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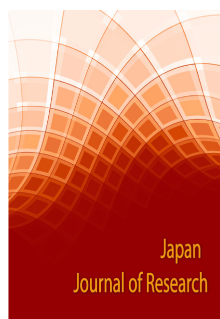


# Mitochondrial DNA Mutations Analysis in Breast, Ovarian and Oral Human Cancers

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## Abstract

*Somatic mutations affecting the mitochondrial DNA (mtDNA) have been frequently observed in human cancers and proposed as important oncological biomarkers. However, the exact mtDNA mutations that is responsible for the pathogenesis of cancer remains unclear. This study aims to identity the somatic mutations shared in breast cancer, ovarian cancer and oral cancer samples from Senegalese patients. We screened the tumor samples from 26 breast cancer, 23 oral cancer, 18 ovarian cancer and 10 healthy controls to detect somatic mutations in D-Loop and MT-CYB regions of mtDNA by direct sequencing. Few mutations were observed in control samples. Pathogenic MT-CYB mutations were observed in oral and ovarian cancers, and many of them in breast cancer. We also show that, while the exact mutation locations differ in each tumorous patient, the global D-Loop mutation profile of ovarian and breast cancers are very similar, but it differs from the one of oral cancer. This might suggest a common pathologic process in ovarian and breast cancers.*

## Introduction

Since mitochondria play a fundamental role in energy production by oxidative phosphorylation (OXPHOS) in human cells, it is not surprising that their role in the molecular level of the tumorigenesis started being investigated. The mode of inheritance of mitochondrial genome is unique because it is inherited maternally. The entire human mitochondrial DNA (mtDNA) sequence has been determined [1]. Mammalian mitochondria contain about 1000 proteins [2] but only 13 of them are encoded by the mitochondrial genome [3]. The other mitochondrial proteins are encoded by nuclear DNA, synthesized in the cytoplasm, and imported by the mitochondria.

The rate of mtDNA mutations is several times higher than the rate for nuclear DNA [4-6]. This is likely explained by two reasons. First, the mismatch reparation proteins existing in the mitochondria are less efficient than those in the nuclear genome [6]. Second, the production of reactive oxygen species by the mitochondrial oxidative phosphorylation system favors mutations [7,8]. Increased accumulation of mtDNA somatic mutations has been reported in aging tissues such as brain, skeletal muscle, and fibroblasts [5,9] and in many pathological conditions including neurologic, metabolic, and age-related disorders. These alterations are especially prevalent in preneoplastic lesions and in human cancers, including breast cancer, ovarian cancer, colorectal cancer, gastric cancer, hepatic cancer, esophageal cancer, prostate cancer, oral cancer and thyroid cancer. Most studies on mtDNA mutations have focused on the D-Loop region. Thus, many studies have proposed that mtDNA mutations might serve as both biomarkers of carcinogenesis and as predictive factors for the disease course.

In this paper, we analyzed the DNA sequence of the *MT-CYB* and D-Loop regions in breast, ovarian and oral cancers samples from 67 Senegalese patients. We focused on the *MT-CYB* gene, because it is highly variable both among species and among human individual, and the D-Loop gene, because this region is crucial for replication and expression of the mitochondrial genome and is the leading-strand origin of replication. We study on the differences in the nucleotide substitution patterns between these two genes.

## Materials and methods

### Population study

This study was approved by the ethical committee of Cheikh Anta Diop University. Tissues samples of 26 breast cancers, 18 ovarian cancers and 23 oral cancers were obtained from surgery, at Joliot Curie Institute and Department of Stomatology and Maxillofacial Surgery, affiliated Hospital Aristide Le Dantec (Dakar, Senegal). 10 blood samples were used for control. We thus collected a total of 77 samples.

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## DNA isolation, PCR amplification and sequencing

Total DNA was isolated from the samples cancerous tissues and blood samples by QIAamp DSP DNA Blood Mini kit (Qiagen) and Quick-DNATM Miniprep Plus Kit (Zymo Research). Extracted DNA was tested using 1.5% agarose gel electrophoresis. We used the primer sequences and amplification of D-Loop and *MT-CYB* previously described by [10,11]. PCR products were purified and sequenced with an ABI Big Dye Terminator cycle sequencing ready reaction kit and an ABIPRISM 3730xl sequencer (Applied Biosystems, Foster City, CA).

