RESEARCH ARTICLE

Is there an association between *NC_012920.1: m.8277T> C* mitochondrial variation the *mt-NC7* locus, and migraine with aura?

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Abstract

Background: The molecular basis of migraines is still not completely understood. Over the last 30 years, mitochondrial dysfunction has been postulated as a potential mechanism in migraine pathogenesis. This study aimed to determine whether maternal mitochondrial variation was associated with migraines with aura.

Methods: In this cross-sectional study, 50 individuals, who had been diagnosed with migraines with aura between January 2016 and July 2018 in the Neurology Department of the University Medical Faculty, and 50 healthy controls were recruited. Genomic DNA was isolated from the Ethylenediaminetetraacetic acid (EDTA) blood samples of the patients and the controls using the Easy One automated DNA isolation system. Mitochondrial DNA (mtDNA) libraries were prepared according to the Nextera XT DNA library-preparation protocol, and they were sequenced on the MiSeq platform (Illumina Inc., San Diego, CA, USA).

Results: In the patient and control groups' analysis, 13 mtDNA variations were determined to be significantly different ($p < 0.05$). The CC genotype for *NC_012920.1: m.8277T>C* variation was found to be higher in the patient group than the control group ($p = 0.001$). The *mtDNA NC_012920.1: m.8277T>C* variation was significantly associated with the presence of neurological disease in the patient's family ($p = 0.043$).

Conclusions: The present study is the first to demonstrate an association between mitochondrial dysfunction and the susceptibility to migraine with aura in individuals carrying the *NC_012920.1: m.8277T>C* variation. Knowing the level of cytochrome C oxidase and oxidative phosphorylation corruption in these patients may be predictive in understanding the phenotype/genotype relationship. Thus, mtDNA variations may contribute to the pathogenesis of migraines with aura. HIPPOKRATIA 2020, 24(2): 59-65.

Keywords: Aura, migraine, molecular basis, mitochondrial DNA, mitochondrial dysfunction

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Introduction

Over the last 30 years, mitochondrial dysfunction has been proposed as a potential mechanism in migraine pathogenesis. The relationship between some subtypes of migraine and mitochondrial DNA (mtDNA) mutations has been demonstrated. It has been suggested that abnormal oxidative metabolism in the brain, due to mutations in *mt*DNA, may increase sensitivity to migraines[1].

Mitochondrial dysfunction is associated with a diversity of nervous system diseases. It has been declared that mitochondrial function is frequently impaired in patients with migraines, and mitochondrial dysfunction is contained in the pathophysiology of migraines[2-4]. In the 1980s, scientists first hypothesized a connection between migraines and mitochondrial disorders. Recently studies have proposed that at least some subtypes of migraines may be related to mitochondrial disturbance[2]. Maternal inheritance of migraines also makes mitochondrial etiology especially interesting. Indeed, lactic acidosis develops due to the impaired function of pyruvate in the Krebs cycle. Consequently, the reduction of pyruvate to lactate by lactate dehydrogenase leads to increasing concentrations of lactate[5]. The increased use of glycogen and glycolytic activity and flawed anaerobic metabolism results in increased lactate levels in tissues. Thus, depending on the degree of oxidative metabolic dysfunction, lactic acidosis is the first sign of generalized metabolic acidosis in patients with migraines and may occur between or during attacks[5].

Nuclear DNA and mtDNA have distinctive features. mtDNA is small, circular, and sensitive to oxidative stress. mtDNA and nuclear DNA mutations are seen in diseases such as Leigh syndrome, "mitochondrial encephalopathy, lactic acidosis, stroke-like episodes" (MELAS), and Kearns-Sayre syndrome. Some of these diseases are associated with the migraine phenotype[2,6]. mtDNA variations (16.519 CT and 3010 G-A) have also been

reported to be associated with periodic cyclic vomiting syndrome and migraines. One of the main symptoms of mitochondrial disease is episodic headache[2]. Therefore, mitochondria are considered to play an important role in the pathogenesis of headaches, particularly migraines. In experimental and clinical studies, mitochondria were shown to play a role in migraines, causing depression and forming the clinical cascade of a migraine. As a result, it is extremely important to identify the potential mtDNA variations of the primary common headaches that cause disability in peoples' daily lives, such as migraines.

