Identification of Mitochondrial DNA Gene Mutations in a Turkish Head and Neck Squamous Cancer Patient Group

Pelin MUTLU[1], Murad MUTLU[2], Serap YALCIN AZARKAN[3], Kemal KESEROGLU[2], Omer BAYIR[2], Guleser SAYLAM[2], Mehmet Hakan KORKMAZ[4]

[1] Ankara University, Biotechnology Institute, Department of Biotechnology Ankara
[2] University of Health Sciences, Dışkapı Yıldırım Beyazıt Training and Research Hospital, Department of Otorhinolaryngology, Ankara
[3] Ahi Evran University, Faculty of Arts and Science, Deparment of Molecular Biology and Genetics, Kirsehir
[4] Yildirim Beyazit University, Faculty of Medicine, Department of Otorhinolaryngology, Ankara, TURKEY

ABSTRACT

Besides the variations in genomic DNA, mitochondrial DNA (mtDNA) mutations are also responsible for many diseases, including cancer. MtDNA among individuals from the same and different ethnic groups is highly polymorphic. In the present study, we screened mitochondrial CO-1 and ND4 gene sequences of Turkish head and neck squamous cell carcinoma (HNSCC) patient group and examined the possible relationship between CO-1 and ND4 gene mutations and the development of the disease. Sixty unrelated Turkish HNSCC patients and thirty six unrelated healthy volunteers from different geographic regions of Turkey were included in this study. Total DNA isolation from blood samples were carried out and amplification of CO-1 and ND4 gene regions of mtDNA were performed by PCR reaction. PCR products were purified and sequencing was carried out by Sanger sequencing. Two mutations in CO-1 gene were identified and among them A6272d mutation was found as statistically significant in the studied HNSCC patient group with respect to control group ($p< 0.05$). Also differences in the alpha helix structure of the protein in patients with mutations were observed. Two mutations (A11251G and T11017TA) in the ND4 gene region were identified, however, none of these mutations were seem to be responsible for the disease development ($p>0.05$). As a conclusion, for the studied Turkish patient group we showed that A6272d mutation in CO-1 gene can be related to HNSCC development ($p< 0.05$). However, we cannot detect a statistically significant alteration between patient and control groups for ND4 gene ($p>0.05$). These differences can be due to ethnic differences.

Keywords: HNSCC, Mitochondrial DNA, CO-1 gene, ND4 gene, Turkish patient group

INTRODUCTION

Squamous cell carcinoma of the head and neck (HNSCC) is a heterogenous malignancy being the seventh cause of cancer related deaths worldwide.[1]

Several causes, such as smoking, chewing tobacco, alcohol intake, and HPV infection, cause HNSCC. Alterations in the genomic DNA structure is the main reason for developing HNSCC.[2,3] So far, several mutations have been identified in different proto-oncogenes that result in either oncogene activation or inactivation.[2,3] While several studies are dealing with alterations in the genomic DNA and HNSCC development, complex genomic molecular events are still not fully understood.[2,3]

Mitochondrial DNA (mtDNA) mutations are also responsible for many disorders, including cancer, in addition to changes in genomic DNA. Moreover to its major function in promoting aerobic respiration in eukaryotes, it has also been shown that mitochondria play a significant role in apoptotic cell death.[4,5] mtDNA codes for 22 tRNAs, 2 rRNAs and for 13 proteins.[6]

The respiratory chain and oxidative phosphorylation system subunits are all 13 proteins encoded by mtDNA (OXPHOS) including Complex I (NADH dehydrogenase; ND1-4, 4L, 5 and 6), Complex III (cytochrome b), Complex IV (cytochrome c oxidase; COI-III) and Complex V (ATPase synthase; ATPase6, ATPase8). Moreover, mtDNA contains a non-coding control region including D-loop.[7,8]

Mitochondrial ND4 and ND5 genes encode core subunits of Complex I of respiratory chain and oxidative phosphorylation system and being the main entry location of electrons in aerobic cellular respiration, Complex I defects could result in impairment of the entire process. In addition, NADH dehydrogenase complex has an important role in the metabolic processing of carcinogen products so alterations in the genes of this complex could contribute to mutagen accumulation.[9] Mutations in mt-ND4 and mt-ND5 have been previously associated with tumorigenesis in different types of cancer.[10,11] However, there is still limited information exists regarding mt-DNA mutations in head and neck cancer, in some of the studies ND4 gene mutations were reported.[9,12] Allegra et al., also showed that 50% of the studied head and neck cancer patients presented polymorphism of this gene.[9]