## Molecular analysis

The sequences were observed and aligned using BioEdit sequence Alignment Editor V.7.1.9 [12]. Tumor mtDNA sequences were compared with mtDNA sequences from controls. Any sequence variation present in the controls samples was considered as a polymorphism. On the contrary, any mutation observed on tumor samples and never found in control samples was regarded as a somatic mutation. mtDNA somatic mutations were compared with the mitochondrial genome database of world population by using Mitomap (www.mitomap.org). Those not found in the Mitomap database were recorded as new mutations in human cancers. Pathogenicity assessment of protein-coding variants were performed using Polyphen-2 [13], mutationAssessor (mutationassessor.org) and Provean [14]. A mutation was considered as pathogenic when at least two methods indicated that there ought to be a deleterious effect. To shed more light on the potential effect of data quality on our understanding of mtDNA mutation patterns in cancer studies, ratios of the number of somatic mutation (Ns) to the number of individuals with somatic mutations (Ni) were calculated.

# Results

## Somatic mtDNA mutations status in human cancers

The sequencing results from the *MT-CYB* and the D-Loop regions of 26 breast cancer, 23 oral cancer and 18 ovarian cancer samples were compared with those obtained from 10 blood controls. 67.16% (45/67) and 100% (67/67) of the cancerous samples had one or more somatic mutations in *MT-CYB* and D-Loop gene, respectively. In total, 31.88% (427/1339) of somatic mutation in the human cancer cases occurred in the D-Loop region of the mitochondrial genome and 68.11% (912/1339) occurred in the *MT-CYB* gene. *MT-CYB* gene was altered in 52.2% (12/23) oral cancer samples, 50% (9/18) ovarian cancer samples and 92.3% (24/26) breast cancer samples. The D-Loop region was altered for all samples in all cancers (table 1). In oral and ovarian cancers, the relative mutation frequency (mutations/nucleotides) for the D-Loop region was 2.01-fold and 6.52-fold higher than the *MT-CYB* gene, respectively, while, in breast cancer, the relative mutation frequency for the *MT-CYB* gene was 8.06-fold higher than the D-Loop region.

In the *MT-CYB* gene, the vast majority of somatic mutations were singletons, observed in only one sample. Breast cancer samples harbored multiple substitutions, ranging from 1 to 88. The number of nucleotide polymorphisms per ovarian cancer sample ranged from 0 to 9 and from 0 to 6 for oral cancer.

Asymmetry mutation was noted for breast cancer and both gene. The most common mutation type was base substitutions and G to A or C to T transition mutations accounted for the most mutations [(54.87%; *MT-CYB*) (61.61%; D-Loop)] followed by A to G or T to C mutations [(39.63%; *MT-CYB*) (38.38%; D-Loop)]. Among ovarian and oral cancers, this asymmetry was a favor to G-A or C-T only D-Loop gene.

Overall, there were 97 missense and false sense mutations in 61 unique nucleotide positions in *MT-CYB* coding region (table 2). The majority of non-synonymous somatic mutations were recorded in breast cancers (75/97, 77.31%), followed by oral cancer (11/97, 11.34%). Ovarian cancer is associated with the lowest mutation rate (8/97, 8.24%). Among these mutations, 45 are pathogenic. Only L82M (non-pathogenic), L82V (pathogenic) and I163F (non-pathogenic) are common to breast and ovarian cancer. These mutations are novel: they were not previously reported in the Mitomap database.

## Analyses mutation co-occurences

Samples are grouped by health status (cancer types or control). Samples are ordered by similarity, i.e. samples presenting similar mutations tend to be placed close to each other's. We can see that few mutations were observed in control samples, and in particular no Cytochrome b mutations were found pathogenic by Provean, Mutation Assessor or PolyPhen2. This was expected and confirms the quality of these three methods.

Regarding oral cancer samples, few mutations were observed on Cytochrome b, and a higher number in D-Loop. No pathogenic mutations of *MT-CYB* gene are shared between oral cancer and another cancer, but some locations are shared (e.g. R→P at position 76, while R→Q is observed in breast cancer). Two samples (labeled CB16 and CB19) have a very high number of mutations in the second half of the D-Loop, a high proportion of them being transversions, compared with other samples. Three samples (CB2, CB17 and CB8) have very

