDNA mutations in tumors have also been reported as polymorphisms in the general population. mtDNA mutations occur in many diseases. This disorder is particularly associated with metabolism and energy [16].

For a long time, most genetic studies of prostate tumors have focused on mutations occurring in the nuclear genome, even though cells contain another genome: the mitochondrial genome. The same observation holds true for both types of tumors. Very few studies on the functional and structural effects of mitochondrial genome mutations in BPH are available. It has been noted that, in recent years, studies on somatic mutations in mtDNA have received particular attention. However, the impact of germline variants on mitochondrial dysfunction and the development of tumors, including cancer, has been the subject of less research [17]. Indeed, the few studies conducted on mitochondrial mutations in various tumors, including prostate cancer, are insufficient to understand their overall frequency and clinical impact [18 ; 19].

To date, our understanding of the genetic risk of prostate cancer in men of African descent has been based primarily on studies conducted mainly among African American men [3 ;19]. Given the importance of mitochondrial markers in tumorigenesis, studies evaluating the mutational profile of the mitochondrial genome in prostate tumors are needed to contribute to a better understanding of these tumors in Senegalese men. Thus, this study aimed to characterize the spectrum of somatic mutations in the *MT-CYB* gene in prostate tumor tissues while identifying those that could serve as candidate biomarkers for predicting prostate cancer risk.

2. Materials and Methods

2.1 Study Population and Data Collection

Twelve patients with benign prostatic hyperplasia and fifteen men with prostate cancer were selected for the study. As a reminder, participants were recruited after obtaining informed consent at the urology department of the El Hadji Ahmadou Sakhir NDIEGUENE Regional Hospital in Thiès, Senegal. Prostate tissue was obtained via biopsy from patients diagnosed with a benign or malignant tumor following histological confirmation (examinations: digital rectal exam combined with a PSA level > 4 ng/ml). A total of 27 samples were collected. Inclusion criteria included any patient with either benign prostatic hyperplasia or prostate cancer, following histopathological confirmation.

2.2 DNA Extraction, Polymerase Chain Reaction, and Sequencing

For each prostate tissue sample, DNA was extracted using the Zymo Research kit according to the manufacturer's instructions. The *MT-CYB* gene was amplified using the following primers: sense primer (F): 5'-CGGACTACAACCACGACCAA-3' and antisense primer (R): 5'-TCCGGTTTACAAGACTGGTGT-3'. The reaction volume used was 25 µl, containing 9.5 µl of PCR water (nuclease-free water), 12.5 µl of master mix, 0.5 µl of forward primer (F), 0.5 µl of reverse primer (R), and 2 µl of DNA. The PCR was performed in a thermocycler (Eppendorf Mastercycler® Nexus PCR thermocycler) under the following conditions : an initial denaturation at 95°C for 3 min, 35 cycles of denaturation at 95°C for 45 s, annealing at 57°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 5 min. The PCR products from each sample were subjected to electrophoresis on a 2 % agarose gel for 30 minutes using 5 µl of amplicons and a 100 molecular weight marker (PurpleLadder). After visualization, they were purified and sequenced using an ABIPRISM BigDye TM Terminator sequencing reaction kit in an MJ Research PTC-225 Peltier-type thermocycler.

2.3 Genetic Analyses

2.3.1 Mutational Profile of the MT-CYB Gene

To identify mutations in the *MT-CYB* gene, the

obtained sequences were analyzed using Mutation Surveyor version 5.0.1 (Softgenetics, State College, Pennsylvania, United States) (www.softgenetics.com) and compared to the reference sequence with the number NC_012920_14174 available on GenBank (https://www.ncbi.nlm;nih;gov/genbank/). The mutations selected were those with a Phred score ≥ 20. The identified variants were also checked against a set of databases to verify whether they had already been reported. These databases are dbSNP (Single Nucleotide Polymorphism) and ClinVar, managed by the NCBI (National Center for Biotechnology Information). A mutation is considered new if it has never been reported in either of these two databases.

2.3.2 Frequencies of missense mutations shared by both prostate tumors

To assess the possibility of any association between the occurrence and development of benign and malignant prostate tumors, the frequency of missense mutations was evaluated by tumor type.

2.3.3 Predicting the Potential Impact of missense mutations on the structure and function of mutated proteins

To explore the effects of detected missense mutations on the pathogenicity of prostate tumors, computational prediction bioinformatics tools were used. These include Polyphen-2, Provean, and SNP & GO. The Polyphen-2 predictive tool was used to predict the potential impact of an amino acid substitution on the function and structure of the protein under study. Mutations with a score of ≥ 0.95 are considered likely to be deleterious, those with a score between 0.5 and 0.95 are considered potentially deleterious, and those with a score of ≤ 0.5 are considered benign. As for the Provean server, it is a predictive tool that uses the primary sequence of the target protein and searches for its homologs by performing a sequence alignment via BLAST in the NCBI nr database (a protein database for BLAST searches). A score is calculated upon submission of the amino acid residue under study. The threshold value is -2.5. When an amino acid substitution has a score greater than -2.5, it is considered deleterious [20]. As for SNP & GO, it is used to accurately predict variants associated with the disease. It calculates a score while evaluating the association of each mutation with the disease. If a missense mutation has a score of ≥ 0.5, it is considered to be involved in the disease; however, if it has a score of ≤ 0.5, it is considered to have a neutral effect.

2.3.4 Prediction of the three-dimensional structural stability of mutated proteins

Two prediction servers were used to evaluate the three-dimensional structural stability associated with mutations:

- I-Mutant (https://folding.biofold.org/cgi-bin/i-mutant2.0.cgi):

It was used to estimate the potential effects of missense mutations on the protein's structural stability and the change in free energy (ΔΔG). When ΔΔG is > 0 kcal/mol, the mutation is stabilizing; when ΔΔG is < 0 kcal/mol, the mutation is destabilizing.

- MuPro (http://mupro.proteomics.ics.uci.edu/): was useful for predicting energy changes and how variants affect protein stability. Protein stability is predicted based on a confidence score (score < 0: decreased stability; score > 0: increased stability).

2.3.5 Prediction of the effects of missense mutations on physicochemical properties and mapping of high-risk mutations

Two servers were used (MutPred2: (http://mutpred2.mutdb.org/) and Mutation3D: http://mutation3d.org/). MutPred2 enabled the prediction of the molecular causes of the disease by assessing the impact of amino acid substitutions. It uses a probability score based on the gain or loss of 14 different structural and functional properties. The five most affected properties are listed along with their p-values, thereby measuring the significance of their disruption. As for the Mutation3D tool, it was used to identify groups of amino acid substitutions via the 3D clustering method. This tool enables the functional prediction and visualization of mutations in order to study the spatial configuration of amino acid substitutions in protein models and structures, thereby mapping high-risk mutations.