Cytochrome c oxidases (COI, COII and COIII), due to their antioxidant effects, have function to decrease the ROS levels. Thus, defects in those genes can cause a subsequent damage in mtDNA. Cytochrome c oxidase mutations have been shown in different types of cancers such as pancreatic, prostate, colon and thyroid.[13-15] The relation between cytochrome c oxidase mutations and HNSCC susceptibility was not directly shown yet. However, Challen et al. reported that H314 SCC cell line had a complex mtDNA genotype with nine homoplastic point mutations in which three of them located in COI gene.[16]

MtDNA is highly polymorphic among people from the same and different ethnic groups.[17] Alonso et al. indicated that in different ethnic groups there are different polymorphisms, as well as different percentages of mutations, and this important ethnic variation in mitochondrial DNA mutation patterns may influence the susceptibility to environmental factors.[18] In comparison to nuclear DNA, since mtDNA lacks histone proteins and an efficient DNA repair mechanism, it is more sensitive to oxidative damage.[19]

In a variety of cancer types, including HNSCC, mtDNA modifications such as deletions, point mutations and variations in copy number have been shown to be associated.[18,20-28] Mitochondrial dysfunction can be observed in tumors due to different mtDNA alterations, particularly in those that are more aggressive.[29] In HNSCC, the association between mtDNA changes, elevated ROS levels and hypoxia may be important because of the relationship between hypoxia and disease aggressiveness.[30]

Therefore, the evaluation of mitochondrial DNA mutations that may cause the development of HNSCC may be important in the early diagnosis and prognosis of the disease. In the present study, we have selected two of the mtDNA genes (ND4 and COI), which have major roles in the metabolic processing of carcinogen products, screened these gene sequences in a Turkish HNSCC patient group and compared them with healthy individuals. ND4 gene alterations were shown in HNSCC in other populations however up to our knowledge there are no reports in Turkish population. On the other hand, COI mutations were only reported in a SCC cell line and the relation between cytochrome c oxidase mutations and HNSCC susceptibility was not directly shown yet. We aimed to find a possible relationship between CO-1 and ND-4 gene mutations and the development of the disease in Turkish HNSCC patients.

PATIENTS AND METHODS

Study Population

Sixty unrelated Turkish HNSCC patients who were clinically diagnosed at Dışkapı Yıldırım Beyazıt Training and Research Hospital, Department of Otorhinolaryngology and 36 unrelated healthy volunteers from different geographic regions of Turkey were included in this study. Control group was selected to match the patients in terms of demographic data including age and gender. All individuals in the study groups gave informed consent and approval of the local ethics committee was obtained from Dışkapı Yıldırım Beyazıt Training and Rese-arch Hospital [2018.11.12; #56/23]. The study was conducted by guidelines of the Declaration of Helsinki. Clinicopathological parameters of HNSCC patients and control groups were given in Table 1.

Table 1. Clinicopathological parameters of HNSCC patients and control groups

	Control group (n= 36)	Patient group (n= 60)
Gender (Male/Female)	32/4	50/10
Age [Median (Range)]	58 [45-72]	60 [49-74]
Tumor type	Larynx (41)	
	Tongue (5)	
	Hypopharynx (4)	
	Buccal (3)	
	Lip (2)	
	Auricular (1)	
	Retromolar/oral (1)	
	Paranasal sinus (1)	
	Nasopharynx (1)	
	Parotis (1)	
Tumor stage	T1: 9	
	T2: 15	
	T3: 28	
	T4: 8	

Determination of Mutations of CO-1 and ND4 Gene Regions Using DNA Sequencing

Total DNA isolation from blood samples were carried out by using QIAamp® DNA Blood Kit (Qiagen, Germany) according to manifacturer's instructions and whole genomic DNA was used in PCR to amplify targeted mitochondrial regions.

Amplification of CO-1 and ND4 gene regions of mtDNA were carried out on a ThermoPCR system in a 50 μl reaction mixture Easytag (TransGen Biotech, China) containing 10 pmol of forward and reverse primers (Table 2), and 50 ng DNA. The PCR cycling conditions consisted of an initial denaturation step at 95^{0}C for 5 min followed by 40 cycles of 94^{0}C for 1 min, 60^{0}C (CO-1) and 62^{0}C (ND4) for 1 min, 72^{0}C for 1 min and final extension step at 72^{0}C for 5 min.

PCR products were separated on a 1.5% agarose gel electrophoresis, visualized by ethidium bromide staining under an ultraviolet illuminator, scanned and photographed using an imaging system (Bio-RAD).

PCR products were purified and sequencing was carried out by Sanger sequencing protocol of BM Laboratory Systems, Turkey. Sequences were analyzed by MITOMAP and compared with CO-1 and ND4 NCBI reference sequences.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS) version 16.0 software was used for statistical analysis. The genotype frequencies of mutations were evaluated by chi-square analysis. A p value lower than 0.05 was considered as statistically significant.

