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HETEROPLASMY-ASSOCIATED MITOCHONDRIAL DNA VARIANTS IN HUMAN BLOOD AND SKELETAL MUSCLE SAMPLES

İNSAN KAN VE İSKELET KASI ÖRNEKLERİNDE HETEROPLAZMİYLE İLİŞKİLİ MİTOKONDRİYAL DNA VARYANTLARI

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ABSTRACT

Objective: Mitochondrial heteroplasmy, a recognized trait in eukaryotic cells, plays a pivotal role in complex disorders like mitochondrial diseases. High-throughput sequencing has improved precision in detecting low-level heteroplasmy and can identify ultra-low-level variants (<1%) associated with heteroplasmy attributes. We aimed to investigate potential genetic and demographic factors associated with heteroplasmy levels in mitochondrial variants by analyzing both blood and muscle tissues in individuals, regardless of their phenotypes.

Material and Methods: High-throughput sequencing was conducted on the mitochondrial genomes of 10 individuals, with an equal gender distribution. Variants with heteroplasmy ratios both ranging from 5% to 95% and out of this range were used for statistical analysis.

Result: A total of 194 heteroplasmic variants were identified, of which 13 displayed lower heteroplasmy ratios in both blood and skeletal muscle samples from females, while the mitochondrial control region (D-Loop) exhibited higher ratios.

Conclusion: The study findings confirm the correlation between the m.10398A>G variant and mitochondrial heteroplasmy levels, consistent with prior research. Additionally, we identified the m.1811A>G variant in *MT-RNR2* and the m.12308A>G vari-

ÖZET

Amaç: Ökaryotik hücrelerin tanımlanmış bir özelliği olan mitokondriyel heteroplazmi, mitokondriyel hastalıkların fenotipik çeşitliliğinde önemli bir rol oynar. Düşük düzeydeki heteroplazminin tespitindeki hassasiyeti artıran yeni nesil dizileme (YND) teknolojisi, heteroplazmi özellikleri ile ilişkili ultra-düşük düzeydeki (%<1) varyantları saptayabilmektedir. Çalışmamız, fenotiplerine bakılmaksızın, bireylerdeki mitokondriyel varyantların heteroplazmi düzeyleri ile ilişkilendirilebilecek potansiyel genetik ve demografik faktörleri incelemeyi amaçlandı.

Gereç ve Yöntem: Cinsiyet dağılımı eşit olan 10 bireyin mitokondriyel genomları üzerinde, yüksek-çıktılı yeni nesil dizileme yöntemi uygulandı. Heteroplazmi oranları %5 ile %95 arasında değişen ve bu aralığın dışında kalan varyantlar, istatistiksel analizler için kullanıldı.

Bulgular: Toplamda 194 heteroplazmik varyant tanımlandı, bunlardan 13'ü dişi bireylerin hem kan hem de iskelet kası örneklerinde daha düşük heteroplazmi oranları sergilerken, mitokondrial kontrol bölgesi (D-ilmiği) daha yüksek oranlara sahipti.

Sonuç: Çalışma bulguları, önceki araştırmalarla uyumlu olarak m.10398A>G varyantı ile mitokondriyel heteroplazmi düzeyleri arasındaki korelasyonu doğruladı. Ayrıca, *MT-RNR2* genindeki m.1811A>G varyantının ve *MT-TL2* genindeki m.12308A>G

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ant in *MT-TL2*, both associated with higher heteroplasmy. Conversely, the m.582T>C variant in *MT-TF*, m.3260A>G in *MT-TL1*, m.3302A>G in *MT-TL1*, m.4409T>C in *MT-TM,* and m.4267A>G in MT-TI were linked to lower heteroplasmy, all involving transition-type alterations. Furthermore, our study hinted at a potential age-related threshold for variant accumulation in the control region. Future studies, involving larger cohorts and advanced expression analysis methods, will further contribute to the validation and enhancement of these findings.

Keywords: Mitochondrial heteroplasmy, *MT-TL1, MT-TL2, MT-RNR2, MT-TM, MT-TI, MT-TF*

varyantının da yüksek heteroplazmi ile ilişkili olduğu gösterildi. Bunun yanı sıra, hepsi tranzisyon tipinde olan, *MT-TF* genindeki m.582T>C, *MT-TL1* genindeki m.3260A>G, *MT-TL1* genindeki m.3302A>G, *MT-TM* genindeki m.4409T>C ve MT-TI genindeki m.4267A>G varyantlarının ise düşük heteroplazmi oranı ile ilişkili olduğu bulundu. Çalışmamız ayrıca, kontrol bölgesindeki varyant birikimi için potansiyel bir yaş sınır eşiği olabileceğini de işaret etmiştir. Gelecekte, daha büyük örnek sayısı ve gelişmiş analiz yöntemlerinin kullanılacağı çalışmalar, bu bulguların doğrulanması ve geliştirilmesine katkı sağlayacaktır.

Anahtar Kelimeler: Mitokondriyel heteroplazmi, *MT-TL1, MT-TL2, MT-RNR2, MT-TM, MT-TI, MT-TF*

INTRODUCTION

Mammalian mitochondrial DNA (mtDNA) is a double-stranded circular molecule of about 16.5 kb long in the mitochondrial matrix. It encodes two rRNAs (12S rRNA, 16S rRNA), 22 tRNAs, and 13 subunits of the electron transport chain (ETC) complexes I, III, IV, and V (1, 2). Regulation of their transcription relies on a specific region named the control region, also called the displacement loop (D-loop) region (3, 4).