***Table 1.*** *Summary of the observed mutation frequencies in the three cancers and the control samples, on the two genes*

| | Cancer type | Oral (23) | Ovarian (18) | Breast (26) | Total (67) | Control (10) | |
|---|---|---|---|---|---|---|---|
| **MT-CYB** | somatic mutations | 24 | 35 | 853 | 912 | polymorphisms | 9 |
| | unique positions | 17 | 23 | 183 | 199 | unique positions | 7 |
| | patients | 12 (52.2%) | 9 (50.0%) | 24 (92.3%) | 45 (67.2%) | patients | 6 (60.0%) |
| **D-Loop** | somatic mutations | 199 | 91 | 137 | 427 | polymorphisms | 4 |
| | unique positions | 109 | 25 | 28 | 114 | unique positions | 4 |
| | patients | 23 (100%) | 18 (100%) | 26 (100%) | 67 (100%) | patients | 1 (10.0%) |
| **Total** | somatic mutations | 223 | 126 | 990 | 1339 | polymorphisms | 13 |
| | unique positions | 122 | 47 | 198 | 277 | unique positions | 11 |
| | patients | 23 (100%) | 18 (100%) | 26 (100%) | 67 (100%) | patients | 7 (70.0%) |

***Table 2.** Functional impact of amino-acids substitutions in Cytochrome b proteins*

| Mutations | P00156 position | Mutation Assessor | Polyphen-2 | Provean | Protein binding site | Codons changes | Trs/Trv | Patients |
|---|---|---|---|---|---|---|---|---|
| L52P | 258 | Neu | PoD | Neu | | CTA->CCA | 1 trs | CB9, CB13, CB2 |
| L52R | 258 | Neu | benign | Neu | | CTA->CGA | 1 trv | CB17 |
| A53D | 259 | high | PD | Del | 1 | GCC->GAC | 1 trv | CB18 |
| N54S | 260 | Med | PD | Del | 1 | AAC->AGC | 1 trs | CB18 |
| P55L | 261 | high | PD | Del | 1 | CCC->CTA | 1 trs 1 trv | CS19 |
| L56M | 262 | | PD | Neu | 1 | TTA->ATG | 1 trs 1 trv | CS19 |
| L56P | 262 | | PD | Del | 1 | TTA->CCA | 2 trs | CS21 |
| N57* | 263 | (stop) | | (stop) | | AAC->AGG | 1 trs 1 trv | CS19 |
| N57K | 263 | Neu | benign | Neu | 1 | AAC->AAG | 1 trv | CS6, CS7, CS8, CS10, CS11, CS12, CS13, CS1, CS14, CS15, CS16, CS17, CS2, CS20, CS22, CS3, CS4, CS5 |
| N57T | 263 | Neu | benign | Neu | 1 | AAC->ACC | 1 trv | CB21 |
| N57T | 263 | Neu | benign | Neu | 1 | AAC->ACG | 2 trv | CS24, CS26 |
| T58D | 264 | | PoD | Del | 1 | ACC->GAC | 1 trs 1 trv | CS21 |
| T58I | 264 | high | PoD | Del | 1 | ACC->ATT | 2 trs | CS22 |
| H61P | 267 | high | PD | Del | 1 | CAC->CCC | 1 trv | CS21, CS24 |
| I62A | 268 | | PD | Del | 1 | ATC->GCT | 3 trs | CS26 |
| I62F | 268 | high | PD | Del | 1 | ATC->TTT | 1 trs 1 trv | CS1 |
| I62S | 268 | high | PD | Del | 1 | ATC->AGT | 1 trs 1 trv | CS21 |
| I62V | 268 | high | benign | Neu | 1 | ATC->GTT | 2 trs | CS6, CS7, CS8, CS10, CS11, CS12, CS13, CS14, CS15, CS16, CS17, CS2, CS19, CS20, CS22, CS3, CS4, CS5 |
| P64T | 270 | high | PD | Del | 1 | CCC->ACC | 1 trv | CS24 |
| E65D | 271 | high | PD | Neu | 1 | GAA->GAT | 1 trv | CB19 |
| E65G | 271 | high | PD | Del | 1 | GAA->GGA | 1 trs | CS1 |
| Y67C | 273 | high | PD | Del | 1 | TAT->TGC | 2 trs | CS6, CS7, CS8, CS10, CS11, CS12, CS13, CS1, CS14, CS15, CS16, CS17, CS2, CS19, CS20, CS22, CS3, CS26, CS4, CS5 |
| Y67C | 273 | high | PD | Del | 1 | TAT->TGT | 1 trs | CS21 |
| F68L | 274 | Med | benign | Del | 1 | TTC->CTT | 2 trs | CS21 |
| L69F | 275 | | PD | Del | 1 | CTA->TTT | 1 trs 1 trv | CS21 |
| F70L | 276 | Med | PD | Del | 1 | TTC->TTG | 1 trv | CS15, CS22, CS3 |
| F70W | 276 | | PD | Del | 1 | TTC->TGG | 2 trv | CB20 |
| A71G | 277 | high | PD | Del | 1 | GCC->GGT | 1 trs 1 trv | CS20 |
| Y72H | 278 | high | PD | Del | 1 | TAC->CAC | 1 trs | CS1 |
| T73A | 279 | Neu | benign | Neu | | ACA->GCA | 1 trs | CS6, CS7, CS8, CS10, CS11, CS12, CS13, CS14, CS15, CS16, CS17, CS2, CS19, CS21, CS22, CS3, CS26, CS4, CS5 |
| I74L | 280 | high | PoD | Neu | | ATT->CTT | 1 trv | CS1 |
| L75F | 281 | high | PD | Del | 1 | CTC->TTC | 1 trs | CS21, CS3 |
| L75V | 281 | high | PoD | Del | 1 | CTC->GTG | 2 trv | CO5 |