In Silico Structural Analyses

The obtained data from sequence analysis were converted to amino acid sequences using NCBI (National Center for Biotechnology Information) program. The secondary structure (2D) and three-dimensional (3D) protein structure were created and analyzed using the I-TASSER (Iterative Threading Assembly Refinement) and Phyre2 (Protein Fold Recognition Server) online servers programs. The raw amino acid sequences of CO-1 and ND4 were uploaded in FASTA format to online servers. 3D structures were predicted in PDB format.

Table 2. CO-1 and ND4 gene primer squences on mtDNA

Gene	Annealing Temperature	Primer Sequences	The of size PCR product
CO-1	60^{0}C	Forward: 5'-TCTCCTACTCCTGCTCGCAT-3' Reverse: 5'- AGGCCACCTACGGT-GAAAAG-3'	735 bp
ND-4	62^{0}C	Forward: 5'-TTCCTCCGACCCCCTAACAA-3' Reverse: 5'-TTGTCGTAGGCAGATGGAGC-3'	670 bp

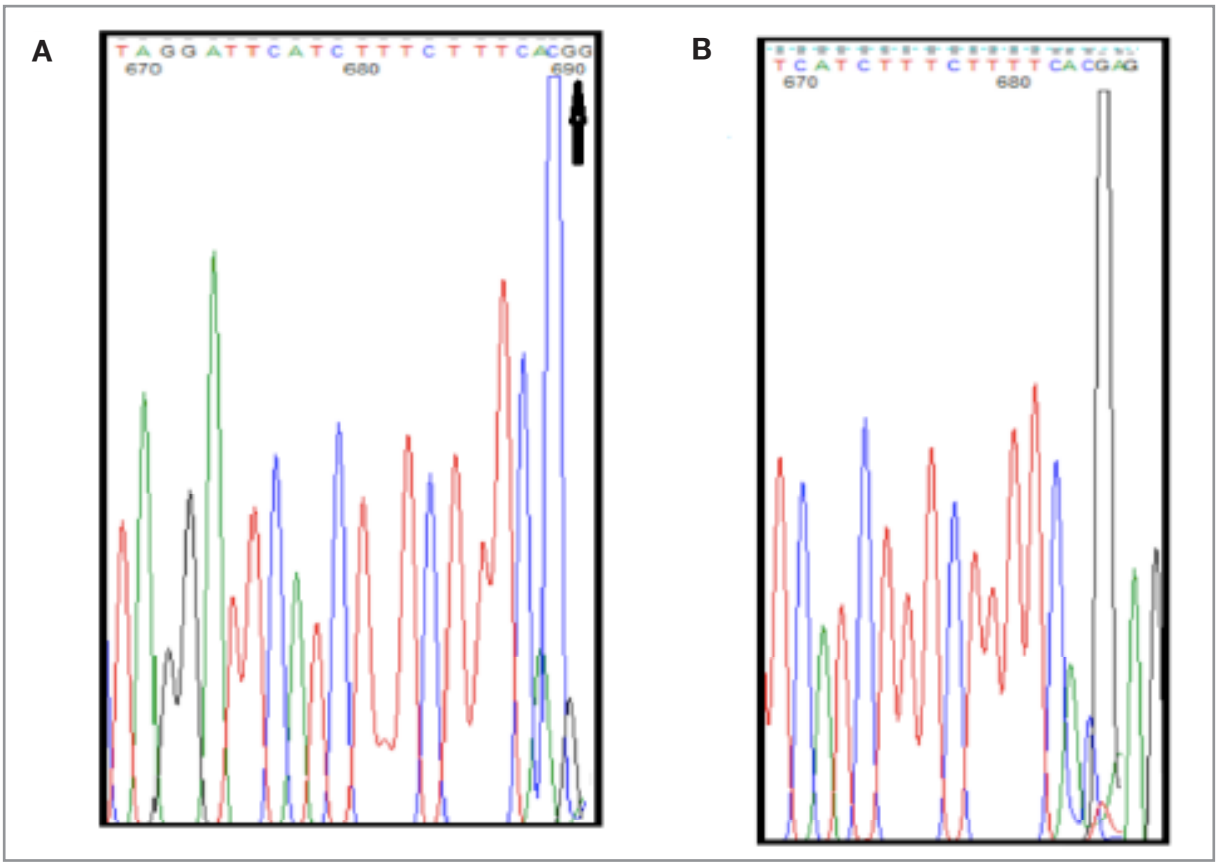

Figure 1. DNA sequencing diagram of CO-1 gene; **(A)** HNSCC patient (A6272d mutation), **(B)** Control

RESULTS

Two of the PCR amplifications from patient group and 6 of the PCR amplifications from control group for CO-1 and ND-4 genes respectively, have not been successfully performed so they were excluded from the study. Since none of the PCR products do not contain non-specific bands, they were purified and sequenced for genotyping analysis.

