MUTATION ANALYSIS OF THE *MT-ATP6* GENE IN BREAST CANCER TISSUE SAMPLES OF BANGLADESHI WOMEN

Marufa Akter Mim[2], Sumaiya Farah Khan[2], Md. Aminul Islam[3], Rokeya Begum[1] and Gazi Nurun Nahar Sultana[1]*

1 Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka -1000, Bangladesh.
2 Department of Genetic Engineering and Biotechnology, Jagannath University, Dhaka -1100, Bangladesh
3 Department of Genetic Engineering and Biotechnology, East West University, Dhaka -1212, Bangladesh.

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ABSTRACT

Background and aim: Mitochondria are crucial for cellular energy production and apoptosis. It plays a crucial part in the development of cancer. This pilot study looked at mitochondrial *ATP6* polymorphisms as a potential preliminary indicator for breast cancer in Bangladeshi women. **Materials and Methods:** The MT-*ATP6* gene of mitochondrial DNA was sequenced from 13 breast cancer tissue samples. Sequencing data were compared with the Revised Cambridge Reference Sequence (rCRS) to detect polymorphisms. Blood samples from 33 healthy women were also analyzed as controls to account for variations. **Results:** A total of five mutations were identified by analysing the sequences of patient samples. Three of the five mutations (m.8701A>G, m.8860A>G, and m.9094C>T) were nonsynonymous, and two (m.8772T>C and 8790G>A) were synonymous. The m.8701A>G and m.8860A>G mutations were detected in both cancer patients and healthy controls and no statistical significance was calculated due to the small sample size. The m.8701A>G mutation was observed in 4 out of 13 (30.77%) cancer patients, and the m.8860A>G mutation was observed in 11 out of 13 (84.61%) cancer patients. Focus was placed on the m.9094C>T mutation, which was observed in two out of thirteen (15.38%) breast cancer patients, and was entirely absent in the control group (0/33). This mutation in Bangladeshi women results in a Leucine (L) to Phenylalanine (F) substitution at codon 120. According to PolyPhen-2 analysis, this variant is "possibly damaging," with a score of 0.855. Structural analysis using the HOPE tool revealed alterations in protein structure, while DynaMut confirmed impacts on structural features. These findings suggest that the m.9094C>T mutation may be indirectly linked to an increased risk of breast cancer. **Conclusion:** The detection of m.9094C>T exclusively in two patient samples, coupled with its predicted pathogenicity, identifies this variant as an interesting candidate for future, large-scale investigation. These preliminary findings do not establish a definitive association or heritability but suggest that variants in the *MT-ATP6* gene warrant further functional and epidemiological investigation as potential susceptibility factors for breast cancer.

KEYWORDS: Breast Cancer, Mitochondria, MT-ATP6 gene mutation, ATP synthase, Polymerase chain reaction

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***CORRESPONDING AUTHORS: Gazi Nurun Nahar Sultana, Centre for Advanced Research in Sciences (CARS), University of Dhaka, Dhaka -1000, Bangladesh. Email: nngazi@gmail.com**

Introduction

Breast cancer is a type of cancerous growth that starts in the milk-producing lobules or the milk-transporting ducts (Touhidul Islam *et al.*, 2021). It is a complex illness that is influenced by both environmental and genetic factors (Barzaman *et al.*, 2020). Breast cancer affects 1 in 8 women globally and is the most common cancer type among females (Shamsi, 2021). According to GLOBOCAN (Global Cancer Observatory) 2020 data, breast cancer is one of the most commonly diagnosed cancers worldwide, with approximately 2.3 million new cases reported, and is the fifth leading cause of cancer-related deaths (Sung *et al.*, 2021). In 2020 specifically, an estimated 685,000 women died from breast cancer, representing about 16% or one in every six cancer deaths among women (Arnold *et al.*, 2022). Both developed and developing nations have different incidence and fatality rates of breast cancer (Bray *et al.*, 2018). While developed nations like Australia, Europe, and the United States have the highest incidence of breast cancer, developing nations in Africa, Asia, and the Caribbean have comparatively higher death rates (Story *et al.*, 2012). The number of American women with a history of breast cancer was estimated to be 4.1 million as of January 1, 2022. Of these women, over half had initial cancer diagnoses in the early stages (I–III). Approximately 4% of them now have metastatic disease (Gallicchio *et al.*, 2022). Bangladesh is among the developing nations with the highest rate of inconsistency; the GLOBOCAN report from 2020 states that breast cancer is the most common cancer among women in Bangladesh (19% of all cancers) and the leading cause of death (6.2% of all cancers) (Ferlay *et al.*, 2021). Research has indicated a potential link between breast cancer and novel mitochondrial mutations in the *ATP6* gene (Grzybowska-szatkowska *et al.*, 2014). In 14/15 cases of breast cancer, at least one somatic mutation was found in the mtDNA (Zhu *et al.*, 2005). Based on our recent findings and those reported by other

