The mitochondrial ATPase6 gene is more susceptible to mutation than the ATPase8 gene in breast cancer patients

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Abstract

Background: Breast cancer is the most common malignancy in women throughout the world. Mitochondria play important roles in cellular energy production, free radical generation and apoptosis. Identification of mitochondrial DNA mutations and/or polymorphisms as cancer biomarkers is rapidly developing in molecular oncology research.

Methods: In this study, the DNA alterations of the mitochondrial ATPase 6 and 8 genes were investigated in 49 breast cancer patients using PCR amplification and direct DNA sequencing on mtDNA. A possible association between these variants and tumorigenesis was assessed. Furthermore, the impact of non-synonymous substitutions on the amino acid sequence was evaluated using the PolyPhen-2 software.

Results: Twenty eight distinct somatic mitochondrial DNA variants were detected in tumor tissues but not in the corresponding adjacent non-tumor tissues. Among these variants, 9 were observed for the first time in breast cancer patients. The mtDNA variants of A8384 (T7A), T8567C (I14T), G8572A (G16S), A9041G (H172R) and G9055A (A177T) showed the most significant effects probably due to damaging changes to the resulting protein. Furthermore, non-synonymous amino acid changing variants were more frequent in the ATPase6 gene compared to the ATPase8 gene.

Conclusion: Our results showed that the ATPase6 gene is more susceptible to variations in breast cancer and may play an important role in tumorigenesis by changing the energy metabolism level in cancer cells.

Keywords: MtDNA, ATPase6, ATPase8, Breast cancer
non-tumor tissues in breast cancer patients. We also investigated the correlation between the variants in these genes and the clinico-pathological features in these breast cancer patients.

Materials and Methods
Tumor tissue collection
Forty-nine breast cancer patients (34–75 years of age with a median age of 52.43 years) took part in this study. The patients were referred to the National Cancer Institute (NCI) at Imam Khomeini Hospital Complex, Tehran, Iran, from Oct. 2007 to Oct. 2009. Tumor tissue and adjacent non-tumor tissue samples were obtained from the Iranian National Tumor Bank (INTB) at NCI. Each specimen was immediately frozen following resection and stored at −80°C until DNA extraction. The pathologic changes in tumor samples were confirmed by two expert pathologists as adenocarcinomas according to the American Joint Committee on Cancer [22]. None of the patients received chemotherapy or radiotherapy treatment before they underwent surgery. All patients were informed on the aim of the study and signed an informed consent approved by the INTB Ethical Committee for the genetic analysis.

DNA extraction and PCR
In order to identify the alterations in the mtDNA ATPase6 and ATPase8 genes, PCR-sequencing was performed as described previously with some modifications [23]. Total genomic DNA was extracted from fresh tumor samples containing at least 90% neoplastic cells, as well as their adjacent non-tumor tissues, using the QIAamp Mini Kit (USA). The sequences of the primers were as follows: F-ATPase: 5′- CTACGGTCAATGCTCTGAAA -3′ (Accession No. NC_012920.1, 8161–8180). R-ATPase: 5′- TACT ATATGATAGGCATGTGA-3′ (9219–9239). PCR amplification was performed using a ready-to-use PCR master mix (Sinaclon LTD, Tehran, Iran) in a final volume of 50 μl containing 5 ng of genomic DNA and 0.10 μM of each primer in a MJ Mini Gradient Thermal Cycler PTC-1148 (Bio-Rad, USA). PCR amplification was carried out with the following program: a 5-min pre-PCR incubation step at 95°C, 35 cycles of 95°C for 60 s, annealing temperature at 55°C for 1 min and 72°C for 2 min, and a final extension of 72°C for 10 min. The amplified fragment (1078 bp) was observed on 1.5% agarose gel.

Sequencing analysis
The PCR products were sequenced using the previously reported primers [23] on a ABI Prism 3700 automated sequencer (Applied Biosystems, USA). Sequence analysis was carried out using the FinchTV 1.4 software (Geospiza, Inc., USA). The sequences were compared to the human mtDNA reference sequence (Gene Bank ID: NC_012920.1) using the BLAST sequence analysis tool (NCBI, Bethesda, USA). The Mitomap database was used to identify mitochondrial genome sequence variants.