In the mitochondrial CO-1 gene region, we have observed mainly two mutations as A6272d and A6281d. Among these mutations, A6272d was found as statistically significant (p= 0.013) since none of the individuals in the control group have this mutation where as 16% of the HNSCC patients carry it. Figure 1 shows the DNA sequencing diagrams of CO-1 gene from both a HNSCC patient and a healthy individual for A6272d mutation.

The ratios of the A6281d mutation were found as 14% in control group and 21% in HNSCC patient group, however it was not statistically significant among the studied groups. Table 3 shows the frequencies of A6272d and A6281d mutations in CO-1 gene among HNSCC patient and control groups.

Table 3. Frequencies of A6272d and A6281d mutations in CO-1 gene among HNSCC pati-ent and control groups

	CO-1 gene	Control n= 36 (n (%))	HNSCC Patients n= 58 (n (%))	P value
A6272d	0 (0)	9 (16)	0.013*	
A6281d	5 (14)	12 (21)	0.405	

Table 4. Frequencies of A11251G and T11017TA mutations in ND4 gene among HNSCC patient and control groups

ND4 gene	Control n= 30 (N (%))	HNSCC Patients n= 60 (N (%))	P value
A11251G	3 (10)	11 (18)	0.304
T11017TA	2 (7)	13 (22)	0.072

Figure 2. (A) 3D protein structure prediction of CO-1 protein. Image coloured by rainbow N to C terminus. Model dimensions (Å): X: 46.739, Y: 62.339, Z: 53.112; **(B)** 3D protein structure prediction of CO-1(A6272d) protein. Image coloured by rainbow N to C terminus. Model dimensions (Å): X: 47.468, Y: 62.265, Z: 54.076; **(C)** 3D protein structure prediction of CO-1(A6281d) protein. Image coloured by rainbow N to C terminus. Model dimensions (Å): X: 48.147, Y: 62.265, Z: 54.737

We have also searched for the mitochondrial ND4 gene region in our study. Among the studied sequences, we have found A11251G and T11017TA mutations which may have possible correlation with the HNSCC development. Statistically significant difference was not found for the ratios of A11251G mutation between patient and control groups being 18% and 10% respectively. On the other hand, we have observed a difference between control (7%) and patient (22%) groups for T11017TA mutation, however it was not statistically significant (p= 0.072). This mutation may be further analyzed with the large patient and control groups in the future studies. Table 4 shows the frequencies of A11251G and T11017TA mutations in ND4 gene among HNSCC patient and control groups.

The data from sequence analysis were converted to amino acid sequences for 2D and 3D structure prediction by in silico structural analyses in order to determine whether gene mutations cause changes in the structure of CO-1 and ND-4 proteins.

Table 5 shows 2D structure prediction of CO-1 and ND4 proteins. There was no change in beta-sheet pattern of CO-1 and ND-4 model proteins compared to the control group. The ratios of alpha helix structure of ND-4 were same when we compare patients and control group for both of the mutations A11251G and T11017TA. However, there was approximately 5% change in the alpha helix structure of the CO-1 protein when we compare patient and control groups for both A6272d and A6281d mutations. Figure 2 shows the 3D structure of CO-1 protein models.

DISCUSSION

A main approach that could be used to enhance HNSCC patient management is the scope for prognostic and predictive biomarkers.[27] Somatic mutations in mtDNA have been frequently observed in several human cancers in addition to genomic DNA mutations and have been suggested as significant oncological biomarkers.[31]

Table 5. 2D structure prediction of CO-1, CO-1(A6272d), CO-1(A6281d) and ND4, ND4(A11251G), ND4(T11017TA) proteins

2D structure	Control	Patient (A6272d)	Patient (A6281d)
CO-1 alpha helix percentage	69%	73%	74%
	Control	**Patient(A11251G)**	**Patient (T11017TA)**
ND4 alpha helix percentage	84%	84%	83%

In various tumors and different investigations, the occurrence of mtDNA mutations and forms of mutations vary.[32-34] The hypothesis that a substantial ethnic difference in the pattern of DNA mutations could influence susceptibility to environmental factors was advanced by Alonso et al. The observation of multiple polymorphisms and varying percentages of mutations in different ethnic groups confirms this hypothesis.[18] Therefore, determining the polymorphisms in mtDNA for different ethnic groups may be important for proposing oncological biomarker for the diagnosis and prognosis of HNSCC disease. In this study, we have tried to determine possible mtDNA mutations related to HNSCC development for a selected Turkish patient group.