3. Results

3.1 Mutational Profile of the *MT-CYB* Gene in Prostate Tumors

A total of 27 samples were included in the study, including 12 from BPH and 15 from PCa. All patients carried at least 3 mutations, with an average of 6 mutations. Thus, the results showed a total of 125 mutations detected in the two tumor types. Of these mutations, 55 (44 %) are listed in the dbSNP database and 86 (56 %) are new ; in contrast, in the ClinVar database, only 23 (18.40 %) are listed and 102 (81.6 %) are new. The breakdown by mutation type is as follows: 91 (74.32 %) non-synonymous mutations,

21 (16.8 %) synonymous mutations, and 13 (10.4 %) nonsense mutations. Regarding the status of these variants, 90 (72 %) are heterozygous mutations and 31 (24.8 %) are homozygous. Furthermore, based on tissue type, mutations are more prevalent in malignant tissues than in benign tissues, with rates of 58.78 % and 32.43 %, respectively. Thirteen mutations are shared by both tumor types, representing 10.4 %. These results are presented in Table 1.

Table 1. *List of MT-CYB Gene Mutations in Prostate Tumors*

Mutations (GVG)	Affected amino acids	dbSNP	ClinVar	Nature of mutations	Histology	Status
m.14766 C>T	p.T7I	rs 193302980	140587	Non-synonymous	BT and MT	homozygous
m.14769A>G	p.N8S	rs 28357679	693761	Non-synonymous	BT and MT	homozygous
m.14869G>A	p.L41L	rs 879218419	New	Synonymous	BT	homozygous
m.14905G>A	p.M53M	rs 193302983	143919	Synonymous	BT and MT	homozygous
m.14911C>T	p.Y55Y	rs 28357683	235655	Synonymous	BT and MT	homozygous
m.15007C>T	p.A87A	rs 879113703	New	Synonymous	BT	homozygous
m.15110G>A	p.A122T	rs 28357685	693826	Non-synonymous	BT and MT	homozygous
m.15115T>C	p.T123T	rs 879035822	New	Synonymous	BT and MT	homozygous
m.15203A>AC	p.I153I/L	New	New	Non-synonymous	MT	heterozygous
m.15212A>AG	p.I156I/V	rs 1603225150	693841	Non-synonymous	MT	heterozygous
m.15217G>A	p.G157N	rs 193302989	235375	Non-synonymous	BT	Homozygous
m.15221G>GC	p.D159D/H	New	New	Non-synonymous	MT	heterozygous
m.15242G>GA	p.G166G/X	rs207459999	9680	Nonsense	MT	heterozygous
m.15244A>G	p.G166G	rs 28357369	New	Synonymous	MT	Homozygous
m.15255T>TA	p.V170V/D	New	New	Non-synonymous	MT	heterozygous
m.15260A>AC	p.S172T	New	New	Non-synonymous	MT	heterozygous
m.15262T>C	p.S172D	rs 1057520097	376959	Non-synonymous	MT	homozygous
m.15266A>AC	p.T174T/P	New	New	Non-synonymous	MT	heterozygous
m.15266A>AC	p.T174T/H	New	New	Non-synonymous	BT	heterozygous
m.15268C>CT	p.T174H	rs 2068746252	New	Non-synonymous	MT	heterozygous
m.15272A>AC	p.T176T/P	New	New	Non-synonymous	MT	heterozygous
m.15274A>AG	p.T176H	rs 1603225190	New	Non-synonymous	MT	heterozygous
m.15276G>C	p.R177P	New	New	Non-synonymous	MT	homozygous
m.15278T>TA	p.F178F/I	New	New	Non-synonymous	MT	heterozygous
m.15287T>TC	p.F181F/L	rs 527236044	140590	Non-synonymous	MT	heterozygous
m.15295C>CT	p.F183F/F	rs 1603225204	New	Synonyms	MT	heterozygous
m.15296A>AC	p.I184I/L	New	New	Non-synonymous	MT	heterozygous
m.15299T>TC	p.L185L/L	rs 878871827	New	Synonymous	MT	heterozygous
m.15301G>A	p.L185L/T	rs 193302991	New	Non-synonymous	BT and MT	homozygous
m.15312T>TA	p.I189I /N	New	New	Non-synonymous	MT	heterozygous
m.15314G>A	p.A190T	rs 527236176	143879	Non-synonymous	BT	homozygous
m.15316A>AG	p.A190A/A	rs 2520749058	New	Synonymous	MT	heterozygous
m.15316A>AG	p.A190S	rs 2520749058	New	Non-synonymous	BT	heterozygous
m.15326A>G	p.T194T/A	rs 2853508	140592	Non-synonymous	BT and MT	homozygous
m.15328A>AG	p.T194T	rs 527236178	143881	Synonyms	BT	heterozygous
m.15348A>AG	p.H201H/R	New	New	Non-synonymous	MT	heterozygous
m.15348A>AG	p.H201A	New	New	Non-synonymous	BT	heterozygous
m.15358A>AG	p.G204G/G	rs 1603225245	New	Synonymous	MT	heterozygous
m.15358A>AG	p.G204I	rs 1603225245	New	Non-synonymous	BT	heterozygous
m.15359T>TG	p.S205S/A	New	New	Non-synonymous	MT	heterozygous
m.15377A>AG	p.I211I/V	rs 1603225248	693872	Non-synonymous	MT	heterozygous
m.15392G>GT	p.D216E/X	New	New	Nonsense	MT	heterozygous
m.15394T>GA	p.D216E/E	New	New	Non-synonymous	MT	heterozygous
m.15394T>G	p.D216X	New	New	Nonsense	MT	homozygous
m.15407C>CT	p.H221H/Y	New	New	Non-synonymous	MT	heterozygous