| Mutations | P00156 position | Mutation Assessor | Polyphen-2 | Provean | Protein binding site | Codons changes | Trs/Trv | Patients |
|---|---|---|---|---|---|---|---|---|
| R76P | 282 | high | PD | Del | 1 | CGA->CCA | 1 trv | CB17, CB20, CB23 |
| R76Q | 282 | high | PD | Del | 1 | CGA->CAG | 2 trs | CS6, CS7, CS8, CS10, CS11, CS12, CS13, CS1, CS14, CS15, CS16, CS17, CS2, CS19, CS22, CS3, CS26, CS4, CS5 |
| S77C | 283 | high | PD | Del | | TCC->TGT | 1 trs 1 trv | CS14 |
| S77F | 283 | high | PD | Del | | TCC->TTT | 2 trs | CS1, CS2, CS3 |
| V78F | 284 | low | PoD | Del | 1 | GTC->TTC | 1 trv | CS1, CS20, CS3 |
| V78I | 284 | Neu l | benign | Neu | 1 | GTC->ATC | 1 trs | CS6, CS7, CS8, CS10, CS11, CS12, CS13, CS14, CS15, CS16, CS17, CS2, CS19, CS22, CS26, CS4, CS5 |
| V78S | 284 | | PD | Del | 1 | GTC->TCC | 1 trs 1 trv | CO7, CO8 |
| N80D | 286 | Med | PoD | Del | 1 | AAC->GAC | 1 trs | CS20 |
| N80K | 286 | high | PD | Del | 1 | AAC->AAA | 1 trv | CO7 |
| N80T | 286 | high | PD | Del | 1 | AAC->ACA | 2 trv | CS1 |
| N80Y | 286 | high | PD | Del | 1 | AAC->TAC | 1 trv | CO11 |
| L82M | 288 | | PD | Neu | 1 | CTA->ATA | 1 trv | CO5, CO12, CS1 |
| L82V | 288 | Med | PD | Del | 1 | CTA->GTA | 1 trv | CO13, CS20 |
| G84* | 290 | (stop) | | (stop) | | GGC->AGG | 1 trs 1 trv | CS15, CS16, CS17, CS22, CS3 |
| G84S | 290 | high | PD | Del | | GGC->AGC | 1 trs | CS6, CS7, CS8, CS10, CS11, CS12, CS13, CS14, CS2, CS19, CS4, CS5 |
| L86H | 292 | high | PD | Del | | CTT->CAT | 1 trv | CO12 |
| L86Q | 292 | | PoD | Del | | CTT->CAG | 2 trv | CO6 |
| L89W | 295 | | PD | Del | | TTA->TGA | 1 trv | CS20 |
| L93F | 299 | Med | PD | Del | | CTC->TTT | 2 trs | CS8, CS16, CS22 |
| L93V | 299 | Med | PD | Neu | | CTC->GTT | 1 trs 1 trv | CS14 |
| M97A | 303 | | benign | Neu | 1 | ATA->GCA | 2 trs | CS19 |
| M97A | 303 | | benign | Neu | 1 | ATA->GCC | 2 trs 1 trv | CS7, CS8, CS10, CS12, CS14, CS16, CS22 |
| I100A | 306 | | benign | Neu | 1 | ATC->GCA | 2 trs 1 trv | CS7, CS12 |
| I100S | 306 | Med | benign | Del | 1 | ATC->TCA | 1 trs 2 trv | CS22 |
| I100T | 306 | Neu | benign | Neu | 1 | ATC->ACA | 1 trs 1 trv | CS14 |
| M103T | 309 | Neu | benign | Neu | 1 | ATA->ACA | 1 trs | CS7, CS10, CS12, CS16, CS22 |
| S117* | 323 | (stop) | | (stop) | | TCA->TAA | 1 trv | CS7 |
| S117L | 323 | Neu | benign | Neu | 1 | TCA->TTA | 1 trs | CB18 |
| S117Y | 323 | | benign | Neu | 1 | TCA->TAC | 2 trv | CS12, CS22 |
| L118F | 324 | low | PoD | Del | | CTT->TTT | 1 trs | CB6 |
| L121P | 327 | Med | PD | Neu | 1 | CTC->CCC | 1 trs | CS7, CS12 |
| L121S | 327 | | PoD | Neu | 1 | CTC->TCC | 2 trs | CS22 |
| A123V | 329 | Neu | benign | Neu | 1 | GCC->GTC | 1 trs | CS7, CS10, CS12, CS14, CS16, CS22, CS4 |
| A124T | 330 | low | benign | Neu | 1 | GCA->ACC | 1 trs 1 trv | CS7, CS12, CS22 |
| D125N | 331 | Neu | benign | Neu | 1 | GAC->AAC | 1 trs | CS7, CS22 |
| L126S | 332 | | PD | Del | 1 | CTC->TCC | 2 trs | CS7 |