Prediction of pathogenicity by protein modeling analysis
The impact of non-synonymous (coding) substitutions in the resulting protein was assessed using PolyPhen-2 (v. 2.2.2) software, a tool for predicting the possible impact of an amino acid substitution variant on the structure and function of the corresponding protein, which is interpreted as benign and damaging effects [24].

Statistical analysis
The correlation between each alteration in the ATPase6 and ATPase8 genes in tumor samples and their adjacent normal tissue were analyzed by Fisher’s exact test using statistical package SPSS (v.16.1). The correlation between the groups was considered statistically significant if the p-value was less than 0.05. Additionally, for each variant the odds ratio (OR) and 95% confidence interval (95% CI) were calculated in order to determine its association to the increased risk in breast cancer patients. The association between mtDNA alteration and clinico-pathological characteristics of breast cancer patients with more than one missense mutation was evaluated using One-way ANOVA analysis.

Results
In this study, the complete sequences of the ATPase6 and 8 genes of 49 tumor tissues and adjacent non-tumor tissues were analyzed in a cohort of breast cancer patients. The clinico-pathological characteristics of the patients are summarized in Table 1. From 49 breast cancer cases, 28 mtDNA variants were found in tumor tissues, which were not present in their adjacent normal tissues. From 28 variants, 23 (82.14%) were found in the ATPase6 gene and the remaining 5 sequence variants were detected in the ATPase8 gene. All cases showed variants in the ATPase6 gene, whereas only 8.16% (4 of 49) cases had variants in the ATPase8 gene. Among 28 mtDNA alterations, 26 were at the homoplasmic state and the remaining 2 variants were at the heteroplasmatic state (Table 2). However, there was no significant correlation (P > 0.05) between the ATPase6 and 8 gene variants and the clinico-pathological characteristics of the patients (Table 1). Our results indicated that the A8860G variant was detected in 100% of tumor tissue samples compared to adjacent non-tumor tissues, showing that this alteration may significantly increase breast cancer risk (P < 0.05). However, the patients’ survival was shorter in cases with more than one mtDNA non-synonomous ATPase variant compared to the patients with only one mtDNA non-synonomous ATPase variant (A8860G) (P =0.051, Table 1).

Furthermore, the damaging impact of an amino acid substitution on the structure and function of the ATPase6
Table 1 Characterization of clinico-pathological parameters and the frequency of cases with more than one somatic mtDNA (ATPase6/8) mutation in breast cancer patients