laboratories (Ferlay *et al.*, 2010; Touhidul Islam *et al.*, 2021) we suggest that mtDNA mutations may contribute to cancer development as follows: during the early stages, cancer cells experience high levels of mutagenesis, either due to exposure to carcinogens or impaired DNA repair mechanisms, making mt DNA particularly susceptible to mutations at this point. The handling of programmed cell death, calcium homeostasis, reactive oxygen species (ROS) generation, and ATP production are all correlated with the morphology of the mitochondria. Moreover, modifications to mitochondrial morphology are linked to changes in metabolism. Higher ATP production is linked to mitochondrial fusion, while reduced OXPHOS(Oxidative phosphorylation), mtDNA depletion, and ROS generation result from repression of fusion (Wai and Langer, 2016). Research has indicated that cells exposed to an abundance of nutrients preserve their mitochondria in a fragmented form, whereas cells experiencing starvation preserve their mitochondria in an elongated and concatenated form (Gomes, Benedetto and Scorrano, 2011). Since the subunits of the electron transport chain are encoded by mtDNA, mutations in these subunits can impact electron transport efficiency and lead to the production of reactive oxygen species (ROS). However, ROS has the ability to encourage more mtDNA mutations. A vicious cycle like this lends credence to the theory that mtDNA mutations could be a precursor to tumorigenesis. As a result, there is interest in tracking the number of mtDNA mutations as a predictive indicator for breast cancer (Mcmahon and Laframboise, 2014). The elevated levels of ROS in cancer cells are attributed to mutations in mitochondrial proteins encoded by both the nuclear and mitochondrial genomes. While an increase in ROS encourages cell division, it also causes protein oxidation and misfolding. Mitochondria are vulnerable to proteotoxic stress because they are both the primary source and target of reactive oxygen species (Routhier A *et al.*, 2010). Structural alterations in mtDNA-encoded protein subunits may reduce electron transport, increasing electron leakage and reactive oxygen species (ROS) production. These factors then heighten the level of oxidative stress and damage to mitochondria during cell transformation, ultimately fueling the carcinogenesis cycle (Zhu *et al.*, 2005; Kobori *et al.*, 2007). Certain mtDNA polymorphisms have been linked to both familial (G9055A, A10398G, and T16519C) and sporadic (G10398A) breast cancer (Bai *et al.*, 2007).

Severe pathological states leading to typical mitochondrial diseases are linked to a generalized decrease in the content of human ATP synthase or modification of its structure and function. Oxidative stress-mediated damage to mtDNA can initiate a cycle of ROS (Reactive Oxygen Species) production and additional mtDNA damage. The "mitochondrial catastrophe hypothesis" or "toxic oxidative stress" refers to this cycle of mtDNA damage, loss of electron transport enzyme function, and increased ROS generation until the antioxidant systems are overwhelmed and cell death occurs. Despite the membrane's impermeability, mitochondria can experience a permeability transition that activates the caspase cascade and releases cytochrome c and apoptosis-inducing factor, which leads to programmed cell death or apoptosis (Fariss *et al.*, 2005). The mtDNA *ATP6* and *ATP8* genes, which code for the human and A6L subunits, respectively, are mutated in the better-characterized ATP synthase diseases. Since the open reading frames of these two subunits overlap for 46 nucleotides, changes in this area may have an impact on how both subunits are expressed. The majority of the mutations causing ATP synthase effects are related to the *ATP6* gene (Ware *et al.*, 2009). In patient cells, the capacity of mitochondria to produce ATP is reduced due to ATP synthase disorders. As oxidative metabolism is the primary energy source for the brain and heart, which are the primary tissues affected, a decrease in aerobic energy provision is predicted to have a negative impact on their morphology and function. A decrease in aerobic energy provision, coupled with increased ROS production, are the two main pathogenic components of isolated ATP synthase defects(Houštěk *et al.*, 2006). These defects are linked to a range of severe pathological states, often correlated with the mutation load (Dautant *et al.*, 2018). Based on these observations, we were inspired to conduct a similar study among Bangladeshi breast cancer patients to investigate the potential association between breast cancer and mtDNA encoding ATP synthase. For this research, a total of 13 breast cancer tissue samples were collected. The primary objective was to sequence the *ATP6* gene from these samples and compare it with the *ATP6* gene sequences from control blood samples (n=33) to determine whether any polymorphisms exist that are positively or negatively associated with breast cancer. Specifically, this study aims to identify various mutations in the *MT-ATP6* gene in breast cancer patients, evaluate their association with breast cancer, and predict their impact on protein structure.