A unique aspect of mtDNA and mitochondria is their presence in multiple copies within a cell, and the structure of multiplicity is influenced by factors like metabolism and exposure to stressors such as reactive oxygen species (ROS) (5). This multi-copy nature leads to a wide range of ratios (from 5% to 95%) of wild-type and variant alleles at specific mtDNA positions, a phenomenon called heteroplasmy (6). Heteroplasmy, combined with somatic mosaicism resulting from post-zygotic changes in mtDNA, contributes to the varying impact of pathogenic mtDNA variants. Notably, pathogenic mtDNA changes are anticipated to predominantly affect neural and skeletal muscle cells due to mitochondria's primary role in providing adenosine triphosphate (ATP) for cellular processes and metabolites for macromolecule synthesis (7). Heteroplasmy varies among tissues due to energy needs and changes over time due to relatively more primitive repair mechanisms (8). While the differences in tissue and age-related heteroplasmy have been recognized for a long time, the prevalence of these differences in mitochondrial diseases has now been confirmed through advanced sequencing techniques, also known as next-generation sequencing (NGS). This confirmation is supported by studies and relevant to the various clinical presentations observed in both rare and common genetic disorders (9, 10).

Acknowledging the pivotal contribution of NGS in elucidating the mutational landscape of mtDNA and its dynamic heteroplasmic profiles across diverse tissue types, our primary objective rests in the comparative assessment of heteroplasmic ratios. In this study, our primary objective was to investigate the potential genetic and demographic factors that may modify the level of heteroplasmy in mitochondrial variants across two distinct tissue types. To achieve this, we performed NGS analyses of mitochondrial genomes extracted from blood and skeletal muscle samples, without regard to individuals' disease status or clinical phenotypes.

MATERIAL and METHODS

Ethical approval was obtained from the Istanbul Faculty of Medicine Ethics Council at Istanbul University (Date: 23.11.2018, No: 1626 and 872). Written informed consent was obtained from the participants or their legal guardians. DNA was isolated from blood and residual biopsy samples were obtained from the leg region for S1-S3 and from the shoulder and arm area (*M. deltoideus*) for S4-S10. mtDNA (16,569 bp) was sequenced using Ion Torrent's Ion PGM by generating amplicon libraries of 400 bp-length of fragmented long-PCR products and aligned with the corrected Cambridge reference sequence (11). Variant alleles with a minimum of three reads from both forward and reverse strands were chosen for further analysis, and heteroplasmy levels were evaluated based on established cut-off values from prior studies, with lower and upper thresholds set at 0.05 and 0.95, respectively (12). Mann-Whitney U test or Student's t-test for mean comparisons and the Pearson test for numeric data correlations were performed for statistical investigation.

RESULTS

The study subjects were composed of five females and five males, with an average age of 36.6 years. All mtDNA was verified to be encompassed by the sequenced reads. The mean sequence depth was 1648±512 (range: 171 to 2000). There was no significant difference (p=0.94) in sequence depth between blood (1675±488) and skeletal muscle (1627±528). mtDNA was totally covered, unveiling 194 variants.

Initially, we compared the mtDNA variants present in the blood and skeletal muscle samples from our cohort of 10

subjects. This comparative analysis unveiled 48 variants exclusively detected in blood samples, 67 variants exclusively found in skeletal muscle, and 79 variants that were present in both blood and skeletal muscle samples.

Statistical analysis

The heteroplasmy ratio of the variants identified in both blood and skeletal muscle was lower in females.

Heteroplasmy ratios were examined in both blood and skeletal muscle to explore potential influencing factors. This involved comparing tissue origin, and subject characteristics such as gender and age. Analysis of gender distribution revealed a lower heteroplasmy ratio in females (p=0.022) compared to males (Figure 1A). Overall heteroplasmy ratio comparisons between tissue types did not yield any significant differences (p=0.44) (Data not shown). However, the heteroplasmy ratio in variants observed across both tissues was lower than those found exclusively in blood or skeletal muscle (Figure 1B). This difference showed statistical significance for blood (p=0.0063) and borderline significance for skeletal muscle (p=0.055). Given the observed gender-related variation in heteroplasmy ratio, we examined its interplay with tissue origin. This assessment revealed that the reduction in heteroplasmy ratio among females was specific to variants present in both tissues (p=0.011) (Figure 1C). Furthermore, when the heteroplasmy ratios of the 194 variants presented in our study were individually assessed in each sample (total of 472), it was observed that transitions (Ti) were notably lower than the transversion (Tv) (p=0.017) (Figure 1D).

Variants detected in both tissues (blood and skeletal muscle) are more likely to be shared among individuals, regardless of their heteroplasmy ratio

Since the notable difference in heteroplasmy ratio was specifically observed in variants read across both tissues, rather than solely in blood or skeletal muscle, we proceeded to assess the variants based on their sharing ratio across two tissues and among 10 subjects. Our findings indicated that over half of the variants (52.58%) were detected in a single subject and within only one tissue type. Conversely, the variants identified in more than half of the subjects were found in both tissue types. This positive correlation between variant sharing ratio across tissues and subjects appeared unrelated to the heteroplasmy ratio (Supplementary data).