| Mutations | P00156 position | Mutation Assessor | Polyphen-2 | Provean | Protein binding site | Codons changes | Trs/Trv | Patients |
|---|---|---|---|---|---|---|---|---|
| S138T | 344 | Neu | PoD | Neu | 1 | AGC->ACC | 1 trv | CO1 |
| Y139H | 345 | Neu | benign | Neu | 1 | TAC->CAC | 1 trs | CS7, CS12, CS16, CS22, CS5 |
| P140S | 346 | high | PD | Del | 1 | CCT->TCC | 2 trs | CS12, CS22 |
| P140S | 346 | high | PD | Del | 1 | CCT->TCT | 1 trs | CS16 |
| F141W | 347 | | PD | Del | 1 | TTT->TGG | 2 trv | CS14 |
| T142I | 348 | Neu | benign | Neu | | ACC->ATC | 1 trs | CS14 |
| T142I | 348 | Neu | benign | Neu | | ACC->ATT | 2 trs | CS12, CS22 |
| I143T | 349 | Neu | benign | Del | | ATC->ACC | 1 trs | CS12, CS14, CS16, CS22 |
| A148V | 354 | Med | PoD | Del | 1 | GCA->GTA | 1 trs | CS12 |
| A148V | 354 | Med | PoD | Del | 1 | GCA->GTG | 2 trs | CS14 |
| V150M | 356 | low | benign | Neu | | GTA->ATA | 1 trs | CS12 |
| L151V | 357 | low | PD | Neu | 1 | CTA->GTA | 1 trv | CS7, CS10, CS12, CS16, CS22 |
| T155M | 361 | | benign | Neu | | ACA->ATA | 1 trs | CS22 |
| I156V | 362 | Med | PoD | Neu | | ATC->GTC | 1 trs | CS2 |
| L157R | 363 | high | PD | Del | 1 | CTA->CGA | 1 trv | CS2 |
| I158F | 364 | Med | benign | Neu | 1 | ATC->TTC | 1 trv | CS19, CS20 |
| I158L | 364 | low | benign | Neu | 1 | ATC->CTC | 1 trv | CS18 |
| I158P | 364 | | PoD | Del | 1 | ATC->CCC | 1 trs 1 trv | CS12 |
| M160F | 366 | | benign | Neu | 1 | ATA->TTC | 2 trv | CS18 |
| M160K | 366 | high | benign | Neu | 1 | ATA->AAA | 1 trv | CS22 |
| P161T | 367 | high | PD | Del | | CCA->ACA | 1 trv | CS22 |
| T162N | 368 | Med | benign | Neu | | ACT->AAC | 1 trs 1 trv | CO5, CO6 |
| T162N | 368 | Med | benign | Neu | | ACT->AAT | 1 trv | CS23 |
| T162S | 368 | low | benign | Neu | | ACT->TCG | 2 trv | CS12 |
| I163F | 369 | low | benign | Neu | | ATC->TTC | 1 trv | CO5, CO6, CS18, CS19 |
| I163N | 369 | Med | benign | Neu | | ATC->AAC | 1 trv | CS14 |
| I163S | 369 | Neu | benign | Neu | | ATC->AGC | 1 trv | CS22 |
| I163V | 369 | Neu | benign | Neu | | ATC->GTC | 1 trs | CB10 |

*Pathogenic mutations are shown in bold. PD: Probably damaging; PoD: Possibly Domaging; Del: deleterious; Neu: Neutral; Med: Medium*

similar mutations on the D-Loop. We also observe an interesting pattern involving two mutations: a G→A transition at position 477 and a T→C transitions at position 606. This applies for 19 samples; 2 samples have both mutations and the last 2 have none. The second mutation (at 606) was also found in one control.