m.15415C>CT	p.Y223Y/Y	New	New	Synonymous	MT	heterozygous
m.15418C>CG	p.Y224Y/X	New	New	Nonsense	MT	heterozygous
m.15428G>GA	p.D228D/N	rs 1603225270	693882	Non-synonymous	MT	heterozygous
m.15440T>TG	p.L232L/V	New	New	Non-synonymous	BT and MT	heterozygous
m.15449T>TG	p.F235F/V	New	New	Non-synonymous	BT and MT	heterozygous
m.15472A>AG	p.L242L/L	rs 2068747274	New	Synonymous	BT and MT	heterozygous
m.15477T>TC	p.L244L/P	New	New	Non-synonymous	BT and MT	heterozygous
m.15484A>AG	p.S246S/S	rs 1603225308	New	Synonymous	BT	heterozygous
m.15506G>GC	p.D254D/H	New	New	Non-synonymous	MT	heterozygous
m.15515A>AT	p.T257T/S	New	New	Non-synonymous	MT	heterozygous
m.15521G>GA	p.A259A/T	rs 1603225327	693898	Non-synonymous	MT	heterozygous
m.15533A>AG	p.N263N/D	rs 1556424601	693903	Non-synonymous	BT	heterozygous
m.15555C>CG	p.P270P/R	New	New	Non-synonymous	MT	heterozygous
m.15563T>TA	p.Y273Y/N	New	New	Non-synonymous	BT	heterozygous
m.15568C>T	p.F274F	rs 2520750683	New	Synonymous	BT	homozygous
m.15575G>GC	p.A277A/P	New	New	Non-synonymous	BT	heterozygous
m.15576C>T	p.A277V	rs 1603225369	New	Non-synonymous	MT	homozygous
m.15581A>AG	p.T279T/A	rs 1603225359	693909	Non-synonymous	MT	heterozygous
m.15586T>C	p.I280I	rs 878971797	New	Synonymous	BT	homozygous
m.15590C>CT	p.R282R/W	New	New	Non-synonymous	MT	heterozygous
m.15596G>A	p.V284I	rs 1603225369	693911	Non-synonymous	BT	homozygous
m.15601T>TG	p.P285P/A	New	New	Non-synonymous	MT	heterozygous
m.15604C>CA	p.N286K	New	New	Non-synonymous	BT	heterozygous
m.15606A>AG	p.K287K/X	New	New	Nonsense	MT	heterozygous
m.15608C>CA	p.L288K	New	New	Non-synonymous	BT	heterozygous
m.15610A>AT	p.L288X	New	New	Nonsense	BT	heterozygous
m.15611G>GA	p.G289G/X	New	New	Nonsense	BT	heterozygous
m.15616C>CG	p.G290E	New	New	Non-synonymous	BT	heterozygous
m.15617G>GA	p.V291V/I	rs 1556424625	693915	Non-synonymous	MT	heterozygous
m.15619C>CG	p.V291V/E	New	New	Non-synonymous	MT	heterozygous
m.15644A>G	p.I300V	rs 1603225400	693920	Non-synonymous	MT	homozygous
m.15648T>TC	p.L301L/P	rs 1603225403	New	Non-synonymous	MT	heterozygous
m.15654T>TG	p.M303M/X	rs 1556424638	New	Nonsense	MT	heterozygous
m.15662A>AC	p.I306I/L	New	New	Non-synonymous	MT	heterozygous
m.15682A>G	p.Q312Q/H	rs 527236192	143897	Non-synonymous	BT	heterozygous
m.15687G>GA	p.S314K	New	New	Non-synonymous	BT	heterozygous
m.15690T>A	p.M315Q	New	New	Non-synonymous	BT	homozygous
m.15693T>TA	p.M316M/K	New	New	Non-synonymous	BT	heterozygous
m.15705T>TG	p.L320L/R	New	New	Non-synonymous	BT	heterozygous
m.15706A>AG	p.L320X	rs 2068748491	New	Nonsense	MT	heterozygous
m.15707A>CG	p.S321R/G	rs 1603225431	New	Non-synonymous	MT	heterozygous
m.15710C>A	p.Q322Q	New	New	Synonymous	BT	homozygous
m.15715A>AG	p.S323H	rs 386829258	New	Non-synonymous	BT	heterozygous
m.15725C>CT	p.L327E	rs 1603225438	693937	Non-synonymous	BT and MT	heterozygous
m.15741T>TC	p.L332P	New	New	Non-synonymous	BT	heterozygous
m.15742C>CG	p.L332T	New	New	Non-synonymous	BT	heterozygous
m.15744T>TA	p.L333A	New	New	Non-synonymous	BT	heterozygous
m.15744T>TA	p.L333P	New	New	Non-synonymous	MT	heterozygous
m.15744T>TA	p.L333L/H	New	New	Non-synonymous	MT	heterozygous
m.15749C>CT	p.L335Y	rs 1603225461	New	Non-synonymous	MT	heterozygous
m.15752A>C	p.T336N	New	New	Non-synonymous	MT	homozygous
m.15754C>T	p.T336X	rs 1603225464	New	Nonsense	MT	homozygous
m.15755T>G	p.W337X	New	New	Nonsense	MT	homozygous

m.15755T>TC	p.W337P	rs 2520751779	New	Non-synonymous	BT	heterozygous
m.15755T>TG	p.W337Q	New	New	Non-synonymous	MT	heterozygous
m.15761G>GT	p.G339Q	New	New	Non-synonymous	BT	heterozygous
m.15767C>CA	p.Q341Q/K	New	New	Non-synonymous	MT	heterozygous
m.15774T>G	p.V343X	New	New	Nonsense	MT	homozygous
m.15784T>C	p.P346P	rs 527236194	New	Synonymous	MT	homozygous
m.15791A>T	p.I349P	New	New	Non-synonymous	MT	homozygous
m.15793C>CT	p.I349X	rs 1603225497	New	Nonsense	MT	heterozygous
m.15794A>AT	p.I350S	New	New	Non-synonymous	MT	heterozygous
m.15798G>A	p.G351N	rs 1603225502	New	Non-synonymous	MT	homozygous
m.15800C>CA	p.Q352K	New	New	Non-synonymous	MT	heterozygous
m.15803G>GT	p.V353K	New	New	Non-synonymous	MT	heterozygous
m.15817A>AT	p.L357M	New	New	Non-synonymous	BT	heterozygous
m.15817A>AT	p.L357L/L	New	New	Synonymous	MT	heterozygous
m.15818T>TA	p.Y358K	New	New	Non-synonymous	BT	heterozygous
m.15818T>TA	p.Y358Y/N	New	New	Non-synonymous	BT	heterozygous
m.15824A>AC	p.T360S	New	New	Non-synonymous	BT	heterozygous
m.15824A>AC	p.T360T	New	New	Synonymous	MT	heterozygous
m.15825C>CA	p.T360K	New	New	Non-synonymous	BT	heterozygous
m.15834T>TG	p.L363Q	New	New	Non-synonymous	BT	heterozygous
m.15839C>CT	p.L365Q	New	New	Non-synonymous	MT	heterozygous
m.15840T>A	p.L365P	New	New	Non-synonymous	MT	homozygous
m.15843T>TA	p.M366K	New	New	Non-synonymous	BT	heterozygous
m.15845C>A	p.P367T	New	New	Non-synonymous	MT	homozygous
m.15853C>CT	p.I369I/I	rs 1603225546	New	Synonymous	MT	heterozygous
m.15853C>CT	p.I369Y	rs 1603225546	New	Non-synonymous	MT	heterozygous
m.15854T>TC	p.S370S/P	rs 1603225547	New	Non-synonymous	MT	heterozygous

Note: BT = *benign tumor; MT = malignant tumor; dbSNP = Database of Short Genetic Variations*

3.2 Frequency of *MT-CYB* Gene Mutations Shared by Prostate Tumors

An analysis to identify frequent mutations was conducted to determine the most highly expressed variants of the *MT-CYB* gene in Senegalese men with prostate tumors. Of the 125 mutations detected, 13 (10.4 %) were observed in both benign and malignant prostate tumors at different frequencies (Table 2). The frequency of most of these mutations, depending on tissue type, is higher in benign tumors than in malignant tumors.