In the non-coding regulatory D-loop region, mitochondrial DNA mutations that have been observed in HNSCC so far are most commonly detected.[12,28,35] However, the biological significance of mtDNA mutations in cancer still remains unclear. Lievre et al. reported that there was no association in the HNSCC patient group between D-loop mutations and prognosis or response to chemotherapy.[36] On the other hand, another study revealed that there were no major variations in demographic and tumor-related features between the mutation group and the non-mutation group, while patients with D-loop mutations had higher survival rates.[37]

Both the non-coding and coding regions of mtDNA may be involved in mtDNA mutations. The majority of coding mtDNA mutations in HNSCC were non-synonymous in nature and affected primarily by cytochrome c oxidase (Complex IV).[28] Subunits I to III of cytochrome c oxidase form the enzyme's catalytic center are all synthesized from mtDNA.[38] In another study, it was shown that H134 SCC cell line had three base pair changes in the CO-1 gene. However, the base changes were all synonymous and there was no predicted change in the amino acid sequence of the codon.[27] The remaining subunits (IV–VIII) are synthesized from cellular nuclear DNA.[38] In prostate cancer and other mitochondrial diseases, cytochrome c oxidase dysfunction due to mutations in different subunits has been reported.[38]

In the present study, we examined two of the coding mtDNA gene regions which have functions for oxidative phosphorylation system. First gene was CO-1 which belongs to OXPHOS Complex IV. According to our results, we have identified two mutations in CO-1 gene and among them we found that A6272d mutation was statistically significant in the studied HNSCC patient group with respect to control group. It was suprising that none of the individuals in the control group have this mutation. We have also made in silico structural analysis for this mutation and when we converted sequence analysis to amino acid sequence, we observed differences in the alpha helix structure of the protein in patients having mutations. This result may indicate that A6272d mutation may be associated with the HNSCC development.

Second gene that we have studied in this study was ND4, an nicotinamide adenine dinucleotide hydrogen (NADH) dehydrogenase subunit, which belongs to OXPHOS Complex I. In the metabolic processing of carcinogenic materials, this complex has a significant role. NADH structure changes might thus lead to an increased probability of mutagen accumulation.[12] Allegra et al. indicated that 50 percent of HNSCC cell lines had ND4 polymorphisms.[9]

On the other hand, in cancer patients, mtDNA anomalies influence the response and outcome of therapies. Mutations in NADH dehydrogenase subunit 4 (MT-ND4) have been reported to lead to acquired chemoresistance during treatment of paclitaxel carboplatin in ovarian cancer.[39]

In HNSCC, ND4 polymorphisms and mutations are frequent. Changes in ND4 gene have been reported in head and neck cancer cell lines. In ten HNSCC cell lines, 8 somatic mutations and 5 polymorphisms were found. Among them, all of the polymorphisms were silent and 3 of the mutations were reported as altering the amino acid sequence.[9]

In our study, we detected two mutations (A11251G and T11017TA) in the ND4 gene region. However, according to the statistical analysis none of these mutations were seem to be responsible for the disease development.

The mitochondrial genome differs from the nuclear genome since it is maternally inherited. In addition, mtDNA heteroplasmy including a mixture of mutant and normal mtDNA in cells accounts for a considerable level of variation during embryonic

development of somatic tissue.[40] Following the first report of somatic mtDNA mutation associated with human cancer by Polyak et al. in 1998[41], mtDNA alterations associated with cancer development and progression have been shown in many different cancer types. Numerous somatic mtDNA mutations were reported in a wide variety of tumors such as, colorectal, breast, bladder, esophageal, head and neck, ovarian, renal, leukemia, lung and thyroid, so far.[12,37,42,43]

Somatic mutations are the ones that cannot be inherited by offspring but found in afterwards proliferating populations of cells. Besides somatic variations, germline mutations which are heritable from mother to offspring are found throughout the body of the offspring and are useful for assigning individuals to certain haplogroups.[44] This haplogrouping is generally used for identifying relationships among individuals and populations, but it can also possess sequence variants that can contribute to cancer susceptibility.[45] Today, the association among several germline mtDNA mutations and a broad spectrum of human malignancies has been widely reported.[46-48] The important role of germline mtDNA variants in tumors has been noted conferring risk for cancer, metastasis and in response to treatment.[49] In the literature, there are also indications that different germline mtDNA mutations can contribute to the development of a certain cancer type in a specific population. Different human populations can be distinguished by different mtDNA haplogroups, reflecting mutations accumulated by a discrete maternal lineage that are associated with genetic drift and/or adaptive selection.[50] Booker et al., showed that a certain haplogroup was related with an increased risk for developing renal cancer in Caucasian American men and also for development of prostate cancer.[51] On the other hand, mtDNA muatations arise in oral cancer either in the germline or in the mtDNA of the tissues indicate that mtDNA mutations may confer selective advantage.[52]

Thus, the interaction between the somatic and germline mtDNA mutations synergistically increases the effect, which accounts for the progressive course of mitochondria-associated diseases, including cancer.[53]