| Variable                              | Patients with more than one somatic mtDNA (ATPase6/8) mutation | OR (95% CI)* | P value |
|---------------------------------------|----------------------------------------------------------------|-------------|---------|
| Frequency of patients in each group   |                                                                  |             |         |
| Total number of patients              | 49                                                              |             |         |
| Age at diagnosis (Yrs)                |                                                                  | 1.482(0.403-5.451) | 0.746  |
| <50                                   | 19(42.2)                                                        | 5(26.3)     |         |
| ≥50                                   | 26(57.8)                                                        | 9(34.3)     |         |
| Histological grade                    |                                                                  |             | 0.121   |
| I                                     | 13(29.5)                                                        | 1(7.7)      |         |
| II                                    | 24(54.5)                                                        | 9(37.5)     |         |
| III                                   | 7(15.9)                                                         | 3(42.9)     |         |
| TNM(AJCC) stage                       |                                                                  |             | 0.680   |
| I                                     | 3(6.7)                                                          | 1(33.3)     |         |
| II                                    | 10(22.2)                                                        | 2(20)       |         |
| III                                   | 3(6.7)                                                          | 0(0)        |         |
| IV                                    | 64(64.4)                                                        | 11(37.9)    |         |
| Tumor size(cm)                        |                                                                  |             | 0.889   |
| <2                                    | 5(11.1)                                                         | 2(40)       |         |
| 2-5                                   | 30(66.7)                                                        | 9(30)       |         |
| >5                                    | 10(22.2)                                                        | 3(30)       |         |
| Lymph node status                     |                                                                  |             |         |
| Negative                              | 12(30.8)                                                        | 4(33.3)     |         |
| Positive                              | 27(69.2)                                                        | 10(37)      |         |
| Lymphatic invasion                    |                                                                  |             | 1.000   |
| Negative                              | 18(47.4)                                                        | 6(33.3)     |         |
| Positive                              | 20(52.6)                                                        | 7(35)       |         |
| Vascular invasion                     |                                                                  |             | 1.000   |
| Negative                              | 13(32.5)                                                        | 4(30.8)     |         |
| Positive                              | 27(67.5)                                                        | 10(37)      |         |
| Estrogen receptor status              |                                                                  |             | 0.222   |
| Negative                              | 8(17.4)                                                         | 4(50)       |         |
| Positive                              | 38(82.6)                                                        | 10(26.3)    |         |
| Progesterone receptor status          |                                                                  |             | 1.000   |
| Negative                              | 22(48.9)                                                        | 7(31.8)     |         |
| Positive                              | 23(51.1)                                                        | 7(30.4)     |         |
| Her-2/neu receptor                    |                                                                  |             | 1.000   |
| Negative                              | 30(65.2)                                                        | 9(30)       |         |
| Positive                              | 16(34.2)                                                        | 5(31.3)     |         |
| PS3                                   |                                                                  |             | 0.526   |
| Negative                              | 23(53.50)                                                       | 8(34.8)     |         |
| Positive                              | 20(46.5)                                                        | 5(25)       |         |
| Cancer metastasis                     |                                                                  |             | 0.191   |
| Negative                              | 18(38.3)                                                        | 3(16.7)     |         |
| Positive                              | 29(61.7)                                                        | 11(37.9)    |         |
| Overall survival (5 yr%)              | 18 of 41(43.9)                                                  | 3(16.7)     |         |