Table 2. *Percentage distribution of mutations shared by both types of prostate tumors*

Mutations (HGVS notation)	Affected amino acids	Benign tumors (%)	Malignant tumors (%)	Total patient frequency (%)
m.14766 C>T	T7I	91.67	100	96.30
m.14769A>G	N8S	33.33	33.33	33.33
m.14905G>A	M53M	16.67	6.67	11.11
m.14911C>T	Y55Y	16.67	6.67	11.11
m.15110G>A	A122T	41.67	13.33	25.93
m.15115T>C	T123T	33.33	33.33	33.33
m.15301G >A	L185L	58.33	33.33	51.85
m.15326A>G	T194T/A	66.67	66.67	66.67
m.15440T>TG	L232L/V	8.33	6.67	11.11
m.15449T>TG	F235F/V	8.33	6.67	11.11
m.15472A>AG	L242L/L	8.33	6.67	7.41
m.15477T > TC	L244L/P	8.33	6.67	7.41
m.15725C > CT	L327E	16.67	6.67	11.11

3.3 In Silico Prediction of the Pathogenicity of Nonsynonymous Mutations

The functional effect of all nonsynonymous mutations was predicted using predictive software. Table 3 summarizes all the prediction servers used and the non-synonymous mutations to estimate the deleterious impact of these variants on the mutated cytochrome b protein. Of the 91 non-synonymous mutations detected, 77 are potentially pathogenic (84.62 %) and 14 are benign (15.38 %). With regard to tissue type, mutations originating from malignant tumors are the most frequent (51, or 56.04 %).

Table 3. *Prediction of the pathogenicity of non-synonymous mutations in the MT-CYB gene in prostate tumors*

Affected amino acids	Polyphen-2 (score)	Provean (score)	SNPs&GO (Probability)	Histology
p.T7I	Benign (0.000)	Neutral (-2.354)	Neutral (0.321)	BT and MT
p.N8S	probably damaging (0.990)	Neutral (-1.354)	Neutral (0.376)	BT and MT
p.A122T	Benign (0.000)	Neutral (0.045)	Neutral (0.318)	BT and MT
p.I153I/L	Benign (0.003)	Neutral (-1.366)	Sick (0.789)	MT
p.I156I/V	Potentially damaging (0.780)	Neutral (-0.318)	Neutral (0.126)	MT
p.G157N	probably damaging (1,000)	Deleterious (-4.867)	Sick (0.907)	BT
p.D159D/H	Potentially damaging (0.682)	Neutral (-1,121)	Sick (0.658)	MT
p.V170V/D	probably damaging (1,000)	Deleterious (-5.655)	Sick (0.897)	MT
p.S172T	Benign (0.016)	Neutral (-0.936)	Neutral (0.434)	MT
p.S172D	Benign (0.066)	Neutral (-0.398)	Sick (0.547)	MT
p.T174T/P	probably damaging (1,000)	Deleterious (-4.932)	Sick (0.841)	MT
p.T174T/H	probably damaging (1,000)	Deleterious (-5.728)	Sick (0.862)	BT
p.T174H	probably damaging (1,000)	Deleterious (-5.728)	Sick (0.862)	MT
p.T176T/P	probably damaging (1,000)	Deleterious (-4,640)	Sick (0.829)	MT
p.T176H	probably damaging (1,000)	Deleterious (-5.363)	Sick (0.846)	MT
p.R177P	probably damaging (1,000)	Deleterious (-5.750)	Sick (0.883)	MT
p.F178F/I	Potentially damageable (0.753)	Deleterious (-4.917)	Sick (0.815)	MT
p.F181F/L	Benign (0.001)	Deleterious (-2.691)	Neutral (0.255)	MT
p.I184I/L	Benign (0.005)	Neutral (-0.509)	Neutral (0.255)	MT
p.L185T	Probably damageable (1,000)	Deleterious (-3.361)	Sick (0.776)	BT and MT
p.I189I/N	Probably damageable (0,999)	Deleterious l (-5.585)	Sick (0.894)	MT
p.A190T	Benign (0.000)	Neutral (-1.675)	Neutral (0.468)	BT
p.A190S	Benign (0.001)	Neutral (-1.669)	Sick (0.576)	BT
p.T194T/A	Benign (0.000)	Neutral (0, 312)	Neutral (0.197)	BT and MT
p.H201H/R	Probably damageable (0.990)	Deleterious (-6.574)	Sick (0.769)	MT
p.H201A	Probably damageable (1,000)	Deleterious (-8,217)	Sick (0.802)	BT
p.G204I	Probably damageable (1,000)	Deleterious (-8,226)	Sick (0.905)	MT
p.S205S/A	Probably damageable (0.962)	Neutral (-2.466)	Sick (0.739)	BT
p.I211I/V	Potentially damageable (0.945)	Neutral (-0.763)	Neutral (0.144)	MT
p.D216E/E	Probably damageable (0.988)	Deleterious (-3.169)	Sick (0.827)	MT
p.H221H/Y	Probably damageable (0.983)	Deleterious (-4.847)	Sick (0.729)	MT
p.D228D/N	Probably damageable (0.998)	Deleterious (-4.039)	Sick (0.841)	MT
p.L232L/V	Benign (0.271)	Neutral (-0.574)	Neutral (0.214)	BT and MT
p.F235F/V	Benign (0.055)	Neutral (0.414)	Neutral (0.378)	BT and MT
p.L244L/P	Probably damageable (1,000)	Deleterious (-5.347)	Sick (0.753)	BT and MT
p.D254D/H	Benign (0.884)	Deleterious (-3.137)	Sick (0.878)	MT
p.T257T/S	Benign (0.000)	Neutral (-1.994)	Neutral (0.331)	MT
p.A259A/T	Probably damageable (0.997)	Deleterious (-3.013)	Sick (0.878)	MT
p.N263N/D	Benign (0.048)	Neutral (-0.834)	Sick (0.554)	BT
p.P270P/R	Probably damageable (1,000)	Deleterious (-6.551)	Sick (0.916)	MT
p.Y273Y/N	Probably damageable (1,000)	Deleterious (-6,290)	Sick (0.851)	BT
p.A277A/P	Probably damageable (1,000)	Deleterious (-3.493)	Sick (0.881)	BT
p.A277V	Probably damageable (1,000)	Deleterious (-2.783)	Sick (0.754)	MT