Materials and Methods

Sample Collection

Tissue samples from 13 breast cancer patients were collected, typically preserved in a container with a water solution immediately after surgical removal. Blood samples from a total of 33 healthy individuals were collected for comparison. Prior to DNA extraction, tissue samples were stored at -20°C. Within three months of sample collection, DNA extraction was completed. A group of people aged 30 to 65 years had been carefully selected for the research. They were all citizens of Bangladesh and belonged to the same ethnic and national group. Written informed consent was taken from each patient, and ethical approval was obtained from the ethical committees of their respective hospitals. This study would not conclusively categorise the identified variations as somatic (tumor-specific) or germline (inherited) due to the use of patient tumour tissue (not matched normal tissue or germline DNA) and healthy control blood. The findings are consistent with mitochondrial variations present in tumour tissue.

DNA isolation and PCR amplification

To isolate the total genomic DNA, the standard phenol-chloroform extraction method was used. The extracted DNA was visualized by 1% (w/v) agarose gel electrophoresis. DNA was quantified and it's purity was determined with a NanoDrop® spectrophotometer. To amplify the *MT-ATP6* gene, 13F (5′-TTTCCCCCTCTATTGATCCC-3′) and 13R (5′-GTGGCCTTGGTATGTGCTTT -3′) primer pairs were chosen by the literature mining (Rieder *et al.*, 1998). The annealing temperature of the primer pairs is 58 °C, and the amplicon size is 816 bp. For each reaction, 30 ng of template DNA (1 µL) was added with PCR master mixture containing 1 µL of 10× PCR buffer, 0.8 µL of 25 mM $MgCl_2$, 0.25 µL of 3.2 pM forward and reverse primers, 0.6 µL of 10 mM deoxynucleotide

triphosphates (dNTPs) 0.3 µL of 1 U TaqMan™ DNA Polymerase (Applied Biosystems, USA), and 5.8 µL of nuclease-free water, making up a total volume of 10 µL. The polymerase chain reactions (PCR) were carried out in the ProFlex PCR system (Applied Biosystems). The PCR amplification of the specific region of mtDNA was performed under the following thermal cycling conditions: initial denaturation at 95 °C for 5 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, and elongation at 72 °C for 2 min; and final extension at 72 °C for 7 min. The PCR products were purified using FavorPrep™ GEL/PCR Purification Mini Kit (FAGCK 0001, Favorgen Biotech Corp.) by following the manufacturer's protocol.

Genotyping and MT-ATP6 gene mutation detection

The PCR amplicons were cycle sequenced using BigDye® Terminator v3.1 sequencing kit (Applied Biosystems). Applied cycling condition of the sequencing PCR consisted of initial denaturation at 96 °C for 3 min; followed by 30 cycles of 96 °C for 10 s, 58 °C for 5 s, and 60 °C for 4 min. After that, cycle sequencing products were purified by ethanol/EDTA/sodium acetate precipitation and analyzed by capillary electrophoresis in the SeqStudio™ Genetic Analyzer (Applied Biosystems). Sanger sequencing was used on bulk DNA. On bulk DNA, Sanger sequencing was employed. As a result, only high-level variants likely homoplasmic or near-homoplasmic were consistently found. Low-level heteroplasmy was not quantified, nor was a sequencing depth threshold established for variant detection, which is a key limitation in mtDNA analysis. The mtDNA sequences were aligned and compared with the revised Cambridge Reference Sequence (rCRS) (NCBI Reference Sequence: NC_012920.1) using the DNA Baser Assembler v. 5.21.0, Chromas v. 2.6.6, and BioEdit v. 7.0.9.0, were used to view, align, and edit the sequence chromatograms. Genetic variations in *MT-ATP6* gene were identified by the ClustalW implementation in MEGA-X (http://megasoftware.net). Additional data about the detected variants of the *MT-ATP6* gene were retrieved from Mitomap (www.mitomap.org).