*OR; Odds ratio, (95% CI); confidence interval reflects a significance level of 0.05.
| No | Locus       | Allele | Nucleotide position | Nucleotide change | Amino acid change* | Mutation status** | Frequency | OR; 95% CI *** | P Value | Reference                                                                 |
|----|-------------|--------|---------------------|------------------|-------------------|-------------------|-----------|-----------------|---------|---------------------------------------------------------------------------|
| 1  | MT-ATPase8  | A8384G | 8384                | A-G              | T7A               | Hm                 | 1         | 1.021; 0.980-1.063 | 0.315   | NR [**]                                                                   |
| 2  | MT-ATPase6  | T8542C | 8542                | T-C              | F6L               | Hm                 | 1         | 1.021; 0.980-1.063 | 0.315   | NR [**]                                                                   |
| 3  | MT-ATPase8  | T8542C | 8542                | T-C              | C59C              | Hm                 | 1         | 1.021; 0.980-1.063 | 0.315   | NR [**]                                                                   |
| 4  | MT-ATPase6  | G8557A | 8557                | G-A              | A11T              | Hm                 | 1         | 0.980-1.063      | 0.315   | Colonic crypts cancer [34], Breast cancer [27,28]                         |
| 5  | MT-ATPase6  | G8557A | 8557                | G-A              | L64L              | Hm                 | 1         | 0.980-1.063      | 0.315   | Alzheimer's disease [40]                                                 |
| 6  | MT-ATPase6  | T8567C | 8567                | T-C              | I14T              | Hm                 | 1         | 0.980-1.063      | 0.315   | Parkinson's disease [42]                                                 |
| 7  | MT-ATPase6  | T8567C | 8567                | T-C              | S68P              | Hm                 | 1         | OR 1.021; 0.980-1.063 | 0.315   | Parkinson's disease [49]                                                 |
| 8  | MT-ATPase6  | G8572A | 8572                | G-A              | G16S              | Hm                 | 1         | 0.980-1.063      | 0.315   | Thyroid tumor [50]                                                       |
| 9  | MT-ATPase6  | G8572A | 8572                | G-A              | G69S              | Hm                 | 1         | 0.980-1.063      | 0.315   | Colonic crypts cancer [34]                                                 |
| 10 | MT-ATPase6  | C8684T | 8684                | C-T              | T53I              | Hm                 | 1         | 0.980-1.063      | 0.315   | Multiple Sclerosis [51], Ataxia telangiectasia [21], Huntington [52], Autism [53], Osteosarcoma [54], Colorectal adenomatous polyps [40] |
| 11 | MT-ATPase6  | T8697C | 8697                | T-C              | I24T              | Hm                 | 1         | 0.980-1.063      | 0.315   | Thyroid tumor [50], Multiple Sclerosis [51], Ataxia telangiectasia [21], Breast cancer [30], Colorectal adenomatous polyps [38], Osteosarcoma [54] |
| 12 | MT-ATPase6  | A8701G | 8701                | A-G              | T59A              | Hm                 | 2         | 0.984-1.063      | 0.153   | Thyroid tumor [50], Ataxia telangiectasia [21], Breast cancer [27,29], colorectal adenomatous polyps [38], Osteosarcoma [54] |
| 13 | MT-ATPase6  | T8777C | 8777                | T-C              | F117F             | Hm                 | 1         | 0.980-1.063      | 0.315   | NR [**]                                                                   |
| 14 | MT-ATPase6  | C8794T | 8794                | C-T              | H90Y              | Ht                 | 2         | 0.984-1.063      | 0.153   | Exercise Endurance/Coronary Atherosclerosis risk [32]                     |
| 15 | MT-ATPase6  | A8850G | 8860                | A-G              | T112A             | Hm                 | 49        | 0.000            |         | Colorectal cancer [36,38], Ovarian cancer [37], Breast cancer [27,29,34], Human glioma cells [33], Osteosarcoma [54], Leber's hereditary optic neuropathy [35] |
| 16 | MT-ATPase6  | T8877C | 8877                | T-C              | F117F             | Hm                 | 3         | 0.992-1.114      | 0.079   | Leber's hereditary optic neuropathy [55]                                  |
| 17 | MT-ATPase6  | T8881C | 8881                | T-C              | S119P             | Ht                 | 1         | 0.980-1.063      | 0.315   | NR [**]                                                                   |
| 18 | MT-ATPase6  | C8910T | 8910                | C-T              | F128F             | Ht                 | 2         | 0.984-1.105      | 0.153   | The southern belt of Siberia population [56]                              |
| 19 | MT-ATPase6  | G8950A | 8950                | G-A              | V142I             | Hm                 | 2         | 0.984-1.105      | 0.153   | Huntington [54], LDYT [57]                                                |
| 20 | MT-ATPase6  | G8994A | 8994                | G-A              | L156L             | Hm                 | 1         | 0.980-1.063      | 0.315   | Ataxia telangiectasia [21], Breast cancer [27], Colorectal adenomatous polyps [38] |
| 21 | MT-ATPase6  | C9003A | 9003                | C-A              | R159R             | Hm                 | 1         | OR 1.021; 0.980-1.063 | 0.315   | NR [**]                                                                   |
| 22 | MT-ATPase6  | A9007G | 9007                | A-G              | T161A             | Hm                 | 1         | 0.980-1.063      | 0.315   | Deafness associated [58]                                                 |
| 23 | MT-ATPase6  | A9041G | 9041                | A-G              | H172R             | Hm                 | 1         | 0.980-1.063      | 0.315   | NR [**]                                                                   |
| 24 | MT-ATPase6  | G9055A | 9055                | G-A              | A177T             | Hm                 | 3         | 0.992-1.114      | 0.079   | Colorectal cancer [36], Colorectal adenomatous polyps [38], Breast cancer [38,50], Non-muscle invasive bladder cancer [44], Osteosarcoma [54], Pancreatic cancer [43], Parkinson's disease protective factor [45] |
Table 2 Frequency of mtDNA ATPase 6/8 gene sequence alterations in 49 breast cancer patients (Continued)