p.T279T/A	Benign (0.000)	Neutral (2.043)	Neutral (0.150)	MT
p.R282R/W	Probably damageable (1,000)	Deleterious (-5.511)	Sick (0.922)	MT
p.V 284I	Benign (0.000)	Neutral (0.524)	Neutral (0.068)	BT
p.P285P/A	Benign (0.437)	Deleterious (-5.418)	Sick (0.864)	MT
p.N286K	Probably damageable (0.998)	Deleterious (-4.050)	Sick (0.853)	BT
p.L288K	Probably damageable (0.990)	Deleterious (-4.077)	Sick (0.872)	BT
p.G290E	Probably damageable (1,000)	Deleterious (-5.497)	Sick (0.941)	BT
p.V291V/I	Potentially damageable (0.878)	Neutral (-0.675)	Sick (0.757)	MT
p.V291V/E	Probably damageable (1,000)	Deleterious (-4.039)	Sick (0.929)	MT
p.I300V	Potentially damageable (0.945)	Neutral (-0.137)	Neutral (0.178)	MT
p.L301L/P	Probably damageable (1,000)	Deleterious (-4.603)	Sick (0.674)	MT
p.I306I/L	Benign (0.000)	Neutral (0.037)	Neutral (0.170)	MT
p.Q312Q/H	Potentially damageable (0.948)	Neutral (-2.413)	Sick (0.599)	BT
p.S314K	Probably damageable (0.995)	Neutral (-1.873)	Sick (0.677)	BT
p.M315Q	Probably damageable (0.998)	Neutral (-2,454)	Sick (0.674)	BT
p.M316M/K	Benign (0.079)	Neutral (-0.888)	Sick (0.782)	BT
p.L320L/R	Probably damageable (1,000)	Deleterious (-3.135)	Sick (0.907)	BT
p.S321G	Probably damageable (1,000)	Neutral (-2,150)	Sick (0.559)	MT
p.S323H	Potentially damageable (0.783)	Neutral (-0.638)	Sick (0.501)	BT
p.L327E	Probably damageable (0.994)	Neutral (-1.599)	Sick (0.782)	BT and MT
p.L332P	Probably damageable (1,000)	Deleterious (-2.889)	Sick (0.885)	BT
p.L332T	Probably damageable (1,000)	Neutral (-1.663)	Neutral (0.304)	BT
p.L333A	Probably damageable (0.968)	Neutral (2,206)	Neutral (0.336)	BT
p.L333P	Probably damageable (0.998)	Deleterious (-3,472)	Sick (0.859)	MT
p.L333L/H	Probably damageable (0.999)	Deleterious (-3.373)	Sick (0.718)	MT
p.L335Y	Probably damageable (0.996)	Deleterious (-3.032)	Sick (0.815)	MT
p.T336N	Probably damageable (1,000)	Deleterious (-3.022)	Sick (0.825)	MT
p.W337P	Probably damageable (1,000)	Deleterious (-9.017)	Sick (0.909)	BT
p.W337Q	Probably damageable (1,000)	Deleterious (-7.813)	Sick (0.876)	MT
p.G339Q	Probably damageable (1,000)	Deleterious (-4,800)	Sick (0.903)	BT
p.Q341Q/K	Potentially damageable (0.855)	Neutral (-1.643)	Sick (0.871)	MT
p.I349P	Potentially damageable (0.886)	Deleterious (-2.745)	Sick (0.926)	MT
p.I350S	Probably damageable (1,000)	Deleterious (-3.191)	Sick (0.881)	MT
p.G351N	Probably damageable (0.960)	Deleterious (-3.352)	Sick (0.854)	MT
p.Q352K	Probably damageable (0.967)	Neutral (-2.196)	Sick (0.832)	MT
p.V353K	Benign (0.324)	Neutral (-2.418)	Sick (0.683)	MT
p.L357M	Probably damageable (1,000)	Neutral (-0.591)	Neutral (0.394)	BT
p.Y358K	Probably damageable (1,000)	Deleterious (-4.755)	Sick (0.962)	BT
p.Y358Y/N	Probably damageable (1,000)	Deleterious (-4.752)	Sick (0.920)	BT
p.T360S	Benign (0.044)	Neutral (-0.337)	Neutral (0.122)	BT
p.T360K	Benign (0.182)	Neutral (-1.276)	Sick (0.736)	BT
p.L363Q	Probably damageable (1,000)	Deleterious (-2.824)	Sick (0.752)	BT
p.L365Q	Probably damageable (1,000)	Deleterious (-2.554)	Sick (0.689)	MT
p.L365P	Probably damageable (1,000)	Deleterious (-3.008)	Sick (0.860)	MT
p.M366K	Benign (0.065)	Neutral (-1.672)	Sick (0.861)	BT
p.P367T	Probably damageable (0.998)	Deleterious (-3.912)	Sick (0.847)	MT
p.I369Y	Benign (0.002)	Neutral (-0.943)	Neutral (0.459)	MT
p.S370S/P	Probably damageable (0.982)	Neutral (-1,360)	Sick (0.828)	MT

3.4 Prediction of the Impact of Mutations on the Stability of Proteins Encoded by *MT-CYB*

Protein stability represents a delicate balance of forces that determine whether a protein will adopt its native conformation or undergo denaturation. Accordingly, two protein stability prediction tools were used.

After analysis, the prediction of the free energy of stabilization ΔΔG showed that, of the 91 non-synonymous mutations detected, all were predicted to be destabilizing by MuPro that is, they reduce the protein's stability whereas, according to I-Mutant 2.0, 80 mutations are destabilizing and 11 are stabilizing. The results are presented in Table 4.

Table 4. *Effect of non-synonymous variants on the stability of mutated proteins*