Assessment of pathogenic effects of the mutations

The identified mutations were considered disease-causing if the nucleotide changes resulted in nonsynonymous mutations like missense, nonsense, and frameshift. In this study, in silico tools, namely SIFT (https://sift.bii.a-star.edu.sg/), PROVEAN (http://provean.jcvi.org/index.php) MutationTaster (https://www.mutationtaster.org/), PMut (http://mmb.irbbarcelona.org/PMut/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) were used to evaluate the potential pathogenicity of the MT-ATP6 gene.

Results

Multiple polymorphisms in the *ATP6* genes were found by sequencing analysis in both patients and healthy participants. Sequence analysis of patient samples revealed a total of five mutations in the *MT-ATP6* gene. All five mutations have been documented in the human mitochondrial genome database (http://www.mitomap.org). The most notable finding is the m.9094C>T (L190F) variant (Figure 1), which was detected in only 2 of the 13 (15.38%) cancer tissue samples and was absent in all 33 healthy control blood samples.

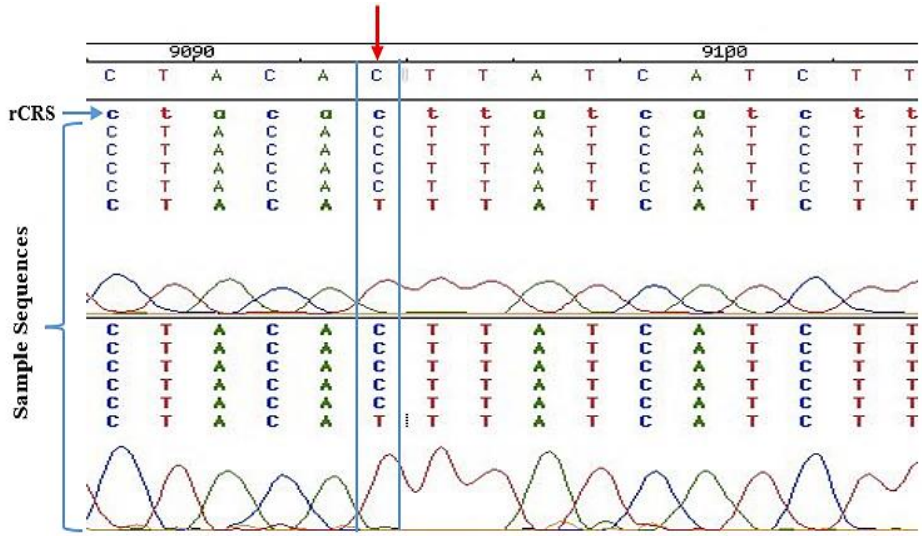

Figure 1. Partial chromatogram of *MT-ATP6* sequences aligned with the revised Cambridge Reference Sequence (rCRS). m.9094 C>T mutation in cancer patient's tissue sample. The red arrow shows the mutated region.

A total of 5 different polymorphisms are detected in 13 breast cancer patients' tissue samples and 6 different polymorphisms in 33 control blood samples. Five polymorphisms found in breast cancer patients' samples are A8701G, T8772C, G8790A, A8860G, C9094T. No formal association statistics were calculated because of the small sample size (n=13), but the raw frequencies are reported. The study focused solely at the three nonsynonymous mutations because of their potential functional impacts. Table 1 summarises the distribution and features of the nonsynonymous variations.

Table 1. Frequency and *In Silico* Pathogenicity of Nonsynonymous *MT-ATP6* Variants in Patient and Control Cohorts

Polymorphism	Amino Acid Change	Patient Frequency (n=13	Control Frequency (n=33)	PolyPhen-2 Score and Impact	Mutation Taster	HOPE
m.9094 C>T	L120F	2 (15.38%)	0 (0%)	Possibly damaging 0.855	Polymorphism and Protein features might be affected	Affect the contacts with the lipid membrane and might lead to bumps.
m.8701 A>G	T58A	4 (30.77%)	23 (69.70%)	Benign 0.002	Polymorphism and no protein features affected	Might lead to loss of interactions and also can result in loss of hydrogen bonds and/or disturb correct folding.
m.8860 A>G	T112A	11 (84.61%)	33 (100%)	Benign 0.00	Polymorphism and Protein features might be affected	The mutated residue is located in a domain that is important for the main activity of the protein. Mutation of the residue might result in loss of hydrogen bonds and/or disturb correct folding.