|   | MT-ATPase6 | G9085A   | 9085 | C-T | P187S | Hm | 1   | 1.021; 0.980-1.063 | 0.315 | NR   |
|---|------------|----------|------|-----|-------|----|-----|------------------|-------|------|
| 25| MT-ATPase6 | G9085A   | 9085 | C-T | P187S | Hm | 1   | 1.021; 0.980-1.063 | 0.315 | NR   |
| 26| MT-ATPase6 | T9090C   | 9090 | T-C | S188S | Hm | 1   | 1.021; 0.980-1.063 | 0.315 | NR   |
| 27| MT-ATPase6 | T9148C   | 9148 | T-C | L208L | Hm | 1   | 1.021; 0.980-1.063 | 0.315 | NR   |
| 28| MT-ATPase6 | C9168T   | 9168 | C-T | F214F | Hm | 1   | 1.021; 0.980-1.063 | 0.315 | NR   |

Abbreviations:
*Missense mutations are in bold.
**Hm: Homoplasmic, Ht: Heteroplasmic.
***OR; Odds ratio, (95% CI); confidence interval reflects a significance level of 0.05.
****NR; Not reported in mitomap website.
and 8 proteins was predicted using PolyPhen-2 software (Table 3). The mtDNA variants A8384 (T7A), T8567C (I14T), G8572A (G16S), A9041G (H172R) and G9055A (A177T) showed significant effects on the resulting protein. Moreover, there was no significant association between mtDNA alterations and the clinico-pathological characteristics of breast cancer patients.

**Discussion**

The identification of mitochondrial DNA mutations and/or polymorphism patterns is rapidly developing in the field of molecular oncology. A large number of somatic mutations in the mitochondrial genome have been recently reported in different types of cancers including breast, colon and ovarian cancers [5,6]. These molecular markers may have potential implication in cancer research.

Mitochondrial complex V genes play an important role in ATP production [25] and the apoptosis pathways [5]. The contribution of mtDNA complex V variants in cell transformation, elevated ROS production, and tumor progression has been described previously [26]. Moreover, efficient programmed cell death needs the molecular machinery of ATP synthase [27].

The ATPase6 gene, one of the complex V genes, contributes to mtDNA maintenance [25]. Furthermore, the ATPase8 variants have been detected in rat and human

**Table 3 Impact of non-synonymous* (coding) substitutions on the ATPase6 and 8 genes**

| Non-synonymous coding substitutions | Damaging score | Benign score |
|-----------------------------------|---------------|--------------|
| **ATPase 6 gene**                 |               |              |
| T8542C (F6L)                      | 0.976         | 0.917        |
| G8557A (A11T)                     | 0.002         | 0.004        |
| T8567C (I14T)                     | 0.617         | 0.280        |
| G8572A (G16S)                     | 0.895         | 0.498        |
| C8684T (T531)                     | 0.005         | 0.005        |
| A8701G (T59A)                     | 0.002         | 0.005        |
| C8794T (H90Y)                     | 0.002         | 0.003        |
| A8860G (T112A)                    | 0.000         | 0.003        |
| T8881C (S119P)                    | 0.325         | 0.149        |
| G8950A (V142I)                    | 0.000         | 0.001        |
| A9007G (T161A)                    | 0.994         | 0.988        |
| A9041G (H172R)                    | 0.854         | 0.331        |
| G9055A (A177T)                    | 0.854         | 0.331        |
| **ATPase 8 gene**                 |               |              |
| A8384G (T7A)                      | 0.845         | 0.399        |
| T8542C (S68P)                     | 0.000         | 0.000        |

Non-synonymous variants were predicted as damaging and benign (With a score of 0 to 1) based on effects on the resulting protein using PolyPhen-2 software. The new variants are in bold format.