Variants	MuPro			I-Mutant2.0	Stability
	ΔΔG (kcal/mol)	Stability	Trust score	ΔΔG (kcal/mol)	
T7I	-0.147	Reduced	-0.702	-1.14	Reduced
N8S	-1.007	Reduced	-0.712	0.61	Increased
A122T	-1.322	Reduced	-0.045	-1.77	Reduced
I153L	-1.181	Reduced	-0.937	-0.34	Reduced
I156V	-1.142	Reduced	-0.550	-1.26	Reduced
G157N	-0.914	Reduced	-0.539	-2.41	Reduced
D159H	-1.166	Reduced	-0.611	-0.75	Reduced
V170D	-1.223	Reduced	-0.867	-2.37	Reduced
S172T	-1.083	Reduced	-0.248	-1.32	Reduced
S172D	-0.928	Reduced	-0.447	-0.27	Reduced
T174P	-0.838	Reduced	-0.609	-1.84	Reduced
T174H	-0.823	Reduced	-1	-1.06	Reduced
T174T/H	-0.823	Reduced	-1	-1.06	Reduced
T176P	-1.059	Reduced	-0.488	-2.04	Reduced
T176H	-0.960	Reduced	-0.751	-1.62	Reduced
R177P	-1.227	Reduced	-0.204	-1.79	Reduced
F178I	-1.037	Reduced	-0.862	-1.05	Reduced
F181L	-0.940	Reduced	0.169	-1.03	Reduced
I184L	-0.366	Reduced	-0.611	0.13	Increased
L185T	-2.293	Reduced	-1	-3.73	Reduced
I189N	-1.127	Reduced	-0.581	-0.77	Reduced
A190T	-0.803	Reduced	-1	-1.08	Reduced
A190S	-0.621	Reduced	-1	0.22	Increased
T194A	-0.428	Reduced	-1	-0.86	Reduced
H201R	-0.916	Reduced	0.392	-0.57	Reduced
H201A	-1.237	Reduced	0.049	-0.37	Reduced
G204I	-0.081	Reduced	-0.044	-0.39	Reduced
S205A	-0.564	Reduced	-0.157	-0.64	Reduced
I211V	-0.772	Reduced	-0.883	-0.48	Reduced
D216E	-1.413	Reduced	-0.479	-0.23	Reduced
H221Y	-0.198	Reduced	0.285	-0.30	Reduced
D228N	-0.685	Reduced	-0.490	0.06	Increased
L232V	-1.098	Reduced	-0.751	-0.11	Reduced
F235V	-1.423	Reduced	0.182	-0.32	Reduced
L244P	-1.707	Reduced	-1	-0.52	Reduced
D254H	-0.809	Reduced	-1	-0.45	Reduced
T257S	-0.671	Reduced	-0.699	-1.38	Reduced
A259T	-0.765	Reduced	-0.271	-1.58	Reduced
N263D	-0.782	Reduced	-0.384	-1.11	Reduced
P270R	-0.604	Reduced	-0.524	-0.83	Reduced
Y273N	-1.194	Reduced	-1	-1.98	Reduced
A277P	-1.428	Reduced	-0.553	-0.50	Reduced
A277V	-0.722	Reduced	-0.116	0.49	Increased
T279A	-0.551	Reduced	1	-0.98	Reduced
R282W	-0.720	Reduced	-0.906	-2.02	Reduced

V284I	-0.623	Reduced	-0.380	-1.12	Reduced
P285A	-1.291	Reduced	-1	-1.04	Reduced
N286K	-1,24	Reduced	0.268	-0.93	Reduced
G290E	-0.362	Reduced	0.405	0.85	Increased
V291I	-0.157	Reduced	-0.734	-0.68	Reduced
V291V/E	-0.933	Reduced	-0.377	-2.01	Reduced
I300V	-0.808	Reduced	-0.361	0.02	Increased
L301P	-1.821	Reduced	-1	0.21	Increased
I306L	-0.082	Reduced	-0.647	-0.35	Reduced
Q312H	-0.583	Reduced	-0.193	-0.69	Reduced
S314K	-0.572	Reduced	0.104	0.16	Increased
M315Q	-1.287	Reduced	-1	-0.26	Reduced
M316K	-1,942	Reduced	-0.999	-0.69	Reduced
L320R	-1.245	Reduced	-0.708	0.02	Increased
S321G	-1.327	Reduced	-0.673	-0.90	Reduced
S323H	-0.877	Reduced	-0.529	-0.62	Reduced
L327E	-1.742	Reduced	-0.644	-0.65	Reduced
L332P	-1.867	Reduced	-1	-0.56	Reduced
L332T	-1.878	Reduced	-1	-3.39	Reduced
L333A	-2.410	Reduced	-0.677	-2.50	Reduced
L333P	-2.254	Reduced	-0.872	-0.88	Reduced
L333H	-1,972	Reduced	-0.916	-1.33	Reduced
L335Y	-1.721	Reduced	-0.846	-1.14	Reduced
T336N	-1.187	Reduced	-0.652	-0.92	Reduced
W337P	-1.663	Reduced	-0.330	-1.63	Reduced
W337Q	-1.377	Reduced	-0.875	-1.87	Reduced
G339Q	-0.839	Reduced	-1	-1.80	Reduced
Q341K	-1.141	Reduced	0.065	-0.38	Reduced
I349P	-1.666	Reduced	-0.601	-3.15	Reduced
I350S	-1.725	Reduced	-1	-4.42	Reduced
G351N	-0.789	Reduced	-0.979	-1.08	Reduced
Q352K	-1,2	Reduced	-0.628	-0.59	Reduced
V353K	-1.369	Reduced	-0.678	-3.6	Reduced
L357M	-0.929	Reduced	-1	-0.42	Reduced
Y358K	-2.127	Reduced	-0.634	0.11	Increased
Y358N	-1,908	Reduced	-0.830	-0.79	Reduced
T360S	-0.988	Reduced	0.502	-0.44	Reduced
T360K	-1.555	Reduced	-0.125	-0.95	Reduced
L363Q	-1.511	Reduced	-1	-1.86	Reduced
L365Q	-1.660	Reduced	-1	-1.9	Reduced
L365P	-1.883	Reduced	-1	-1.29	Reduced
M366K	-2.210	Reduced	-1	-1.43	Reduced
P367T	-1.195	Reduced	-0.904	-0.78	Reduced
I369Y	-1.422	Reduced	-0.018	-1.47	Reduced
S370P	-1.104	Reduced	-0.063	-0.73	Reduced

3.5 Prediction of Changes in Physicochemical Echanisms

An assessment of alterations in molecular and physicochemical mechanisms was conducted to analyze the physicochemical properties induced by nonsynonymous mutations. Of these, 14 exhibited altered physicochemical properties and are the only ones reported in Table 5. The molecular mechanisms associated with these most common substituted residues include alterations in transmembrane proteins (85.71 %), modifications to the ordered interface (71.43 %), loss of a strand or helix (50 % each), and gain of a strand (42.86 %). Similarly, certain variants

predicted more molecular alterations than others, particularly those that predicted more than four modifications for example, variants such as V170D, Y273N, A277P, Y358N, and L365Q.