Two out of thirteen (15.38%) patient samples had the C9094T variant. A8701G and A8860G polymorphisms were found in both of our analyzed control and cancer patients' samples. In 30.77% cases with breast cancer, A8701G was polymorphic. Additionally, the proportion of A8860G polymorphism was 84.61%, indicating a phylogenetic mutation. Here, the m.8701A>G mutation was found in 23 out of 33 controls (69.70%), and the m.8860A>G mutation was found in all 33 controls (100%). The synonymous variants (m.8772T>C and m.8790G>A) were considered neutral polymorphisms and were not further analyzed for disease association. Here, the nucleotide bases Adenine, Thymine, Guanine, and Cytosine are denoted by the letters A, T, G, and C, respectively. The frequency of *MT-ATP6* gene mutations in patients with breast cancer is shown in Figure 2.

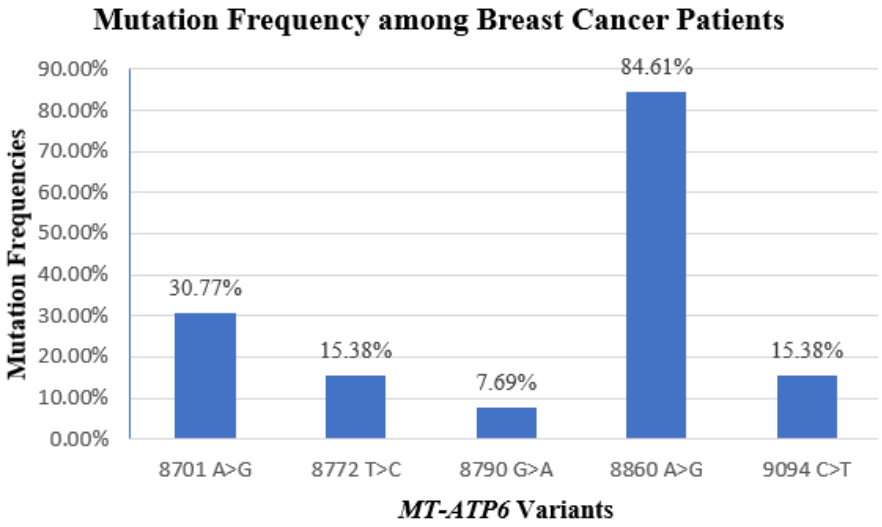

Figure 2. Frequency of *MT-ATP6* gene mutation among breast cancer patients. Here, X axis represents *MT-ATP6* nucleotide variants and Y axis represents mutation frequencies.

PolyPhen-2 software (Polymorphism Phenotyping v2) was used to assess an amino acid substitution in the encoded protein. The structure and functions of the *ATP6* protein were assessed using simple physical and comparative considerations. The analysis predicted that this variant (m.9094C >T) is "possibly damaging" with a score of 0.855 (Figure 4). The second prediction performed by Mutation Taster program indicated that this substitution might affect the protein structure. According to the HOPE program, this mutation has caused the protein to enlarge, which has an impact on the interactions with the lipid membrane and may result in bumps.

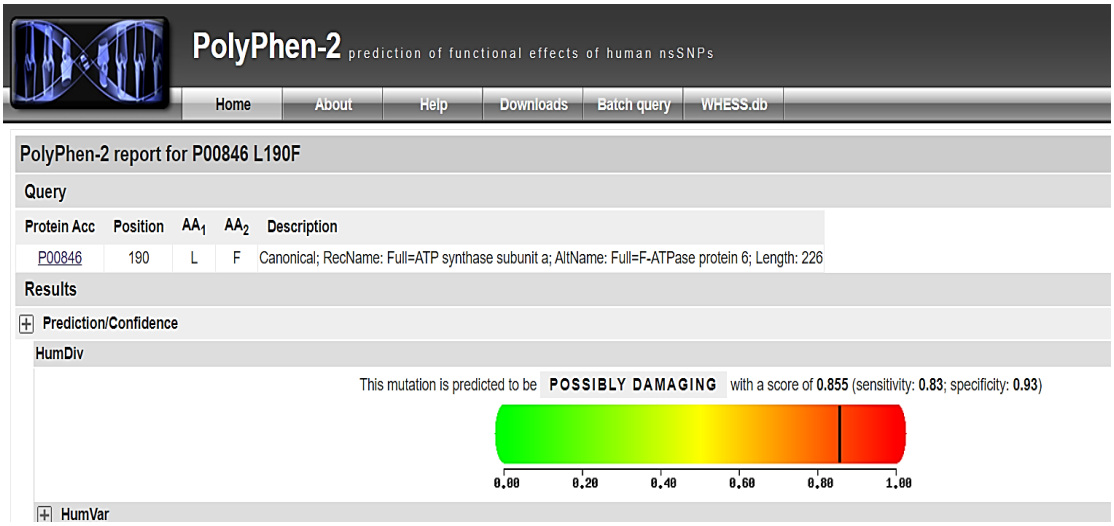

Figure 4. Polyphen-2 analysis prediction for m.9094C >T variation indicates a damaging effect on the translated protein.