The identified mitochondrial DNA mutations and/or polymorphism patterns are rapidly developing in the field of molecular oncology. A large number of somatic mutations in the mitochondrial genome have been recently reported in different types of cancers including breast, colon and ovarian cancers [5,6]. These molecular markers have potential implications in cancer research.

Mitochondrial complex V genes play an important role in ATP production [25] and the apoptosis pathways [5]. The contribution of mtDNA complex V variants in cell transformation, elevated ROS production, and tumor progression has been described previously [26]. Moreover, efficient programmed cell death needs the molecular machinery of ATP synthase [27].

The ATPase6 gene, one of the complex V genes, contributes to mtDNA maintenance [25]. Furthermore, the ATPase8 variants have been detected in rat and human bladder cancer cells developed through chemically-induced carcinogenesis [28]. In a meta-analysis study carried out by Lu et al. a total of 55 variants, comprising 34 missense variants, 20 silent variants and 1 nonsense variant, were found in the ATPase6 gene and a total of 9 variants, including 2 missense variants and 7 silent variants, were detected in the ATPase8 gene [6].

In our study, among 28 distinct somatic variants, 18 were missense variants. Six variants have been previously reported in breast cancer [29-32] and 9 variants were new, including 4 missense and 5 silent variants which were observed for the first time in breast cancer patients. However, 17 variants were previously reported in other types of cancers and diseases (Table 2). In addition, more non-synonymous amino acid changing variants were found in the ATPase6 gene in comparison with the ATPase8 gene (Table 2). Our findings suggest that in breast cancer patients, the ATPase6 gene might be more susceptible to mutation in comparison to the ATPase8 gene. Shidara et al. and Kirches reported that ATPase6 gene variants may enhance cancer progression by preventing apoptosis pathways [6,33].

The functional role of ATPase6/8 variants in tumorigenesis is debatable; however, some of these variants are located in structurally and functionally important regions of the proteins. For instance, the A8860G alteration in ATPase6 has been reported as a polymorphism in different studies [29,31,34-40]. The frequency of this polymorphism has been reported to be from 79–91.66% in breast cancer patients [30,31], 75-100% in other types of cancers [38-40] and 92.85%-100% in neurodegenerative diseases [37,41-43]. Our results indicated that the A8860G variant was present in 100% of tumor tissue samples. Although this variant is located in a poorly conserved protein region with no impact on protein structure based on PolyPhen-2 software (Table 3), the variation may still contribute to other mtDNA and nDNA mutations.

The frequency of the G9055A variation has been reported as either 10.5% [28] or 18.6% [30] in breast cancer patients, indicating that it may increase the risk of breast cancer progression (OR: 3.03, 95% CI: 1.63-5.63, P = 0.0004) [32,44]. This variation is located in a conserved protein region with damaging impact on protein structure (Table 3). Furthermore, the frequency of this polymorphism has been reported as 10% in pancreatic cancer [45] and as 57% and 100% in tubular and villous adenomas, respectively [40]. Moreover, the high frequency of this variation has been shown in non-muscle invasive bladder cancer [46]. In addition, this polymorphism has been reported as a protective factor (OR: 0.46, 95% CI: 0.22-0.91, P = 0.03) in Caucasian women with Parkinson’s disease [47]. From these results, we propose that this mtDNA variation is unfavorable for neurodegenerative disorders, while having a protective effect on cancer. According to our results, the

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frequency of this variation was 6.12% (3 of 49) in tumor samples.

A study by Petros et al. indicated that T8993G in ATPase6 can contribute to tumor growth in nude mice [48]. Another study showed that cybrids with a T8993G or T9176 ATPase6 mutation in nude mice can contribute to tumor development by preventing apoptosis in the early stages of tumor growth [10]. However, we detected none of these mutations in breast cancer patients.