Table 5. *Effect of non-synonymous variants on the properties of molecular mechanisms*

Variants	MutPre2 score	Molecular mechanisms	*P-value*	Histology
V170D	0.577	B-factor gain	3.2e-03	MT
		Modified ordered interface	9.2e-03	
		Loop gain	0.02	
		Strand loss	0.04	
		Gain of proteolytic cleavage at J171	2.2e-03	
		Gain of sulphation at Y168	0.02	
R177P	0.567	Modified ordered interface	1.30E-03	MT
		Altered transmembrane protein	3.70E-04	
		Strand loss	0.05	
Y273N	0.551	Modified ordered interface	1.00E-03	BT
		Helix gain	0.04	
		Strand loss	0.02	
		Allosteric site gain at W272	8.1e-03	
		Altered transmembrane protein	2.2e-03	
A277P	0.518	Modified ordered interface	2.70E-03	MT
		Strand gain	2.4e-03	
		Allosteric site gain at W272	3.00E-03	
		Helix loss	0.02	
		Altered transmembrane protein	9.4e-04	
L301P	0.507	Helix loss	2.3e-03	MT
		Modified signal peptide	6.50E-03	
L332P	0.604	Altered transmembrane protein	9.70E-06	BT
		Helix loss	1.1e-03	
		Strand gain	2.00E-03	
L333P	0.646	Altered transmembrane protein	0.00E+00	MT
		Helix loss	1.1e-03	
		Strand gain	2.00E-03	
W337P	0.828	Modified ordered interface	3.3e-04	BT
		Altered transmembrane protein	9.70E-06	
		Strand loss	0.02	
		Loss of pyrrolidone carboxylic acid at Q341	7.2e-03	
W337Q	0.619	Altered transmembrane protein	0.00E+00	MT
		Modified ordered interface	2.1e-03	
		Strand loss	0.04	
		Loss of pyrrolidone carboxylic acid at Q341	7.4e-03	
Y358K	0.707	Altered transmembrane protein	1.50E-05	BT
		Modified ordered interface	4.2e-03	
		Helix gain	5.2e-03	
		Strand loss	4.3e-03	
Y358N	0.675	Modified ordered interface	1.30E-03	BT
		Altered transmembrane protein	4.90E-05	
		Helix gain	0.02	
		Altered disordered interface	0.04	
		Strand loss	0.02	
		Gain of nitrogen-linked glycosylation at Y358	7.6e-03	

L365Q	0.501	Altered transmembrane protein	0.00E+00	MT
		Helix loss	1.50E-04	
		Strand gain	1.1e-04	
		Modified ordered interface	0.04	
		Gain in solvent accessibility	0.04	
		Modified signal peptide	4.2e-03	
L365P	0.742	Helix loss	7.80E-05	MT
		Altered transmembrane protein	1.50E-05	
		Strand gain	9.3e-05	
		Modified signal peptide	4.2e-03	
M366K	0.679	Altered transmembrane protein	0.00E+00	BT
		Modified ordered interface	0.03	
		Gain in relative solvent accessibility	0.04	
		Modified signal peptide	4.0e-03	

3.6 Prediction of Nonsynonymous Mutations on the 3D Structure of the Altered Protein Using the Mutation3D Tool

Certain protein sites are essential for maintaining the structural or functional integrity of proteins, while others can be altered or even replaced with little or no impact on protein structure or function. For this reason, 3D structure prediction using the mutation3D tool was evaluated to identify non-synonymous variants with negative or positive selection that is, to determine whether they would be pass-through variants or drivers. Thus, 16 out of 91 (17.58 %) were predicted to have a negative cluster selection effect, all of which are located in the critical C-terminal region of cytochrome B (residue positions 258–359). Of these 16 residues, 10 (P270R, A277V, T279A, R282W, L333P, L333H, L335Y, T336N, W341K, and W337Q) are from malignant tumors, while the remaining 6 (Y273N, A277P, L332P, L332T, W337P, and G339Q) are from benign tumors (Figure 1).

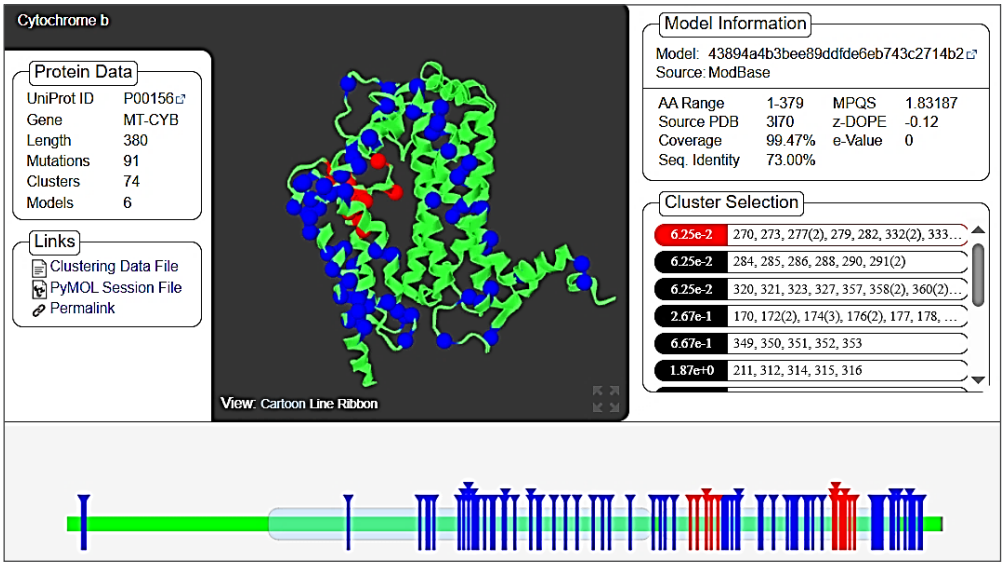

Figure 1. *3D structure of the cytochrome b protein, generated by the Mutation 3D server*

4. Discussion

The aim of this study is to evaluate mutations of interest in the mitochondrial *MT-CYB* gene that may be associated with the origin, development, and aggressiveness of prostate tumors, while seeking to understand the frequencies and mutational links between the two tumors, namely BPH and PCa. The results showed the presence of mutations in both benign and malignant tumors in all individuals. These results do not align with those found in cervical cancer, where the mutation frequency rate for this same gene is 50 % [21]. A total of 125 mutations were detected in 27 patients. This high number of *MT-CYB* gene mutations, despite the small sample size, is not surprising, since mitochondrial DNA mutates 10 to 20 times more frequently than nuclear DNA due to a less efficient mitochondrial DNA repair system [22 ; 23]. Other explanations could account for this high frequency. For example, these tumors are known to be age-related conditions, and mitochondrial mutations have been reported to be strongly associated

with patients diagnosed with prostate tumors at an advanced age [24].

The mutations detected were more prevalent in malignant tissues (58.78 %) than in benign tissues (32.43 %). This mutation rate in malignant tumors is higher than that reported in studies of oral cavity cancers in Senegalese patients, which found a rate of 27.3 % [25]. This higher prevalence in malignant tissues confirms the findings of Issa et al. regarding the role of *MT-CYB* mutations in the genetic progression of breast cancer in Chad [26]. Among the mutations detected, 74.32 % are non-synonymous, with HBP accounting for 77.08 % and PCa for 73.56 %. When comparing these rates for prostate tumors to those for breast tumors, the rate of malignant tissues (PCa) is higher than that reported for breast cancer in Chad, where the rate of non-synonymous mutations was 69.81 % [26]. Similarly, for benign tumors, in addition to being more prevalent than in malignant prostate tumors (perhaps due to the small sample size for BPH compared to that for PCa), it is also high compared to the rate reported by Doupa et al., who found a 50 % rate in studies examining the involvement of *MT-CYB* mutations in benign breast tumors in Senegalese women [27].