The mutant residue is located next to another equally important domain and in a domain that is essential to the function of protein. The functioning of the protein might be impacted by this mutation as it might interfere with the interaction between these domains. Protein sequences were obtained from UniProtKB (P00846) and the Dynamut webserver provided the three-dimensional structure of the protein, which was used to ascertain the structural implications of the modified amino acids. For the changes, m.9094 C>T (L190F), The mutant residue was bigger. There was an addition of water mediated hydrogen bond (in orange dots) with the adjacent residue in Figure 5. This might bring change in the protein structure too.

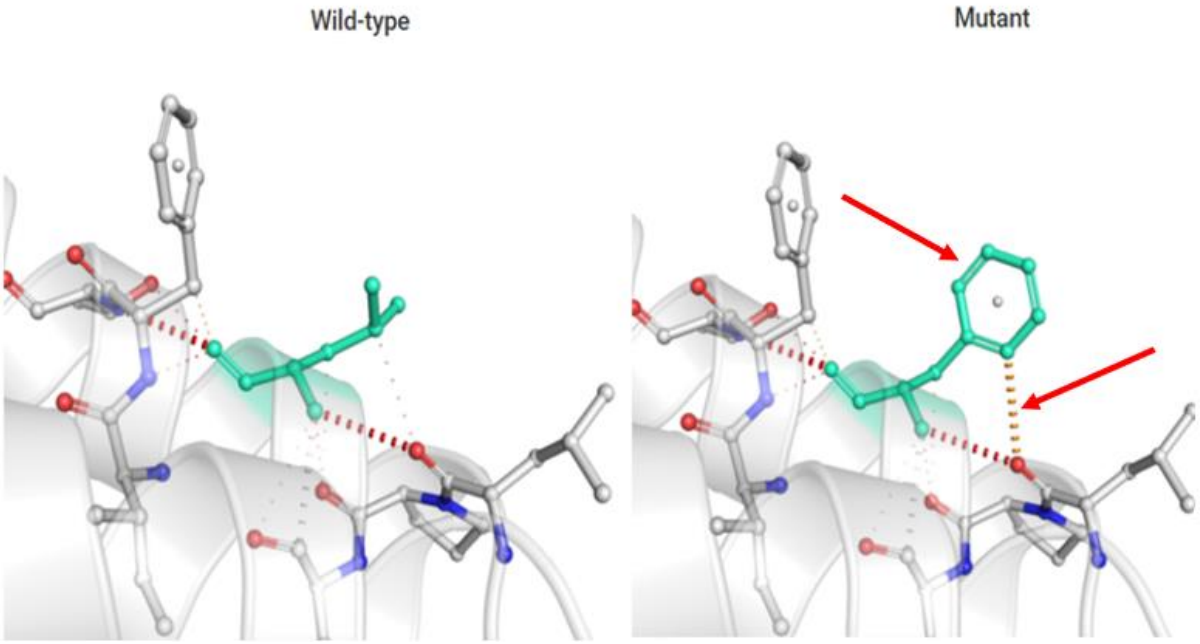

Figure 5. Interatomic interaction variations due to m.9094 C>T (L190F) variation. With the nearby new residue in the mutant type, indicated by the red arrow, a new water-mediated hydrogen bond is formed in this instance.

Discussion

Organelles called mitochondria have a morphology that is related to energetic states and cellular viability (Li *et al.*, 2017). The handling of programmed cell death, calcium homeostasis, reactive oxygen species (ROS) generation, and ATP production are all correlated with the morphology of the mitochondria (Wai and Langer, 2016).The mitochondrial genome is only inherited maternally. mtDNA is subject to a mutation rate 100-times higher than that of the nuclear genome because the mtDNA repair systems are less robust (more error-prone) than nuclear DNA repair systems (Maria Jackson, Leah Marks, 2018). Another reason is the internal environment of mitochondria has reactive oxygen molecules released as a by-product during oxidative phosphorylation in cells and these can damage mtDNA (Meyerson, Van Stavern and McClelland, 2015). The polypeptides encoded by mtDNA are part of the five complexes involved in oxidative phosphorylation (OXPHOS). The complexes can be divided into two parts. Complexes I–IV are part of the electron transport chain, while complex V is