The relative frequency of variants in the control region displayed an age-dependent increase

Due to the recognized distinct variant frequencies in the control region and coding region of mtDNA, with the control region being acknowledged as a polymorphic range that accumulates variants, we examined heteroplasmy distribution in both areas (13, 14).

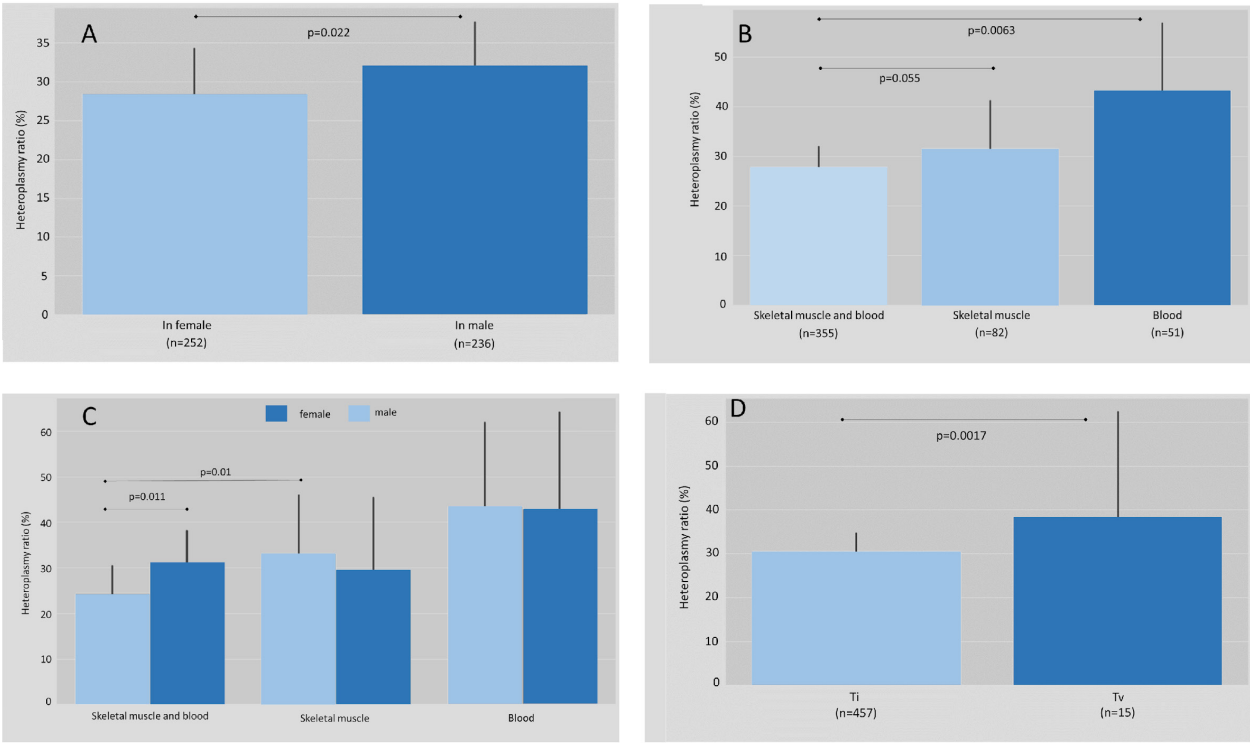

Figure 1: The correlations of the heteroplasmy ratio with gender (A), tissue origin (B), gender-dependent tissue origin (C), and substitution types (D)

The heteroplasmy distribution across mtDNA indicated an accumulation of heteroplasmy and a reduction of homoplasmy in the control region (Data not shown). To explore differences in heteroplasmy ratio or variant frequencies, our findings showed that the variant frequency within the control changed with age. This alteration appeared as an increase at earlier ages, followed by a slight decrease at later stages. The positive correlation between control region variant frequency and age became statistically significant by the age of 42 (r=0.92, p=0.0086). After age 49, there was a tendency of decreasing of the variant frequency, implying an increased frequency in the coding region (Figure 2). However, the status of the variant frequency between 42 and 49 could not be shown due to a lack of samples between these ages. Additionally, the Ti/Tv ratio significantly declined (p=0.033) after age 42 (Figure 3).

The mitochondrial variant m.10398A>G is linked to higher heteroplasmy

A recent study indicated an association between the m.10398A>G variant and overall heteroplasmy levels (14). To investigate further, we compared heteroplasmy levels between subjects with and without the m.10398A>G variant, considering the significant heteroplasmy ratio difference between the coding and control regions (Figure 4A). This comparison highlighted a significantly higher overall heteroplasmy level in the presence of this variant (Figure 4B). This trend was notably pronounced within the coding region (Figure 4C) and among individuals of the female gender (Figure 4D).