Although there have been few studies investigating mtDNA variants and migraines, it is possible that variants in mtDNA could play a significant role in the disorder. Systematic screening studies have failed to demonstrate the exact relationship between migraine headaches and mtDNA abnormalities. We, therefore, consider the possibility that mitochondrial mutations may play some role in migraine with prolonged aura. Therefore, we screened the whole mtDNA of all the patients and controls comprising 16,569 nucleotides. The present study aimed to investigate the frequency of the mtDNA variations in with-aura migraine patients in the Edirne population.

Material and Methods

Participants

This cross-sectional study was approved by the University of Trakya, Medical Faculty Ethics Committee (decision No 2014/26, date: 13/2/2014). All the procedures complied with the 1964 Declaration of Helsinki and its subsequent amendments or comparable ethical standards. Informed consent was obtained from the participants. A total of 100 individuals were recruited: 50 with migraines with aura [12 (24 %) males, 38 (76 %) females] and 50 healthy controls [10 (20 %) males and 40 (80 %) females]. The participants with migraines had all been referred to the Neurology Polyclinic of the Medical Faculty, Trakya University, Edirne, Turkey, between January 2016 to July 2018, and they had been diagnosed according to the International Classification of Headache Disorders (ICHD-II) criteria[7]. The need to recruit a total of 96 cases for the patient and control groups was calculated on the basis that the mtDNA variation might be observed as 0.14 in the control group, with an odds ratio (OR) of 2.21, in order to find an mtDNA variation and migraine association at 80 % power and 5 % probability of error (Medcalc v12.12.0). However, considering possible case losses, 100 people (50 patients and 50 controls) were recruited to adhere to our sample size calculation. Patients with cardiovascular, renal, hepatic, gastrointestinal, pulmonary, endocrine, oncologic, autoimmune, respiratory, or inflammatory diseases were excluded. The controls were selected randomly; the individuals in this group have a similar age and gender, and they have no cardiovascular, renal, hepatic, gastrointestinal, pulmonary, endocrine, oncologic, autoimmune, respiratory, inflammatory, or psychiatric diseases.

mtDNA variation determination

Peripheral blood samples from both patients and controls were drawn into 2 mL EDTA tubes. DNA isolation from peripheral blood samples was performed using Qiagen DNA isolation kits (EZ1® DNA Blood 200 µL Kit) with an EZ1 Advanced XL Nucleic Acid Isolation system (Qiagen, Hilden, North Rhine-Westphalia, Germany). Consequently, DNA concentration and purity of isolated DNA samples were measured using a Nano Drop 2000C device (Thermo Fisher Scientific Inc., Wilmington, MA, USA). In the patient and control groups included in the study, mtDNA sequencing was performed using Next Generation Sequencing (NGS) technology. For this purpose, mitochondrial DNA samples were amplified via a long-range polymerase chain reaction (PCR). PCR primers for long-range amplification were optimized using nested sets of primers previously defined by Tanwar et al[8]. Five single plex long-range PCR reactions were performed for each sample to amplify the whole mitochondrial genome. PCR products were then processed according to Nextera XT DNA Library Preparation (Illumina Inc., San Diego, CA, USA) protocol.

Primary data analysis was performed on Miseq Reporter Software (Illumina Inc., San Diego, CA, USA). Fastq files taken from Miseq Reporter Software were directly submitted to mtDNA server[9]. Visual evaluation of the data was performed using the Integrative Genomics Viewer (IGV, Broad Institute, Cambridge, USA) program. mtDNA variants were compared according to the literature and MitoMap database.