involved in adenosine triphosphate (ATP) generation (Koopman *et al.*, 2013). ATP synthase is a key enzyme in the OXPHOS process. Generalized decreases in the content of human ATP synthase or alteration of its structure and function are associated with several mitochondrial diseases. More than 150 distinct genetic mitochondrial diseases characterized by a diminished OXPHOS capacity have been described (Houštěk *et al.*, 2006).Qualitative defects of ATP synthase are caused by mutations in one of the mtDNA encoded ATP synthase subunits. For example, maternally transmitted mutations in the *ATP6* gene are heteroplasmic and the pathogenic phenotype correlates with their mutation load (Carelli *et al.*, 2002). At a high mutation load, up to approximately 95%, the mutation (m.8993 T > G) manifests mitochondrial diseases such as neuropathy, ataxia, and retinitis pigmentosa (NARP) or as fatal encephalopathy known as Leigh syndrome (DiMauro and Schon, 2001). In a study, 5 changes in the *ATP8* gene (10/50) and 8 changes in the *ATP6* gene were found in 36 out of 50 examined breast cancer cell samples. The majority of them were missense-type homoplasmic alterations. A8439C, G8858C, C9130G, and T9119G are the four changes that had not previously been documented in the literature. The detected polymorphisms and mutations, particularly those of the missense type, can impact mitochondrial functions, particularly when it comes to the protein's conservative domain. One key step in the development of cancer may be the substitution of mutant mtDNA for "wild-type" mtDNA (Grzybowska-szatkowska *et al.*, 2014).

Here, a case-control study was conducted to assess the polymorphisms in the mitochondrial *ATP6* gene as potential risk factors for breast cancer in women from Bangladesh. Our investigation identified 6 polymorphisms in control samples and 5 polymorphisms in cancer patients. Both cancerous and control samples contain A8701G, A8860G polymorphisms.

The most significant finding of this pilot study is C9094T polymorphism which has not been previously reported in any breast cancer studies. C9094T polymorphism was not detected in any control samples, but 02 breast cancer samples out of thirteen (15.38%) samples contained this specific polymorphism. In this mutation, wild-type residue Leucine which was present at the 190th position (in the domain) of the protein was replaced by Phenylalanine. This amino acid change may alter ATPase enzyme activity and facilitate cancer. While C9094T polymorphism was linked to primary ovarian insufficiency according to one earlier study (Venkatesh *et al.*, 2010), its novel association with breast cancer represents an important contribution of this study. Given that the C9094T mutation is absent in healthy individuals and present in breast cancer patients, it is possible that this mutation is the cause of an enzyme malfunction that controls the interaction of enzyme proteins during cofactor binding and transportation. Therefore, in the pathophysiology of breast cancer, there may be a change in cellular chemistry due to the accumulation of hazardous compounds or the reduction of an essential component. This previously unknown variant, m.9094C>T, was found only in a subset of breast cancer tissue samples from this pilot cohort, suggesting that it may be a candidate factor for further, extensive research into Bangladeshi women's susceptibility to breast cancer.

According to a study, the intracellular calcium dynamics were hampered by the A8701G mtSNP (Kato, 2008). Certain diseases' pathogenesis has also been linked to the mutation (Wallace, Brown and Lott, 1999). It has been shown that the *ATP6* gene, which codes for the ATP synthase protein, contains a nucleotide change from A to G at position 8701, which decreases ATP synthesis and seriously compromises the stability or assembly of the ATP synthase (Wallace DC, Fan W, 2010). Both the examined control and cancer patient samples in this study had the m.8701A>G mutation. Four out of thirteen (30.77%) patients with breast cancer had this variation. This nonsynonymous mutation altered the amino acid Threonine to Alanine at position 59. MT-ATPase6 gene, particularly the A8812C and A8701G polymorphisms, can be biomarkers for breast cancer diagnosis, according to one study that demonstrated this by examining blood samples from patients with breast cancer (Islam *et al.*, 2021). However, it is quite likely that m.8701A>G is a common benign polymorphism in the study population rather than a major risk factor for breast cancer, given the variant was found in 23 out of 33 (69.70%) of our healthy control samples. Therefore, despite reports of its pathogenicity, the high frequency in our controls suggests that its role as a primary breast cancer risk factor in this population requires further investigation, including functional assays and correction for underlying haplogroup structure.