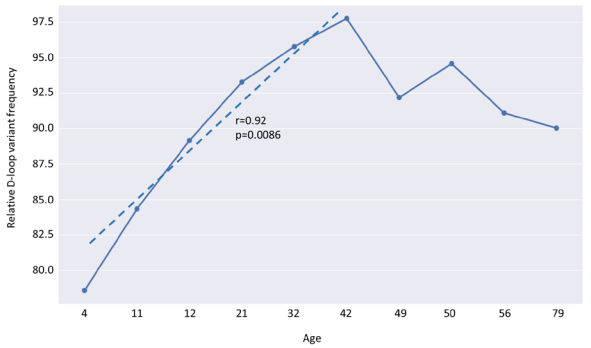

Figure 2: Positive correlation between control region variant frequency and age

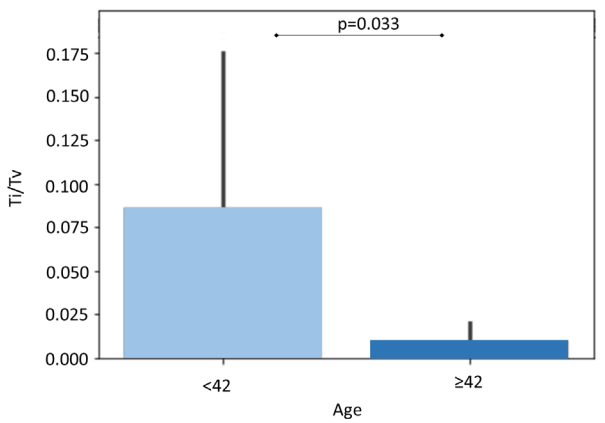

Figure 3: Decreased Ti/Tv ratio observed after age 42

Other heteroplasmy-related mtDNA variants

Supporting the correlation between the presence of the m.10398A>G variant and mitochondrial heteroplasmy ratio, we wondered if other variants could similarly impact heteroplasmy levels. To explore this, we compared heteroplasmy levels in cases carrying each of the 194 variants against non-carriers. Two variants (m.1811A>G and m.12308A>G) exhibited an association with higher heteroplasmy ratios akin to m.10398A>G, while five variants (m.582T>C, m.3260A>G, m.3302A>G, m.4267A>G, and m.4409T>C) appeared to correlate with lower heteroplasmy levels (Figure 5). All these variants were of the transition type and found in both tissues. The mean heteroplasmy ratio for higher-heteroplasmy-associated variants across three cases (97.5±1.68) was higher than that for lower-heteroplasmy-associated variants across eight cases (0.39±0.12). Positive associations of m.1811A>G and m.12308A>G with heteroplasmy ratio were particularly pronounced in the coding region (p=0.00031 for m.1811A>G, p=0.0005 for m.12308A>G). Notably, the higher-heteroplasmy-associated variants were situated in *MT-TL2* and *MT-RNR2* genes, while the lower-heteroplasmy-associated variants were in *MT-TM*, *MT-TI*, *MT-TL1*, and *MT-TF* genes.

DISCUSSION

Mitochondrial heteroplasmy, prevalent in multicellular organisms, is a recognized characteristic of eukaryotic cells. Yet, in a medical context, its significance is amplified due to its pivotal role in the pronounced clinical diversity observed in mitochondrial diseases (15-19).

The clinical severity of mitochondrial disorders is notably modulated by the magnitude and tissue-specific allocation of heteroplasmic mutations (20). In addition to mitochondrial disorders, a multitude of complex diseases including metabolic disorders, neurodegenerative conditions, and diverse forms of cancer have also been demonstrated to exhibit correlations with the heteroplasmy levels of mitochondrial variants (21-25).

While measuring mitochondrial heteroplasmy is of vital significance, it presents challenges due to technical and biological factors. Advances in sequencing technologies have largely overcome technical obstacles, allowing for more accurate measurement of even low-level heteroplasmy. Furthermore, various NGS-based methods, including modified approaches, exhibit varying sensitivity