Statistical analyses

Statistical evaluation was performed using the IBM SPSS Statistics for Windows, Version 21.0. (IBM Corp., Armonk, NY, USA). One-sample Kolmogorov-Smirnov test was used to assess the eligibility for normal distribution of measured data, and since the data did not show a normal distribution, the Mann Whitney U test and the Kruskal-Wallis analysis of variance were used for comparison between groups. Pearson's chi-squared test, Fisher's exact test, and the Kolmogorov-Smirnov two-sample test were used to analyze qualitative data. Median (minimum-maximum) values and mean value ± standard deviation (SD) were determined as descriptive statistics. The significance limit was set at $p < 0.05$ for all statistics.

Results

The mean age of the male and female patients in our migraine with aura group was 35.7 (SD: 1.89) and 37.4 (SD: 1.67), respectively. The mean age of the male and female controls was 40.2 (SD: 1.60) and 38.8 (SD: 1.76), respectively (Table 1). There was no significant difference in terms of average age or body mass index (BMI) ($p = 0.589$) between the patient and control groups. Moreover, there was no statistically significant difference between time after migraine diagnosis, frequency of migraines, family history, stroke, and coronary artery disease. Other clinical features of the individuals in the patient and control groups are summarised in Table 1. The genotypes of the patients were evaluated, and haplotype analysis was performed. Unlike other studies, a total of

16,569 *mt*DNA variations were studied in this research. As a result of the analysis between the patient and the control group, 13 mtDNA variations were determined to be statistically significant (p =0.001, or p =0.012, or p =0.027) (Table 2). Moreover, the CC genotype of the *NC_012920.1: m.8277T> C* variation in the *mt-NC7* locus was found to be higher in the patient group than the control group (Table 2). This result supports the theory that the CC genotype may be associated with migraines with aura. Mutant genotypes of the other variations were higher in the control group than the patient group (Table 2), suggesting that the CC genotype of this variation may protect against migraines with aura. Also, in the human map mtDNA is located in the *mt-NC7* locus (molecular location: base pairs 7,586 to 8,269 on mtDNA)[10] as shown in Figure 1. The clinical characteristics associated with migraine with aura and the *mt-NC7 m.8277T>C DNA* variations are summarised in Table 3. There was no statistically significant difference between coronary artery disease presence in the family and the frequency of the mtDNA variation of *mt-NC7 m.8277T>C DNA* (p =0.056). However, a family history of coronary artery disease may be an important factor in the variation of *mt-NC7 m.8277T>C DNA*. A statistically significant difference was found between the presence of neurological disease in the patient's family and the frequency of the mtDNA variation *mt-NC7 m.8277T>C DNA* (p =0.043), as shown in Table 3. It is important to consider the presence of the *mt-NC7 m.8277T>C DNA* variation in relation to the etiopathogenesis of other neurological diseases (stroke, myopathy, epilepsy, etc.). Although the HVOe haplotype was found to be higher in the patient group, no statistically significant difference was observed (p =0.076). Although the H2a2, T2b, H5, and T haplotypes were found to be higher in the control group, no statistically significant difference was observed (p =0.362, p =0.357, p =0.242, and p =0.495, respectively). The analysis of mtDNA variations' results that were considered to be statistically significant revealed 85 haplogroups in the patient and control groups, as shown in Table 4.

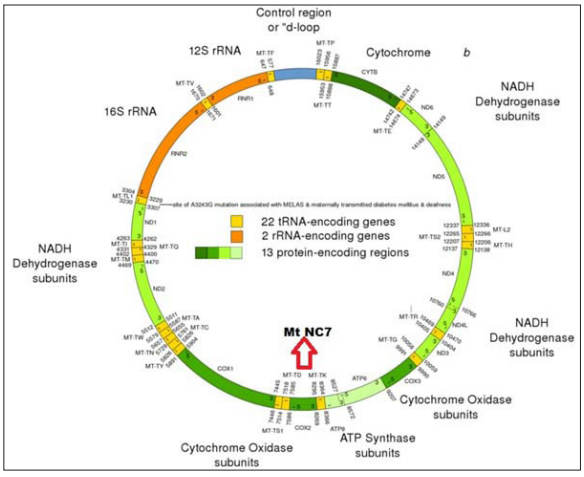

Figure 1: This suggests that the CC genotype of this variation may protect against migraines with aura. The mT-NC7 locus (Molecular Location: base pairs 7,586 to 8,269 on mitochondrial DNA) is shown in the map of human mitochondrial DNA.