Both patients and healthy controls had the same mutation (m.8860A>G). The m.8860A>G variant (T112A) was highly prevalent in this cohort, found in 11 out of 13 (84.61%) of patient samples and 100% (33/33) of controls. Moreover, at position 112, this nonsynonymous mutation changed the amino acid threonine to alanine. The high frequency of this well-established phylogenetic variant indicates that it represents a common maternal lineage within the research population, which restricts its use as a distinct risk factor. However, earlier research indicates that it is a variation of interest. A8860G transition was found in a significant percentage of patients with hypertrophic cardiomyopathy (HCM), which draws the question of whether this polymorphism is connected to the disease as a secondary effect (Houshmand *et al.*, 2011). It was discovered in another population to be one of the potential mutations for the M7 haplogroup-related MELAS illness (Choi *et al.*, 2008). This variation may still contribute to additional mutations in mtDNA and nDNA (Ghaffarpour *et al.*, 2014) even though it is situated in a poorly conserved protein region and has no effect determined by the PolyPhen-2 program but properties of the amino acid were affected which was determined by HOPE tool in our study. According to one study, 70% of cases of breast cancer are linked to the A8860G polymorphism. Protein structures are impacted by the polymorphism A8860G, which results in a shift in the polar threonine at position 112 to a non-polar alanine (Grzybowska-szatkowska *et al.*, 2014).

Conclusion

Understanding the underlying mechanisms of a disease is crucial for proper diagnosis, treatment, and classification. In this pilot study, *MT-ATP6* gene variations were found in tissue samples from Bangladeshi women with breast cancer. In order for the physiological function of the breast to function normally, healthy mitochondria must produce enough energy and fewer reactive oxygen species. *MT-ATP6* gene mutations that affect mitochondrial assembly or function directly or indirectly can lead to breast cancer. Only two of the thirteen

patient samples had the m.9094C>T variation, which has not previously been reported in the literature on breast cancer. The absence of this novel mutation in healthy controls and predictions from structural modelling that point to protein disruption highlight its importance. However, the observed frequency pattern of m.9094C>T can only be classified as a preliminary candidate factor due to the study's significant sample size constraint (n=13). In order to determine the ultimate relevance of this variation in the development of breast cancer, more population-based and functional investigations are required. This finding requires further investigation. Additionally, this study finds that this mutation is a strong candidate for the development of breast cancer in Bangladeshi women. The sample size (n=13) in this study is severely limited. Larger cohort studies are needed to validate the findings and offer strong epidemiological confirmation. As a result, nonsynonymous mutations in the ATP6 gene more especially, m.9094C>T, remain genetic factors that should be studied as potential susceptibility candidates for breast cancer in Bangladeshi women.

Study Strength

Our pilot analysis suggests that this novel variation is a strong candidate genetic factor contributing to breast cancer susceptibility in Bangladeshi women based on the discovery of m.9094C>T alone in patient samples. The use of several in silico prediction methods (PolyPhen-2, Mutation Taster, HOPE, DynaMut), all of which support its predicted pathogenicity and ability to disrupt protein structure. This study establishes a foundation for focused genetic screening by supplying preliminary data for upcoming, extensive research in an under-represented group (Bangladeshi women).

Study Limitations and Future Directions

Variants like m.8701A>G and the highly prevalent m.8860A>G are frequently used as haplogroup markers in mitochondrial DNA studies. Formal haplogroup assignment and population structure correction was not carried out. As a result, even while we find differences in variant frequencies, we cannot exclude the potential that those differences are caused by underlying maternal lineage variation rather than a correlation with the risk of breast cancer. Due to the limited size of the patient group, our current findings are only preliminary. In subsequent investigations, a substantial increase in the number of samples should be used in order to obtain a definitive result. Furthermore, the entire mitochondrial genome should be sequenced in order to properly compare the maternal lineages (haplogroups) of the two groups and ensure that the observed differences are due to cancer rather than just normal population variation.

CRediT authorship contribution statement

Marufa Akter Mim: Data curation, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing – original draft, Writing – review & editing. **Sumaiya Farah Khan:** Methodology, Resources, Supervision, Writing – review & editing. **Md. Aminul Islam:** Investigation, Methodology, Visualization. **Rokeya Begum:** Methodology, Supervision, Writing – review & editing. **Gazi Nurun Nahar Sultana:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of Competing Interest

The authors disclose that they have no conflict of interest related to this article.

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Data availability

The original contributions of the study are included in the article. Further inquiries can be directed to the corresponding authors.

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