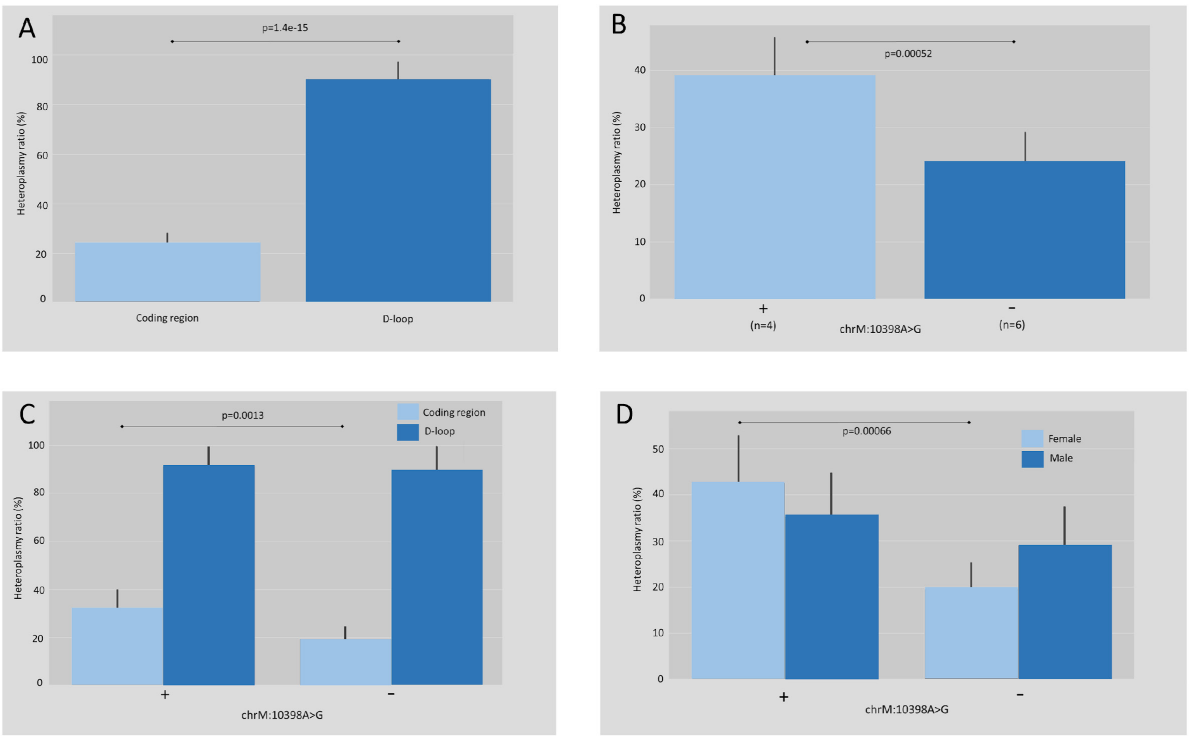

Figure 4: Heteroplasmy ratio alteration influenced by m.10398A>G variant. While both the control region (A) and m.10398A>G variant (B) are tied to increased heteroplasmy ratios, the elevated heteroplasmy linked to the m.10398A>G variant was limited to the coding region (C) and the female gender (D)

to heteroplasmy levels (26, 27). Nevertheless, biological mysteries about mitochondrial heteroplasmy, such as its nature, mechanism, and tissue distribution, remain elusive. In this study, we investigated correlations between mitochondrial heteroplasmy levels and associated features in 10 patients using deep sequencing.

While aligning with prior studies in some aspects, our findings introduced novel insights into the determinants of mitochondrial heteroplasmy ratios. Our investigation identified 13 heteroplasmic variants with heteroplasmy levels ranging from 5% to 95%. Since this count fell short of yielding statistically robust conclusions, we considered low-level heteroplasmies in our subsequent analyses, based on prior studies employing deep sequencing and single-cell analysis that have underscored the prevalence of heteroplasmic variants, often at extremely low levels (<1%), in the tissues of even healthy individuals (10, 15, 28, 29).

Nonetheless, our limited pool of heteroplasmic variants sufficed to reveal that skeletal muscle exhibited a higher heteroplasmic variant ratio compared to blood. This finding can be rationalized by disparities in mitotic activity between the two tissues, as demonstrated for the m.3243A>G variant in *MT-TL1* gene, which displayed a declining heteroplasmy over time in mitotically active blood cells but not in post-mitotic tissues such as skeletal muscle (30).

A significant finding in our study was the gender-related variation in heteroplasmy levels. Within our cohort, females exhibited lower heteroplasmy ratios compared to males. While a urine sample study involving 235 patients found higher heteroplasmy for the m.3243A>G variant in males, a leukocyte DNA investigation of 1035 individuals did not detect notable gender disparities (15, 31). The correlation between mitochondrial heteroplasmy and gender appears contentious in light of prior research. However, even when acknowledging such a correlation, the precise mechanism driving gender-related modulation in heteroplasmy remains enigmatic.

We also observed a more pronounced reduction in heteroplasmy among females, particularly in variants identified within both skeletal muscle and blood. These findings suggest that gender-related distinctions in mitochondrial heteroplasmy might be confined to specific tissue types. Notably, given the elevated heteroplasmy ratios of tissue-specific variants relative to common