Although somatic mtDNA alterations are the primary targets in the diagnosis and prognosis of various cancers as they are only carried in the tumor tissue; the complexities in dealing with the traditional mode of cancer diagnostics have needed a shift into finding out new areas that explores biomarkers that are less or non invasive with easily accessible samples such as blood, urine, saliva etc.[54,55] In the literature, there are several studies that support the relation between somatic and germline mtDNA mutations in both tumor tissues and blood samples of the same patients. Li et al., showed this relation in breast cancer patients by detecting mtDNA mutations by using high-throughput sequencing method in the blood samples of the patients.[56] In addition, Buffet et al., identified a germline mutation in the SLC25A11 gene which codes for mitochondrial 2-oxoglutarate/malate carrier and showed the correlation with metastatic paragangliomas.[57] Non-invasive methods were also reported in patients with mtDNA-mutated head and neck cancers. Fliss et al. reported mtDNA mutations in saliva, thus playing a potential role in early diagnosis.[12]

In this study, we have focused on germline mtDNA mutations that can be associated with HNSCC development in a Turkish patient group. The importance of screening germline mtDNA mutations in HNSCC patient blood samples is to identify a potential marker by using a less invasive method without taking biopsy samples from tissues. According to our results, A6272d mutation in CO-1 gene can be a potential biomarker as a germline mutation in the studied Turkish patient group. However, this study still have some limitations that needs to be addressed in our future studies. First, this germline mutation is going to be matched with the somatic mtDNA mutation from the tissue samples of the HNSCC patients. Secondly, we are going to increase the control and patient group numbers in order to make a more reliable decision about the incidance of the mutation in Turkish population.

Conclusion

In this study we have focused on the alterations of two gene regions (CO-1 and ND4) on mtDNA for their possible role in the development on HNSCC in a Turkish patient group. CO-1 mutations were infrequent in HNSCC where as ND4 mutations were more common. However, for the studied Turkish

patient group we showed that A6272d mutation in CO-1 gene can be related to HNSCC development. On the other hand, we cannot detect a statistically significant alteration between patient and control groups for ND4 gene. These differences can be due to ethnic differences. In future studies, this germline mutation should to be matched with the somatic mtDNA mutation from the tissue samples of the HNSCC patients and also control and patient group numbers need to be increased in order to make a more reliable decision about the incidance of the mutation in Turkish population.