Regarding breast cancer samples, many mutations are observed in Cytochrome b, many of them being highly pathogenic. However, a few samples (e.g. CS9, CS23 and CS25) have very few mutations in Cytochrome b, or even none. Regarding ovarian cancer samples, few mutations are observed in Cytochrome b, most of them being also observed in breast cancer. As said above, mutations in D-Loop in breast and ovarian cancers are very similar. Three mutations were present in all breast and ovarian cancer samples: a G→C or T transversion at position 518, a T→C transition at 606 and an A→G transition at 634. However, the transition at 606 was also observed in one control. We can also identify in Figure 3 a set of 4 G→A transitions around position 275 that are present in many cancer samples, and one control.

## Discussion

mtDNA is the only genetic material of the human genome not contained in the nucleus. In recent years, somatic mutations in the mtDNA have been increasingly studied in human cancers, such as oral cavity squamous cell carcinoma [15], ovarian [16] and breast cancer [17]. Here, we presented a study investigating the mtDNA somatic mutations in human cancers of Senegalese patients.

By direct sequencing, we found that 67.16% (45/67) and 100% (67/67) of human cancer samples carried respectively somatic mutations in the *MT-CYB* and in the D-Loop of mtDNA. *MT-CYB* gene was altered in 52.2% (12/23) oral cancer patients, 50% (9/18) ovarian cancers and 92.3% (24/26) breast cancer patients. The discrepancy might be due to the diverse anatomical origin of cancers. The D-Loop region of mtDNA is a crucial position for replication and expression of the mitochondrial genome because it possesses essential transcriptional promoters and is the leading-strand origin of replication [18]. Furthermore,

the D-Loop region is hypervariable and susceptible to somatic mutations because of its distinctive triple-stranded DNA structure. Mitochondrial mutations may modify the function of normal oxidative phosphorylation chain which operates as a metabolic caretaker to prevent unexpected alterations to the glycolytic metabolic phenotype and also serves as a gatekeeper to avoid improper production of genotoxic reactive oxygen species [19]. In this study, the incidence of D-Loop mutations in human cancers seems higher than the one of Cytochrome b. The mutation rate in the mitochondrial D-Loop region varies between different cancer types; from 4-5% in gastric and oesophageal cancer to 63-70% in breast, head and neck and lung cancers [18,20-21], to 20-26% in ovarian cancer [15,22].

Investigating the possibility that *MT-CYB* and D-Loop mitochondrial gene might be associated with cancer development, we assessed relative mutation frequency in each gene. We found that the D-Loop region had a 2.01-fold and 6.52-fold higher mutation rate than the *MT-CYB* region respectively in ovarian and oral cancer. The majority of somatic mutations occur in the D-Loop region, which is considered as a hot spot for mutations. Other studies reported a 7-fold increase in susceptibility [23]. However, in breast cancer we found the *MT-CYB* mutation 8.06-fold higher mutation rate than the D-Loop region. Each of these mutations may be of functional significance, but more extensive biochemical and molecular studies will be necessary to determine their effects on energy metabolism in malignant cells.

In Senegal, Breast cancer is a major health problem that affects 42% of women [24]. Further-more, among low-income countries including Senegal, individuals have a high risk for developing breast cancer. Given the well-established mitochondrial dysfunction in cancer and the high rate of somatic mutation in mtDNA, the mitochondrial genome is an under-explored avenue for insight into breast cancer pathogenesis, as well as an attractive candidate source for biomarkers. A total of 912 mutations (involving 199 unique positions) were detected in the *MT-CYB* coding region, among which 853 mutations (183 positions) were found in breast cancer. These mutations are the cause of 75 amino acid changes. Multiple mitochondrial genes have documented somatic mutations which may be implicated in tumor formation. Protein-coding genes found in the mitochondria belong to four different complexes of the mitochondrial respiratory chain. Complex III, of which *MT-CYB* is the only gene encoded by mtDNA, contains fewer documented somatic variants. Mutations in the *MT-CYB* gene can cause mitochondrial complex III deficiency. Most *MT-CYB* gene mutations that cause mitochondrial complex III deficiency change single protein building blocks (amino acids) in the cytochrome b protein or lead to an abnormally short protein. These cytochrome b alterations impair the formation of complex III, severely reducing the complex's activity and oxidative phosphorylation.