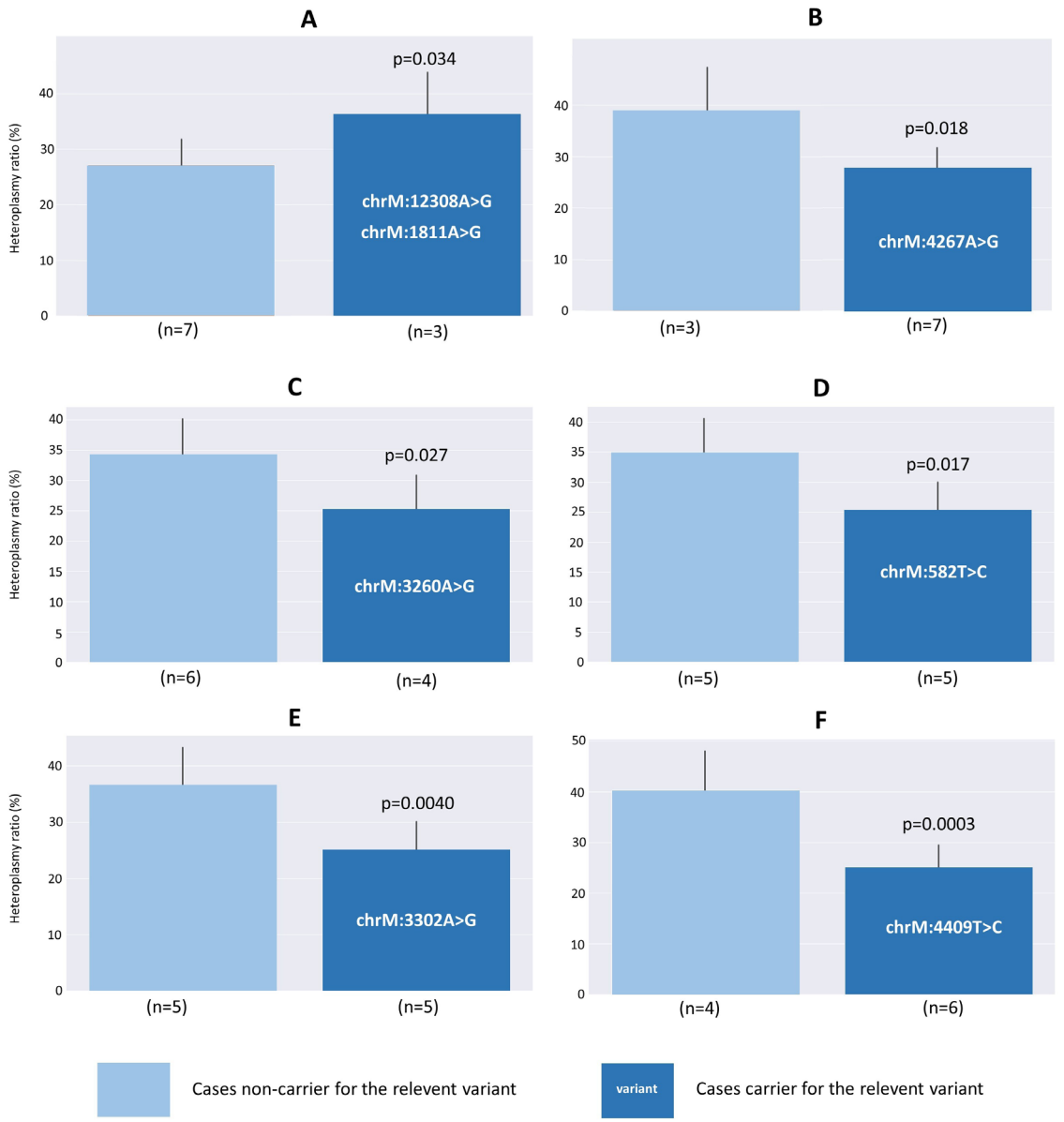

Figure 5: Variants increasing (**A** and **B**) or decreasing (**C** to **G**) the heteroplasmy ratio of total mtDNA variants

variants, we hypothesize that tissue-restricted variants may selectively amplify their heteroplasmy ratios during tissue development. It is established that, contingent on cell type, mitochondrial variations originating from a single molecule within a cell can either diminish or proliferate clonally over time. Given that this process hinges on cell division and fitness, gender-dependent disparities in heteroplasmy ratios can be attributed to gender-driven distinctions in the mitotic activity and fitness of mitochondrial variants within cells of the analyzed tissues across genders.

Our study made an original discovery by establishing a positive relationship between control region variant frequency and age. This finding aligns with prior research, which has emphasized the accumulation of sequence variants within the control region (13, 14). However, our data also suggests that there may be a threshold for such variant accumulation within the control region, possibly occurring between the ages of 42 and 49. It's important to acknowledge that a more precise cutoff age would require a wider age range in our cohort. While this correlation hasn't been reported before, numerous studies have investigated age- and tissue-dependent changes in mito-

chondrial variants. One of these studies measured length heteroplasmy in a polycytosine tract of the mitochondrial HV2 region in blood, brain, heart, liver, skeletal muscle, and hair shaft samples collected during autopsies of 25 individuals and revealed that the frequency of heteroplasmy did not differ significantly with age (32). However, a differentiated distribution pattern was observed across blood and organ tissues over the age of 28 for all heteroplasmic mtDNAs, while in hair shafts over the age of 44 for the homoplasmic individuals in that study. Although our subsequent finding implied a connection between age and mitochondrial variant frequency, the majority of our prior data unraveled the factors influencing the heteroplasmy ratio of mitochondrial variants.

It is noteworthy that mitochondrial heteroplasmy can also be influenced by the nuclear genome (33). A Genome-Wide Association Study (GWAS) identified 20 loci, including the mitochondrial transcription factor A (*TFAM*) gene, as being linked to heteroplasmy (34). However, genetic variants serving as modifier factors for mitochondrial variant heteroplasmy are not confined solely to nuclear genome variants. Recent investigations propose that mitochondrial genome variants may also assume a role as modifiers in mitochondrial variant heteroplasmy levels. Notably, one of these studies demonstrated an association between the m.10398A>G variant and elevated heteroplasmy levels in brain tissue (35). In consonance with this study, our findings also indicate an association of this variant with higher heteroplasmy ratios in both blood and skeletal muscle. However, in contrast to the prior study's observation in the control region, our results highlight this association within the coding region. This variance could potentially stem from variations in cohort size or the nature of the utilized tissue.