REFERENCES

1. Lubov J, Maschietto M, Ibrahim I, et al. Meta-analysis of microRNAs expression in head and neck cancer: Uncovering association with outcome and mechanisms. Oncotarget. 8: 55511-55524, 2017.
2. Nagai MA. Genetic alterations in head and neck squamous cell carcinomas. Braz J Med Biol Res 32: 897-904, 1999.
3. Beck TN, Golemis, EA. Genomic insights into head and neck cancer. Cancers Head Neck 1: 1, 2016.
4. Leonard JV, Schapira AH. Mitochondrial respiratory chain disorders I: mitochondrial DNA defects. Lancet 355: 299-304, 2000.
5. Leonard JV, Schapira AH. Mitochondrial respiratory chain disorders II: neurodegenerative disorders and nuclear gene defects. Lancet 355: 389-394, 2000.
6. Schapira AHV. Mitochondrial disease. Lancet 368: 70-82, 2006.
7. Gu M, Cooper JM, Taanman JW, Schapira AH. Mitochondrial DNA transmission of the mitochondrial defect in Parkinson's disease. Ann Neurol 44: 177-186, 1998.
8. Wallace DC. Why do we have a maternally inherited mitochondrial DNA? Insights from evolutionary medicine. Annu Rev Biochem 76: 781-821, 2007.
9. Allegra M, Garozzo A, Lombardo N, et al. Mutations and polymorphisms in mitochondrial DNA in head and neck cancer cell lines. Acta Otorhinolaryngol Ital 26: 185-190, 2006.
10. Cavalcante GC, Ribeiro-dos-Santos A, de Araujo GS. Mitochondria in tumor progres-sion: a network of mtDNA variants in different types of cancer. BMC Genomic Data 23:16, 2022.
11. Tzen CY, Mau BL, Wu TY. ND4 mutation in transitional cell carcinoma: Does mitochondrial mutation occur before tumorigenesis? Mitochondrion 7:273-278, 2007.
12. Fliss MS, Usadel H, Caballero OL, et al. Facile detection of mitochondrial DNA mutations in tumors and bodily fluids. Science 287: 2017-2019, 2000.
13. Jeronimo C, Nomoto S, Caballero OL, et al. Mitochondrial mutations in early stage prostate cancer and bodily fluids. Oncogene 20: 5195-5198, 2001.
14. Polyak K, Li Y, Zhu H, et al. Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 20: 291-293, 1998.
15. Maximo V, Soares P, Lima J, et al. Mitochondrial DNA somatic mutations (point mutations and large deletions) and mitochondrial DNA variants in human thyroid pathology: a study with emphasis on Hürthle cell tumors. Am J Pathol 160: 1857-1865, 2002.
16. Challen C, Brown H, Cai C, et al. Mitochondrial DNA mutations in head and neck cancer are infrequent and lack prognostic utility. British J Cancer 104: 1319-1324, 2011.
17. Torroni A, Petrozzi M, D'Urbano L, et al. Haplotype and phylogenetic analyses suggest that one European-specific mtDNA background plays a role in the expression of Leber hereditary optic neuropathy by increasing the penetrance of the primary mutations 11778 and 14484. Am J Hum Genet 60: 1107-1121, 1997.
18. Alonso A, Martin P, Albarran C, et al. Detection of somatic mutations in the mitochondrial DNA region of colorectal and gastric tumours by heteroduplex and single-strand conformation analysis. Electrophoresis 18: 682-685, 1997.
19. Dean OM, van den Buuse M, Berk M, et al. N-acetyl cysteine restores brain glutathione loss in combined 2-cyclohexene-1-one and d-amphetamine-treated rats: Relevance to schizophrenia and bipolar disorder. Neurosci Lett 499: 149-153, 2011.
20. Parrella P, Xiao Y, Fliss M, et al. Detection of mitochondrial DNA mutations in primary breast cancer and fine-needle aspirates. Cancer Res 61: 7623-7626, 2001.
21. Hasgood 3rd HD, Liu CS, Rothman N, et al. Mitochondrial DNA copy number and lung cancer risk in a prospective cohort study. Carcinogenesis. 31: 847-849, 2010.
22. Lam ET, Bracci PM, Holly EA, et al. Mitochondrial DNA sequence variation and risk of pancreatic cancer. Cancer Res 72: 686-695, 2012.
23. Petros JA, Baumann AK, Ruiz-Pesini E, et al. mtDNA mutations increase tumorigenicity in prostate cancer. Proc Natl Acad Sci U. S. A. 102: 719-724, 2005.
24. Liu SA, Jiang RS, Wang WY, Lin JC. Somatic mutations in the D-loop of mitochondrial DNA in head and neck squamous cell carcinoma. Head & Neck 37: 878-883, 2015.
25. Burgart LJ, Zheng J, Shu Q, et al. Somatic mitochondrial mutations in gastric cancer. Am J Pathol 147: 1105-1111, 1995.
26. Tamura G, Nishizuka S, Maesawa C, et al. Mutations in mitochondrial control region DNA in gastric tumours of Japanese patients. Eur J Cancer 35: 316-319, 1999.
27. Challen C, Brown H, Cai C, et al. Mitochondrial DNA mutations in head and neck cancer are infrequent and lack prognostic utility. Br J Cancer 104: 1319-1324, 2011.

28. Dasgupta S, Koch R, Westra WH, et al. Mitochondrial DNA mutation in normal margins and tumors of recurrent head and neck squamous cell carcinoma patients. Cancer Prev Res (Phila.) 3: 1205-1211, 2010.

29. Gogvadze V, Zhivotovsky B, Orrenius S. The Warburg effect and mitochondrial stability in cancer cells. Mol Aspects Med 31: 60-74, 2010.

30. Nordsmark M, Bentzen SM, Rudat V, et al. Prognostic value of tumor oxygenation in 397 head and neck tumors after primary radiation therapy. An international multi-center study. Radiother Oncol 77: 18-24, 2005.

31. Jakupciak JP, Wang W, Markowitz ME, et al. Mitochondrial DNA as a cancer biomarker. J Mol Diagn 7: 258-267, 2005.

32. Ivanova R, Lepage V, Loste MN, et al. Mitochondrial DNA sequence variation in human leukemic cells. Int J Cancer 76: 495-498, 1998.

33. Horton TM, Petros JA, Heddi A, et al. Novel mitochondrial DNA deletion found in a renal cell carcinoma. Genes, Chromosome Cancer. 15: 95-101, 1996.

34. Lewis PD, Baxter P, Griffiths PA, et al. Detection of damage to the mitochondrial genome in the oncocytic cells of Wartin's tumour. J Pathol. 191: 274-281, 2000.

35. Tan DJ, Chang J, Chen WL, et al. Novel heteroplasmic frameshift and missense somatic mitochondrial DNA mutations in oral cancer of betel quid chewers. Genes Chromosomes Cancer 37: 186-194, 2003.

36. Lie`vre A, Blons H, Houllier AM, et al. Clinicopathological significance of mitochondrial D loop mutations in head and neck carcinoma. Br J Cancer 94: 692-697, 2006.