Discussion

The mitochondrial component of migraines is suggested to be important in the pathophysiology of migraines with aura[11,12]. Impaired energy metabolism is a common feature of migraines[13]. In experimental and clinical studies, it has been shown that mitochondria play a role in migraine's progress, the cortical spreading of depression, and the generation of the clinical cascade of migraines[2]. It has been stipulated that abnormal oxidative metabolism in the brain due to variations in mtDNA may increase susceptibility to migraines[14]. Mitochondrial dysfunction may be one of the underlying reasons that cortical hyperexcitability has been demonstrated in individuals with migraines[15].

mtDNA variants have been associated with various disorders, including cancer, diabetes, Alzheimer's, Parkinson's, stroke, cardiomyopathy, mental retardation, and other complex diseases[4]. Although there have been few studies investigating mtDNA variants and migraines, it is possible that variants in mtDNA could play a significant role in the disorder. Guo et al[16] noticed a high prevalence of migraine in individuals with the *mtDNA 3243A>G* variation. This evidence suggested a clinical association between a monogenetically inherited disorder of mitochondrial dysfunction and susceptibility to migraine. Also, Guler et al determined significant differences in the distribution of eNOS haplotypes in patients with migraines, with and without aura[17]. Molecular genetic studies have not detected specific mtDNA variations in patients with migraines, although other studies suggest that particular genetic markers (i.e., neuronal polymorphisms or secondary mtDNA mutations) might be present in some migraine sufferers[5]. However, we detected some specific mtDNA variations associated with migraine patients with aura.

Our findings showed that, as expected, females have a higher risk of migraine than men. Our next aim was then to assess if there was a gender-biased transmission. We found that the mothers of probands were more frequently affected than expected. This biased transmission could be explained by a maternally inherited factor, such as mtDNA[18]. One study reported that the ratio of affected probands' fathers is lower than the ratio of affected mothers and siblings, which is evidence in favor of a maternally inherited factor, according to Boles et al[18]. Although mtDNA by itself may not explain the gender differences found since migraine is a complex disease with several genetic factors variants in mtDNA, or in nuclear genes affecting mitochondrial mechanisms, could influence migraine susceptibility. Also, it has been suggested that impairment of mitochondrial metabolism could lower the threshold for migraine attacks[5].

The Mitochondrially Encoded Cytochrome C Oxidase II (*mT-CO2*) gene has been previously associated with encephalomyopathy, late-onset Alzheimer's dis-

Table 1: Clinical characteristics of the 50 patients with migraines with aura and the 50 healthy controls included in this cross-sectional study.

Parameters	Controls	Migraine With Aura(%)	p
Number (n)	50	50	0.691
Age (years,) (Male/Female)	40.2 ± 1.60 / 38.8 ± 1.76	35.7 ± 1.89 / 37.4 ± 1.67	0.61
Gender (Male/Female)	10/40	12/38	0.629
BMI (kg/m^2)	25.72 ± 4.76	25.58 ± 4.92	0.589

Values are presented as means ± standard deviation or number of subjects, BMI: Body Mass Index.

Table 2: Relationship in mitochondrial DNA variations between the 50 patients with migraines with aura and the 50 healthy controls included in this cross-sectional study.