As our findings associated the m.10398A>G variant and mitochondrial heteroplasmy levels, we probed the possibility of additional variants exhibiting similar associations. Subsequent analyses unveiled seven additional mtDNA variants that appear to be correlated with elevated or balanced heteroplasmy levels. Among these, two exhibited higher heteroplasmy levels in mtDNA variants, with a notable concentration within the coding region. Intriguingly, all these variants were characterized as transitions and were present in both tissue types.

The heteroplasmy association of these variants may be attributed to the function of the genes in which they are situated. Nevertheless, a conspicuous common feature between *MT-TL2* and *MT-RNR2* genes for higher-heteroplasmy-associated variants, or between *MT-TM*, *MT-TI*, *MT-TL1*, and *MT-TF* genes for lower-heteroplasmy-associated variants could not be discerned. Notably, the m.1811A>G variant, a higher-heteroplasmy-associated variant, has been reported to induce significant changes in mitochondrial 16S rRNA secondary structure (36). However, whether alterations in the secondary structure of mitochondrial 16S rRNA could influence heteroplasmy remains an area requiring further investigation. Another plausible scenario is that these variants might act as expression quantitative trait loci (eQTL) for specific mitochondrial genes, akin to the demonstrated role of the m.10398A>G variant in a prior study. In that context, the m.10398A>G variant was identified as an eQTL for the *MT-ND3* gene in the brain and the *MT-ND4* gene in lymphoblastoid cell lines (35). Evaluating the potential eQTL role of these variants necessitates expression analysis conducted within the pertinent tissues.

The primary limitation of this study lies in its small sample size. Furthermore, the study population's inherent heterogeneity challenged discerning potential phenotype-genotype correlations pertaining to mitochondrial variants.

CONCLUSION

Our recent study has highlighted that mitochondrial heteroplasmy levels are influenced by both tissue type and gender, aligning with prior research. Additionally, our study suggests a potential age-related threshold for variant accumulation within the control region, alongside identifying additional seven novel association of mtDNA variants that could potentially serve as modifiers for mitochondrial heteroplasmy. Subsequent investigations employing larger cohorts and expression analysis methodologies are anticipated to validate and expand upon our findings.

Ethics Committee Approval: The study has ethical approval from the Istanbul Faculty of Medicine Ethics Council at Istanbul University (Date: 23.11.2018, No: 1626 and 872).

Informed Consent: Written informed consent was obtained from the participants or their legal guardians.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- Z.O.U.; Data Acquisition- A.G., A.D., G.Ş.; Data Analysis/Interpretation- Ç.G., A.G., G.Ş., G.T., Z.O.U.; Drafting Manuscript- Ç.G., Z.O.U.; Critical Revision of Manuscript- Ç.G., A.G., G.Ş., G.T., Z.O.U.; Final Approval and Accountability- Ç.G., Z.O.U.

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Conflict of Interest: The authors have no conflict of interest to declare.

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Data Availability Statement: Data available within the article or its supplementary materials.