Based on our results, the existence of more than one missense variants in some cases with different clinico-pathological features (Table 4) suggests a synergistic effect of different mtDNA variations on carcinogenesis. In conclusion, the high frequency of ATPase6 gene alterations in breast cancer proposes that mitochondrial gene

Table 4 MtDNA alterations and clinico-pathological characteristics of breast cancer patients with more than one missense mutation

| Case | Locus | Variant | Frequency | Age (Yrs) | Grade | Tumor size (cm) | TNM* | Stage |
|------|-------|---------|-----------|-----------|-------|----------------|------|-------|
| BC-6 | ATPase6 | A8384G  | 4         | 44        | III   | 3              | T2N1M0 | II    |
|      | ATPase8 | T8542C  |           |           |       |                |      |       |
|      |        |         |           |           |       | T8542C         |      |       |
|      |        |         |           |           |       | A8860G         |      |       |
| BC-10| ATPase6 | A8860G  | 3         | 55        | III   | 2.5            | T2N0M1 | IV    |
|      |         | G8950A  |           |           |       |                |      |       |
|      |         | A9041G  |           |           |       |                |      |       |
| BC-19| ATPase6 | A8860G  | 2         | 42        | II    | 5              | T3N2M1 | IV    |
|      |         | G9055A  |           |           |       |                |      |       |
| BC-20| ATPase6 | A8860G  | 2         | 68        | III   | 1.8            | T2N1M1 | IV    |
|      |         | A9007G  |           |           |       |                |      |       |
| BC-21| ATPase6 | A8860G  | 2         | 43        | II    | 1.2            | T1N0M1 | IV    |
|      |         | G8950A  |           |           |       |                |      |       |
| BC-23| ATPase6 | A8860G  | 2         | 36        | III   | 10             | T3N3M1 | IV    |
|      |         | G9055A  |           |           |       |                |      |       |
| BC-25| ATPase6 | A8860G  | 2         | 50        | II    | 13             | T4N3M1 | IV    |
|      |         | C8794T  |           |           |       |                |      |       |
| BC-32| ATPase6 | A8860G  | 2         | 74        | I     | 5              | T3N1M1 | IV    |
|      |         | T8881C  |           |           |       |                |      |       |
| BC-35| ATPase6 | C8794T  | 2         | 75        | II    | 5              | T3N3M1 | IV    |
|      |         | A8860G  |           |           |       |                |      |       |
| BC-37| ATPase6 | A8860G  | 2         | 67        | II    | 2              | T1N0M0 | I     |
|      |         | G9095A  |           |           |       |                |      |       |
| BC-38| ATPase6 | A8701G  | 3         | 69        | II    | 3.5            | T2N3M1 | IV    |
|      |         | A8860G  |           |           |       |                |      |       |
|      |         | T9685C  |           |           |       |                |      |       |
| BC-39| ATPase6 | A8701G  | 2         | 59        | III   | 3              | T2N0M0 | II    |
|      |         | A8860G  |           |           |       |                |      |       |
| BC-41| ATPase6 | C8684T  | 2         | 51        | II    | 3.5            | T2N0M1 | IV    |
|      |         | A8860G  |           |           |       |                |      |       |
| BC-48| ATPase6 | T8567C  | 3         | 41        | II    | 4.5            | T2N3M1 | IV    |
|      | ATPase8 | T8567C  |           |           |       |                |      |       |
|      |         | A8860G  |           |           |       |                |      |       |

T1–T4: Size and/or extent of the primary tumor; NX: Regional lymph nodes cannot be evaluated; N0: No regional lymph node involvement (no cancer found in the lymph nodes); N1–N3: Involvement of regional lymph nodes (number and/or extent of spread); M0: No distant metastasis; M1: Distant metastasis (spread of cancer from one part of the body to another). There was no significant association between the mtDNA alterations and clinic-pathological characteristics of breast cancer patients.
variants may play an important role in tumorigenesis, changing the energy metabolism in cancer cells, and may be suggested as molecular biomarkers in breast cancer.

**Competing interests**

The authors declare that they have no competing interests.

**Authors’ contributions**

MG carried out the experimental procedures, participated in the sequence alignment and drafted the manuscript. RM, FF, and NM participated in the alignment and drafted the manuscript. BK wrote his constructive comments and edited the manuscript. MH conceived the project and supervised the study. All authors read and approved the final manuscript.

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