mt DNA Variations	Genotype	MA	Controls	p
mT-DLOOP2 m.73A>G	AA	39 (78)	21 (42)	0.001
	AG	-	-	
	GG	11 (22)	29 (58)	
mT-DLOOP2 m.263A>G	AA	30 (60)	2 (4)	0.001
	AG	-	-	
	GG	20 (40)	48 (96)	
mT-DLOOP2 m.295C>T	CC	50 (100)	43 (86)	0.012
	CT	-	-	
	TT	0 (0)	7 (14)	
mT-DLOOP2 m.310T>C	TT	30 (60)	2 (4)	0.001
	TC	-	-	
	CC	20 (40)	48 (96)	
mT-DLOOP2 m.311C>T	CC	30 (60)	6 (12)	0.001
	CT	-	-	
	TT	20 (40)	44 (88)	
mT-DLOOP2 m.462C>T	CC	50 (100)	44 (88)	0.012
	CT	-	-	
	TT	0 (0)	6 (12)	
mT-DLOOP2 m.489T>C	TT	50 (100)	39 (78)	0.001
	TC	-	-	
	CC	0 (0)	11 (22)	
mT-NC7 m.8277T>C	TT	35 (70)	50 (100)	**0.001**
	TC	-	-	
	CC	**15 (30)**	**0 (0)**	
mT-ND6 m.14470T>C	TT	50 (100)	10 (20)	0.001
	TC	-	-	
	CC	0 (0)	40 (80)	
mT-DLOOP1 m.16069C>T	CC	50 (100)	43 (86)	0.012
	CT	-	-	
	TT	0 (0)	7 (14)	
mT-DLOOP1 m.16126T>C	TT	48 (96)	37 (74)	0.001
	TC	-	-	
	CC	2 (4)	13 (26)	
mT-DLOOP1 m.16278C>T	CC	50 (100)	44 (88)	0.027
	CT	-	-	
	TT	0 (0)	6 (12)	
mT-DLOOP1 m.16519T>C	TT	40 (80)	25 (50)	0.001
	TC	-	-	
	CC	10 (20)	25 (50)	

Values are presented as number and percentage in brackets; Pearson chi-Square test and Kolmogorov-Smirnov two sample test were used. MA: migraine with aura, mT: mitochondrial.

ease[19]. Leber's Hereditary Optic Neuropathy LHON[20], myopathy[21], hypertrophic cardiomyopathy[22], Alpers-Huttenlocher-like disease[23], pseudoexfoliation glaucoma[24], and rhabdomyolysis[25]. The *mt-CO2* gene is a protein-coding gene that encodes for the second subunit of cytochrome C oxidase (complex IV), which is a component of the mitochondrial respiratory chain that catalyzes the reduction of oxygen to water. *mt-CO2* is one of the three subunits responsible for forming the functional core of the cytochrome C oxidase *mt-CO2*. Among its related pathways are gene expression and the epidermal growth factor (EGF)/EGF receptor (EGFR) signaling pathway. Gene Ontology annotations related to this gene include oxidoreductase activity and cytochrome C oxidase activity. *mt-CO2* has been described in relation to cytochrome C oxidase subunit II[26].

On the other hand, the *mt-TK* gene provides instructions for a specific form of tRNA designated as tRNA^{Lys}. The tRNA^{Lys} molecule is involved in the production of proteins that carry out oxidative phosphorylation. A mutation in the *mt-TK* gene was found in maternally inherited diabetes and deafness (MIDD)[10,27]. Several muta-

Table 3: Clinical characteristics of aura migraine and *mt-NC7 m.8277T>C DNA* variations.

Parameters	mt DNA Variations	Migraine with aura genotype		p
Frequency (n,%)		**TT**	**CC**	
1-3/month	mT-NC7 m.8277T>C	18 (85.7)	3 (14.3)	
3-5/month	mT-NC7 m.8277T>C	9 (50)	9 (50)	0.097
5-10/month	mT-NC7 m.8277T>C	7 (70)	3 (30)	
10-15/month	mT-NC7 m.8277T>C	1 (100)	0 (0)	
Intensity (n,%)		**TT**	**CC**	
mild	mT-NC7 m.8277T>C	0 (0)	1 (100)	
moderate	mT-NC7 m.8277T>C	6 (54.5)	5 (45.5)	0.116
severe	mT-NC7 m.8277T>C	29 (76.3)	9 (23.7)	
Duration (n,%)		**TT**	**CC**	
<12 hours	mT-NC7 m.8277T>C	2 (66.7)	1 (33.1)	
12-24 hours	mT-NC7 m.8277T>C	2 (40)	3 (60)	0.294
>24 hours	mT-NC7 m.8277T>C	31 (73.8)	11 (26.2)	
Gender (Male/Female)		**TT**	**CC**	
	mT-NC7 m.8277T>C	7/28	3/12	1.000
Family history of migraine (n, no/yes)		**TT**	**CC**	
	mT-NC7 m.8277T>C	13/22	6/9	0.849
Cigarette use (n, no/yes)		**TT**	**CC**	
	mT-NC7 m.8277T>C	24/11	8/7	0.304
Alcohol use (n, no/yes)		**TT**	**CC**	
	mT-NC7 m.8277T>C	30/5	13/2	1.000
Family history of stroke (n, no/yes)		**TT**	**CC**	
	mT-NC7 m.8277T>C	26/9	11/4	1.000
Family history of CAD (n, no/yes)		**TT**	**CC**	
	mT-NC7 m.8277T>C	25/10	6/9	**0.056**
Family history of ND (n, no/yes)		**TT**	**CC**	
	mT-NC7 m.8277T>C	32/3	10/5	**0.043**