In this study, the lowest rate of mitochondrial genome mutations was noted in ovarian cancer. 50% (9/18) of the ovarian tumors analyzed contained *MT-CYB* mutations and all patients (18/18) have D-Loop mutations ranging from 1 to 11. The frequency of the *MT-CYB* mutations is 3.83% and that the D-Loop gene of 21.31%. Somatic mutation rate of D-Loop is 2.01-fold higher than the one of *MT-CYB* region in ovarian cancer. Liu et al. [25] sequenced the D-Loop region of mtDNA of 15 primary ovarian carcinomas and matched normal control tissues. Their study revealed that 20% of tumor samples carried single or multiple somatic mtDNA mutations. In the same study, a complete sequence analysis of the mtDNA genomes of another 10 pairs of primary ovarian carcinomas as and control tissues showed a high incidence (60%) of somatic mtDNA mutation. The four regions of mitochondrial genome primarily affected by these mutations were the D-Loop, 12S rRNA, 16S rRNA, and Cytochrome b, suggesting that these regions may be mitochondrial hotspots in ovarian cancer.

In oral cancer, Twenty-four (24) different positions of mutations were discovered in the region of *MT-CYB*. The frequency of mutations was much higher in the non-coding D-Loop relative to the *MT-CYB* gene where, in total, 199 positions of mutations were found. A recent study on oral squamous cell carcinoma also found a high rate of somatic mutations in the D-Loop region (85%, 203/240) [15].

The distribution of mutation types may be able to provide some insights into the causing mechanisms of these mutations. The majority of the base substitution mutations were either G to A or C to T transitions, a spectrum characteristic of oxidative DNA damage [26]. Because the C→T transition on the L strand is equivalent to the G→A transition on the H strand, we can regard the difference in the frequencies between the G→A transitions and the C→T transition as an index of mutational asymmetry between the L an H strands. The frequency of G→A transitions was higher than that of C→T transitions among both genes in breast cancer, among D-Loop only in ovarian and oral cancers. These findings suggest that the mutational asymmetry between the L and H strands is marked in the *MT-CYB* and D-Loop genes. This observation can be explained by assuming that deamination of C to T on the H strand occurs more often in the *MT-CYB* and D-Loop region. The deamination frequency may be proportional to the duration of the single stranded state of the parental H strand before it is used as the template for the daughter L strand. The duration of the single stranded state of the parental H strand is longer in two genes breast cancer patients, in D-Loop on ovarian and oral cancers.

The human mtDNA sequence is highly variable. It is necessary to define functionally important somatic mutations that may be deleterious or pathogenic in terms of tumor progression. A key step in the process of annotating sequencing results is the integration of pathogenicity predictions. It uses various tools that aim to predict the effects of substitutions on the structure and / or function of proteins. Several tools are available and use different approaches. Although these methods are useful in practice, their accuracy remains a concern. Therefore, we applied a battery of four tools to determine pathogenic mutations, as described in the materials and methods section, and we found that 3 (L82M; L82V; I163F) shared non-synonymous mutations in breast and ovarian cancers. Among these mutations, L82V was pathogenic. All cancers are thought to have a common pathogenesis. The different visualization approaches we used have shown that breast, ovarian and oral cancers share few mutations, although we found that ovarian and breast cancer samples shares three mutations and have a common mutation profiles on D-Loop. While each individual patient has different mutations, these mutations may differ between cancer types (*MT-CYB*; D-Loop for oral cancer vs ovarian and breast cancers) or be similar (e.g. for D-Loop in ovarian and breast cancer).

## Conclusion

In conclusion, a high rate of somatic mutations in the *MT-CYB* and the D-Loop region of mtDNA were noted in human cancer. In addition, this study reveals that the exact mutation locations differ in human cancer patients. However, the mutation profiles of ovarian and breast cancers were found very similar in D-Loop,

and include three common mutations shared by all patients. Further studies with larger populations and involving the whole mtDNA genome would be required in order to elucidate the relationship between the mtDNA mutations, the pathologic process and the survival rate in human cancer patients.

## Conflicts of interest

The authors declare that they have no conflict of interest.