Thirteen mutations (8,78 %) were observed in both benign and malignant prostate tumors at varying frequencies. This rate is higher than that reported in studies of breast cancer in Chad (27.02 %) [26]. Interestingly, two of these 13 mutations common to both tumor types were also found in his study (T7I and T194A), where their frequencies are highest in both benign and malignant tumors. These observations suggest that *MT-CYB* mutations may be very early events that promote initial clonal expansion and thus act as a metabolic driver of hyperplasia, but they are also considered transient events during tumorigenesis [28].

Mutations affecting the mitochondrial genome are less well characterized. Their role in tumor development and more specifically in prostate tumors remains to be elucidated. Therefore, to understand the impact of the non-synonymous mutations detected in this study on protein structure and function, three prediction tools were used to determine which of these mutations are pathogenic. Consequently, of the 91 non-synonymous mutations detected, 77 (84.62 %) were found to be potentially pathogenic, meaning they could potentially alter the structure and function of the cytochrome b protein and thus have the potential to influence the progression and aggressiveness of prostate tumors. This rate is higher than that reported by Touré et al. in their studies on the impact of *MT-CYB* mutations in oral cavity cancers among Senegalese patients, where they found a 25 % rate of pathogenic mutations [25]. While only 43.95 % of these deleterious mutations originated from BPH, more than half 51 (56.04 %) were found in malignant tumors. These results are consistent with those of Schöpf and colleagues, who suggested that the mutational burden of potentially deleterious mtDNA is higher in tumors and that these types of mutations are associated with adverse risk factors [29].

Although the number of potentially deleterious mtDNA mutations reported in tumor studies is on the rise, their functional and structural impact on the protein specifically its stability and flexibility has yet to be evaluated [28 ; 24]. Consequently, researchers have begun to investigate the effects of these mutations on the protein, seeking to understand the variations in this gene as a potential target, particularly for cancer treatment [30]. All non-synonymous mutations detected in this study are predicted to be destabilizing by one of two protein stability prediction tools for cytochrome b. This could have an impact on the biological process of tumor progression, specifically the progression of prostate tumors. The impact could be structural, disrupting hydrogen bonds and critical domains, triggering overall protein destabilization, or reducing the necessary rigidity and altering multimeric interactions [31 ; 32]. Thus, this could cause local unfolding or a defect in the assembly of Complex III and stiffen the enzyme's structure, which corresponds to a reduction in the activity of this complex [33].

Despite the significant number of mtDNA mutations in various human tumors, these mutations have, to a large extent, previously been considered transient events during tumorigenesis [34 ;35]. Analysis revealed that, out of 91 non-synonymous mutations, 14 substituted residues exhibited only altered physicochemical properties, with transmembrane alterations being the most frequent, accounting for 85.71 %. This high rate of alterations in membrane proteins was also highlighted in a study predicting missense SNPs in the human HLA-G gene and conducting an in silico assessment of their structural and functional consequences [36]. These physicochemical alterations lead to metabolic changes that contribute to tumor aggressiveness and therapeutic resistance in prostate tumors [29]. It has been reported in the literature that polymorphisms located in coding regions are likely to alter protein conformation. This can affect structural function at several levels, notably by promoting the

expression of alternative isoforms, modifying the protein's binding affinity for peptides, or altering its ability to polymerize [37 ; 38].

One of the major challenges following the detection of somatic mutations in various cancers as a result of large-scale sequencing projects (The Cancer Genome Atlas (TCGA)) is to distinguish the rare mutations that functionally contribute to oncogenesis namely, driver mutations from the numerous biologically neutral mutations, such as transient mutations [39 ; 40]. To make this distinction among the detected non-synonymous mutations, the mutation3D tool was used. This tool enabled us to predict which mutations were "driver" mutations and which were "passenger" mutations and to distinguish between them. Thus, 16 out of 91 mutations (17.58 %) are selectively negative, indicating that they are likely to be driver mutations. These mutations are located in the C-terminal region, a critical region of cytochrome b (residue positions 258–359). This is the region richest in pathogenic variants of *MT-CYB*, with a major role in mitochondrial diseases. This phenomenon of very low impact in terms of mutational burden confirms the hypotheses previously put forward by Ju et al., demonstrating that most mitochondrial mutations are considered passenger events during tumorigenesis [28]. Among these 16 mutations predicted to be drivers, 10 (62.5 %) are found in malignant tumors, a frequency that is not negligible. These mutations, which form clusters (hotspots) in the protein structure of cytochrome b, cause alterations in tumor cells by disrupting specific protein functions, such as those predicted earlier. This is the case, for example, with transmembrane alterations that could disrupt complex III or other transient interactions [41].

5. Conclusion

Prostate tumors are the most common tumors in men, particularly prostate cancer. Although previous studies have been conducted on various cancers to investigate the role of mtDNA specifically the *MT-CYB* gene its effects on structure, function, and physiology remain to be explored, especially in the case of prostate tumors. Thus, in this study, an in silico oncogenetic methodology was adopted to establish the mutational profile and predict the effects of mutations on pathogenicity, protein stability, molecular mechanisms, and high-risk mutations in prostate tumors among Senegalese men. To this end, 125 mutations were detected in both benign and malignant prostate tumors. A higher prevalence was observed in malignant tissues, particularly for non-synonymous mutations. Thirteen shared mutations were identified, revealing highly relevant information: namely, the possibility that they serve as potential early biomarkers of tumor risk, and that their presence in prostate tissue could predict tumor aggressiveness and biochemical recurrence. Most of these mutations are potentially pathogenic (capable of leading to the aggressiveness and development of prostate tumors) and cause instability in the mutated proteins, particularly respiratory proteins. These mutations could induce alterations by promoting prostate tumorigenesis via ROS (reactive oxygen species) and metabolic reprogramming. It is clear that most non-synonymous mutations are located in the critical C-terminal region of cytochrome b (which is rich in pathogenic variants and implicated in mitochondrial diseases, including cancers). Although the sample size is small, the results obtained are intriguing and confirm that mitochondrial dysfunction caused by mutations is a factor contributing to the development and progression of cancer. Therefore, expanding the study nationwide by conducting multicenter studies and including healthy control groups would be a highly effective strategy for better understanding the mechanisms by which mitochondrial mutations contribute to tumorigenesis and prostate carcinogenesis. This would also help establish more targeted anticancer therapeutic approaches specifically for the Senegalese population with prostate tumors, where cancers are constantly evolving and genetic approaches are still in their infancy, particularly when it comes to treatment decisions.

6. References

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