Values are presented as number and percentage in brackets or number of having (yes) / not having (no) each symptom. CAD: coronary artery disease, ND: neurological disease.

tions in the *mt-TK* gene were identified in people with myoclonic epilepsy with ragged-red fibers (MERRF). A small number of subjects with a mutation in the *mT-TK* gene demonstrate both features of MERRF and some of MELAS. These affected individuals are said to have MERFF/MELAS overlap syndrome[28].

In addition, the *mt-TK* gene has been identified in Leigh Syndrome. In the literature, the Mt-CO2 7896 variation is associated with the lack of cytochrome C oxidase level[29]. The *mt-NC7* locus is located between *mt-CO2* and *mt-TK* loci even though it is defined as a non-coding nucleotide region in mitochondrial DNA function. In our study, the 8277 variation of the *mt-NC7* locus was first shown to be associated with migraines with aura. Because it is located between *mt-NC7* locus, *mt-CO2*, and *mt-TK* loci, the investigation of the cytochrome C oxidase levels in migraine patients with aura is thought to be predictive in terms of the phenotype/genotype relationship. The *NC_012920.1: m.8277T> C* variation in the *mt-NC7* locus associated with migraines with aura, and this is the first study to show the effect of mtDNA variations on aura migraines' pathogenesis in four different gene variations and 13 different positions. Therefore, it is thought that our work provides significant contributions to the literature.

Until now, no specific mtDNA variation and locus has been associated with aura migraine. In our study, the CC genotype of the *NC_012920.1: m.8277T> C* variation in the *mt-NC7* locus was found to be higher in migraines with aura than in the control group. Hence, it is the first study to indicate the *mt-NC7* locus, which is a non-coding nucleotide region in mitochondrial DNA function, as a possible risk factor for aura migraine. In addition, we found 12 different mtDNA variations that protect against with-aura migraines. Our investigation is the first study showing that four loci *(mt-NC7, mt-DLOOP1, mt-DLOOP2, mt-ND6)* may be important in the pathogenesis of migraines.

Haplotype analysis of genetic markers' combinations within a chromosome cluster location is valuable because it provides a more powerful approach to genetic studies. Because only the analysis of a single nucleotide can be

Table 4: Haplotype frequencies results, with aura-migraine patients and the control group.