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## References

1. Anderson S, Bankier AT, Barrell BG, et al. Sequence and organization of the human mitochondrial genome. Nature. 1981;290(5806):457-465.
2. Goffart S, Wiesner RJ. Regulation and co-ordination of nuclear gene expression during mitochondrial biogenesis. Exp Physiol. 2003;88(1):33-40.
3. Taanman JW. The mitochondrial genome: structure, transcription, translation and replication. Biochim Biophys Acta. 1999;1410(2):103-123.
4. Wallace DC, Brown MD, Lott MT. Mitochondrial DNA variation in human evolution and disease. Gene. 1999;238(1):211-230.
5. Máximo V, Sobrinho-Simões M. Hürthle cell tumours of the thyroid. A review with emphasis on mitochondrial abnormalities with clinical relevance. Virchows Arch. 2000;437(2):107-115.
6. Czarnecka AM, Marino Gammazza A, Di Felice V, Zummo G, Cappello F: Cancer as a "Mitochondriopathy". J Cancer Mol. 2007, 3: 71-79.
7. Hochhauser D. Relevance of mitochondrial DNA in cancer. Lancet. 2000;356: 181-182.
8. Singh KK. Mitochondria damage checkpoint, aging, and cancer. Ann N Y Acad Sci. 2006;1067:182-190.
9. Michikawa Y, Mazzucchelli F, Bresolin N, Scarlato G, Attardi G. Aging-dependent large accumulation of point mutations in the human mtDNA control region for replication. Science. 1999;286(5440):774-779.
10. Mbaye F, Dem A, Fall M, et al. Genetic Diversity of Breast Cancer in Senegalese Women: New Insight from Somatic Mutations. Journal of Health Science. 2014;4(2): 25-33.
11. Mbaye F, Dem A, Sidy KA, et al. Mutation screening in the mitochondrial D-Loop region of tumoral and non tumoral breast tissues in Senegalese patients. International Journal of Cancer Research. 2014;48(2): 1564-1573.
12. Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. Nucleic Acids Symposium. 1999;41: 95-98.
13. Adzhubei I, Jordan DM, Sunyaev SR. Predicting functional effect of human missense mutations using PolyPhen-2. Curr Protoc Hum Genet. 2013;Chapter 7:Unit7.20.
14. Choi Y, Sims GE, Murphy S, Miller JR, Chan AP. Predicting the functional effect of amino acid substitutions and indels. PLoS One. 2012;7(10):e46688.
15. Lai CH, Huang SF, Liao CT, Chen IH, Wang HM, Hsieh LL. Clinical significance in oral cavity squamous cell carcinoma of pathogenic somatic mitochondrial mutations. PLoS One. 2013;8(6):e65578.
16. Guerra F, Kurelac I, Magini P, et al. Mitochondrial DNA genotyping reveals synchronous nature of simultaneously detected endometrial and ovarian cancers. Gynecol Oncol. 2011;122(2):457-458.
17. Cai FF, Kohler C, Zhang B, et al. Mutations of mitochondrial DNA as potential biomarkers in breast cancer. Anticancer Res. 2011;31(12):4267-4271.
18. Fliss MS, Usadel H, Caballero OL, et al. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. Science. 2000;287(5460):2017-2019.
19. Zhou S, Kachhap S, Sun W, et al. Frequency and phenotypic implications of mitochondrial DNA mutations in human squamous cell cancers of the head and neck. Proc Natl Acad Sci U S A. 2007;104(18):7540-7545.
20. Hibi K, Nakayama H, Yamazaki T, et al. Mitochondrial DNA alteration in esophageal cancer. Int J Cancer. 2001;92(3):319-321.
21. Tan DJ, Bai RK, Wong LJ. Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. Cancer Res. 2002;62(4):972-976.
22. Van Trappen PO, Cullup T, Troke R, et al. Somatic mitochondrial DNA mutations in primary and metastatic ovarian cancer. Gynecol Oncol. 2007;104(1):129-133.
23. Zhu W, Qin W, Bradley P, Wessel A, Puckett CL, Sauter ER. Mitochondrial DNA mutations in breast cancer tissue and in matched nipple aspirate fluid. Carcinogenesis. 2005;26(1):145-152.
24. Dem A, Traoré B, Dieng MM, et al. Gynaecological and breast cancers at the Dakar Cancer Institute. Sante. 2008;18(1):25-29..
25. Liu VW, Shi HH, Cheung AN, et al. High incidence of somatic mitochondrial DNA mutations in human ovarian carcinomas. Cancer Res. 2001;61(16):5998-6001.
26. Chatterjee A, Mambo E, Sidransky D. Mitochondrial DNA mutations in human cancer. Oncogene. 2006;25(34):4663-4674.