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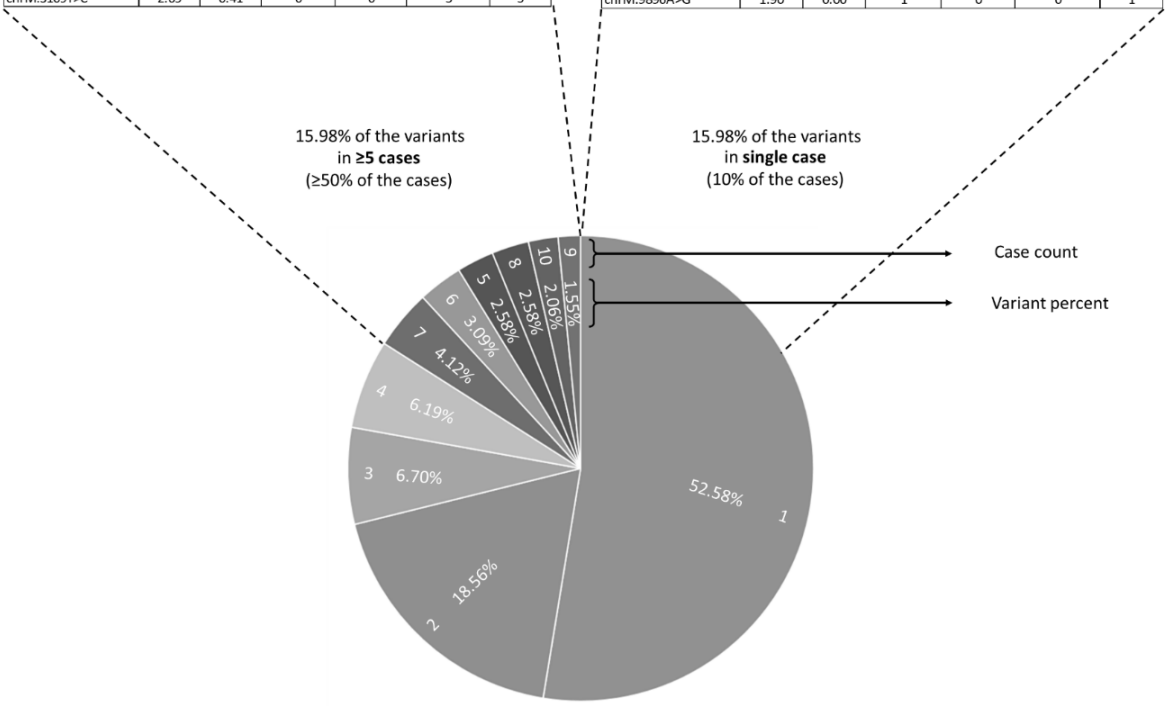
Variant	Heteroplasmy		Tissue origin			Total number
	Mean	Std	only SM	only B	SM and B	
chrM:1630A>G	0.35	0.10	0	0	10	10
chrM:522CAC>C	2.75	0.44	0	0	10	10
chrM:4267A>G	0.45	0.15	0	0	10	10
chrM:3264T>C	1.31	0.64	0	0	10	10
chrM:608A>G	0.43	0.10	0	0	9	9
chrM:4274T>C	0.43	0.10	0	0	9	9
chrM:3291T>C	0.46	0.12	0	0	9	9
chrM:4289T>C	0.37	0.06	0	0	8	8
chrM:3271T>C	0.43	0.11	0	0	8	8
chrM:593T>C	0.39	0.08	0	0	8	8
chrM:4263A>G	0.33	0.07	0	0	8	8
chrM:1616A>G	0.34	0.06	0	0	8	8
chrM:10398A>G	98.80	0.18	0	0	7	7
chrM:4279A>G	0.33	0.09	0	0	7	7
chrM:7028C>T	99.38	0.10	0	0	7	7
chrM:11719G>A	98.17	0.40	0	0	7	7
chrM:5613T>C	0.33	0.06	0	0	7	7
chrM:4409T>C	0.30	0.05	0	0	7	7
chrM:9909T>C	5.09	0.88	0	0	7	7
chrM:15326A>G	99.66	0.10	0	0	7	7
chrM:10415T>C	0.36	0.11	0	0	6	6
chrM:4302A>G	0.33	0.09	0	0	6	6
chrM:1659T>C	0.26	0.05	0	0	6	6
chrM:3302A>G	0.43	0.11	0	0	6	6
chrM:3260A>G	0.38	0.06	0	0	6	6
chrM:12261T>C	0.74	0.36	0	0	6	6
chrM:1438A>G	99.78	0.04	0	0	5	5
chrM:3288A>G	0.43	0.07	0	0	5	5
chrM:582T>C	0.40	0.09	0	0	5	5
chrM:4300A>G	0.26	0.05	0	0	5	5
chrM:3109T>C	2.69	0.41	0	0	5	5

Variant	Heteroplasmy		Tissue origin			Total number
	Mean	Std	only SM	only B	SM and B	
chrM:5728T>C	0.50	0.00	1	0	0	1
chrM:14709T>C	0.25	0.00	0	1	0	1
chrM:642T>C	0.25	0.00	0	1	0	1
chrM:982A>AT	99.45	0.00	1	0	0	1
chrM:7510T>C	0.40	0.00	1	0	0	1
chrM:9698T>C	97.98	0.00	0	1	0	1
chrM:13676A>G	2.50	0.00	1	0	0	1
chrM:14128A>G	19.93	0.00	1	0	0	1
chrM:14723T>C	0.40	0.00	1	0	0	1
chrM:636A>G	0.30	0.00	1	0	0	1
chrM:3480A>G	98.60	0.00	0	1	0	1
chrM:7505T>C	0.45	0.00	1	0	0	1
chrM:14167C>T	99.28	0.00	0	1	0	1
chrM:5692T>C	0.21	0.00	1	0	0	1
chrM:3283G>A	0.40	0.00	0	1	0	1
chrM:9055G>A	98.54	0.00	0	1	0	1
chrM:983C>T	99.45	0.00	1	0	0	1
chrM:199T>C	99.65	0.00	1	0	0	1
chrM:6956T>C	99.75	0.00	1	0	0	1
chrM:16224T>C	98.05	0.00	0	1	0	1
chrM:16002T>C	0.43	0.00	0	1	0	1
chrM:263A>G	99.60	0.00	0	1	0	1
chrM:8276C>T	99.42	0.00	1	0	0	1
chrM:3277G>A	0.20	0.00	0	1	0	1
chrM:14370A>C	4.45	0.00	1	0	0	1
chrM:15812G>A	99.69	0.00	1	0	0	1
chrM:5327C>A	2.36	0.00	1	0	0	1
chrM:625G>A	0.30	0.00	0	1	0	1
chrM:15908T>C	0.22	0.00	0	1	0	1
chrM:9896A>G	1.90	0.00	1	0	0	1



Supplementary Data: Variants' Percentage Distribution and Counts of Cases Sharing the Same Variants. The pie chart shows the total number of samples sharing the same variant (Case count), and the percentage of the shared variant (Variant percent). The tables above the pie chart display the mean and standard derivation of the heteroplasmy ratio of individual variants and the count of the cases sharing this variant, with (Tissue origin) and without (Total number) considering tissue type. While all variants shared by more than half of the cases (SM + B > 5 and Total number > 5) were read in both tissues (left table), all variants unique to a single case, not shared by more than one case (SM + B = 0 and Total number = 1), were read just in one of two tissues (right table).