	Haplotype									
	H	H1	H+16311	HV	HV2	HV4	HV1a1a	HV0e	HV9+152	**p**
Patient	2	2	1	1	1	1	1	**2**	1	
Control	2	2	0	1	0	0	0	0	0	NS
TOTAL	**4**	**4**	1	2	1	1	1	2	1	
	Haplotype									
	H1b1	H1c+152	H1b1+16362	H2a2	H55	H5e1	H5e1a1	H8b	H6a2	
Patient	1	1	1	1	1	1	1	1	1	
Control	0	0	0	**4**	0	0	0	0	0	NS
TOTAL	1	1	1	**5**	1	1	1	1	1	
	Haplotype									
	H13a1a1e	I1	I5a	J1b2	J1c	J1c3k	J1c3g	K1b2a1	K1b1b1	
Patient	1	1	1	1	1	1	1	1	1	
Control	1	0	0	2	0	0	0	0	0	NS
TOTAL	2	1	1	3	1	1	1	1	1	
	Haplotype									
	K2	N1b1a	R2+146	T2a1b2a	T1a2b	T1b1	T2	T2a	T2b	
Patient	1	1	1	1	1	1	1	1	1	
Control	0	0	0	0	0	0	1	0	**4**	NS
TOTAL	1	1	1	1	1	1	2	1	**5**	
	Haplotype									
	T2h	U4a	U4b1b1	U4d2	U5a1a1a	U5b2a5	U5a2b	U5b2a2b	V+@72	
Patient	1	1	1	1	1	1	1	1	1	
Control	0	0	0	0	0	0	1	0	0	NS
TOTAL	1	1	1	1	1	1	2	1	1	
	Haplotype									
	W	W+194	D4	G2a5	H1by	H4	H5	H11a1	H11a2	
Patient	1	1	0	0	0	0	0	0	0	NS
Control	0	0	1	1	1	1	**3**	1	1	
TOTAL	1	1	1	1	1	1	**3**	1	1	
	Haplotype									
	H13a2b2	H14a	H15a1	H40	H76	I1c1	J1b1b1	J1c5a	J1d6	
Patient	0	0	0	0	0	0	0	0	0	NS
Control	1	1	1	1	1	1	1	1	1	
TOTAL	1	1	1	1	1	1	1	1	1	
	Haplotype									
	J1c15a	J2b1h	K1a	K1a4c	K1b1+(16093)	K1b1c	M9a'b	M35b2	R0a1a	
Patient	0	0	0	0	0	0	0	0	0	NS
Control	1	1	1	1	1	1	1	1	**2**	
TOTAL	1	1	1	1	1	1	1	1	**2**	
	Haplotype									
	T	T1a1	U1a1a	U1b	U2e1a1	U3b2a1	U8b1b	W6	W7	
Patient	0	0	0	0	0	0	0	0	0	NS
Control	1	1	1	1	1	1	1	1	1	
TOTAL	1	1	1	1	1	1	1	1	1	
	Haplotype									
	V1a1b	X2	X2n	X2I						
Patient	0	0	0	0						NS
Control	1	1	1	1						
TOTAL	1	1	1	1						

Values are presented as number of subjects. Two-Sample Kolmogorov-Smirnov test was, NS: No Statistics were computed.

performed each time, it is possible to eliminate inconsistencies[30]. Our study found that the H2a2, T2b, H5, and T haplotypes to be high in the control group. However, no statistically significant difference was found between the patient group. It may be shown in future studies whether the presence of these haplotypes is protective against the formation of migraines with aura or not.

One of our study's limitations is that the number of patients was low due to the kit's high cost. Our study found a variation related to migraines with aura, but these results need to be studied in different populations with larger numbers of patients. Similarly, no statistically

significant difference was found between haplotype frequencies. Also, the study design was cross-sectional rather than longitudinal. Following up the subjects' clinical progression with the *NC_012920.1: m.8277T>C* variation CC genotype would be of value to assess whether they developed more severe aura migraines over time. One of our study's strengths is the genetic study's geographic extent for mitochondrial variations associated with aura migraines, as it contains the Edirne provincial center and all of its districts. Edirne is located in a region that has not experienced a massive population displacement or migration. This situation is thought to provide homogeneity of genetic studies. Another strength of our study is that it showed that mainly four loci were important in the pathogenesis of migraines with aura, and three of them were protective against migraines with aura. This study is extremely important because it is the first clinical study that shows that the *mT-NC7* locus may play a role in the pathogenesis of migraine with aura.

In the future, a study that conducts full mitochondrial genome sequencing should prove useful in determining the role of mitochondrial and mitochondria-related genes in the formation of migraines. Moreover, sequencing would determine all the nuclear encoding genes and non-coding nucleotide regions affecting mitochondrial function in a large migraine patient cohort. Such a study would contribute to current knowledge about migraine pathogenesis and identify genetic variants that affect the risk of developing migraines.

Conflict of interest

The authors report no conflicts of interest.

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