37. Liu VW, Shi HH, Cheung AN, et al. High incidence of somatic mitochondrial DNA mutations in human ovarian carcinomas. Cancer Res 61: 5998-6001, 2001.

38. Herrmann PC, Gillespie JW, Charboneau L, et al. Mitochondrial proteome: altered cytochrome c oxidase subunit levels in prostate cancer. Proteomics 3: 1801-1810, 2003.

39. Guerra F, Perrone AM, Kurelac I, et al. Mitochondrial DNA mutation in serous ovarian cancer: implications for mitochondria-coded genes in chemoresistance. J Clin Oncol 30: 373-378, 2012.

40. Bogehan DF, Rousseau D, Burke S. The layered structure of human mitochondrial DNA nucleoids. J Biol Chem 283: 3665-3675, 2008.

41. Polyak K, Li Y, Zhu H, et al. Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 20: 291-293, 1998.

42. Gochhait S, Bhatt A, Sharma E, et al. Concomitant presence of mutations in mitochondrial genome and p53 in cancerdevelopment– A study in north Indian sporadic breast and esophageal cancer patients. Int J Cancer 123: 2580-2586, 2008.

43. Nagy A, Wilhelm M, Sükösd F, et al. Somatic mitochondrial DNA mutations in human chromophobe renal cell carcinomas. Genes Chromosomes Cancer 35: 256-260, 2002.

44. Weissensteiner H, Pacher D, Kloss-Brandstatter A, et al. HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. Nucleic Acids Res 44(W1): W58-63, 2016.

45. Czarnecka AM, Bartnik E. The role of the mitochondrial genome in ageing and carcinogenesis. J Aging Res 2011: 13643, 2011.

46. Larman TC, DePalma SR, Hadjipanayis AG, et al. Spectrum of somatic mitochondrial mutations in five cancers. Proc Natl Acad Sci USA 109: 14087-14091, 2012.

47. Jimenez-Morales S, Perez-Amado CJ, Langley E, et al. Overview of mitochondrial germline variants and mutations in human disease: focus on breast cancer (Review). Int J Oncol 53: 923-36, 2018.

48. Czarnecka AM, Krawczyk T, Plak K, et al. Mitochondrial genotype and breast cancer predisposition. Oncol Rep 24: 1521-1534, 2010.

49. Weerts MJA, Sleijfer S, Martens JWM. The role of mitochondrial DNA in breast tumors. Drug Discov Today 24: 1202-1208, 2019.

50. Torroni A, Huoponen K, Francalacci P, et al. Classification of European mtDNAs from an analysis of three European populations. Genetics 144: 1835-1850, 1996.

51. Booker LM, Habermacher GM, Jessie BC, et al. North American White Mitochondrial Haplogroups in Prostate and Renal Cancer. J Urology 175: 468-473, 2006.

52. Czarnecka AM, Kukwa W, Krawczyk T, et al. Mitochondrial DNA mutations in cancer-from bench to bedside. Front Biosci (Landmark Ed) 15: 437-460, 2010.

53. Bohr VA, Anson RM. DNA damage, mutation and fine structure DNA repair in aging. Mutat Res 338: 25-34, 1995.

54. Clayton DA. Replication of animal mitochondrial DNA. Cell 28: 693-705, 1982.

55. Ling L, Hann HW, Wan S, et al. Cell-free circulating mitochondrial DNA content and risk of hepatocellular carcinoma in patients with chronic HBV infection. Sci Rep 6: 23992, 2016.

56. Li LH, Kang T, Chen L, et al. Detection of mitochondrial DNA mutations by high-throughput sequencing in the blood of breast cancer patients. Int J Mol Med 33: 77-82, 2014.

57. Buffet A, Morin A, Castro-Vega LJ, et al. Germline Mutations in the mitochondrial 2-Oxoglutarate/Malate carrier SLC25A11 gene confer a predisposition to metastatic para-gangliomas. Cancer Res 78: 1914-1922, 2018.

Correspondence:
Pelin MUTLU, Ph.D.
Ankara Universitesi, Biyoteknoloji Enstitusu
Biyoteknoloji Anabilim Dali
Gumusdere Yerleskesi
06135, Kecioren
ANKARA / TURKEY

Tel: (+90-532) 331 37 20
e-mail: pelinkaya78@yahoo.com

ORCIDs:

Pelin Mutlu	0000-0001-7970-880X
Murad Mutlu	0000-0003-0325-5511
Serap Azarkan Yalcin	0000-0002-9584-266X
Kemal Keseroglu	0000-0002-6589-1663
Omer Bayir	0000-0001-9445-6129
Güleser Saylam	0000-0002-6499-7140
Mehmet Hakan Korkmaz	0000-0001-8732-3061