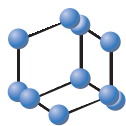


RESEARCH ARTICLE


**BENTHAM
SCIENCE**

Mitochondrial Common Deletion Level in Blood: New Insight into the Effects of Age and Body Mass Index


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Abstract: Background: Age-related decrease in mitochondrial activity has been reported in several tissues. Reactive Oxygen Species (ROS) produced from defected mitochondria lead to aging and accumulate through time. However, studies about the mitochondrial DNA mutation level in blood are contradictory. Other lifestyle factors may modify the effects of age in post-mitotic tissues such as blood. The BMI represents the sum of the various lifestyle factors.

Objective: We proposed that age, obesity and mtDNA deletion are three ROS producing factors, which may interact with each other and induce senescence.

Methods: In a cross-sectional study, 172 male and female volunteers without known mitochondrial diseases were selected and the presence of common mitochondrial 4977bp deletion (Δ mtDNA⁴⁹⁷⁷) evaluated using Nested-PCR.

Results: Our results showed that a high percentage of samples (54.06%) harbor common deletion in blood. Furthermore, both BMI and the Δ mtDNA⁴⁹⁷⁷ levels significantly decrease with age. The chronological age, BMI and Δ mtDNA⁴⁹⁷⁷ reciprocally affect each other.

Conclusion: Our data suggest that age affects purifying selection and BMI, which may influence the relative level of the mtDNA common deletion in blood.

ARTICLE HISTORY

Received: June 04, 2018
Revised: January 06, 2019
Accepted: January 25, 2019

DOI:
10.2174/1874609812666190201163421



CrossMark

Keywords: Aging, mitochondrial common deletion, BMI, ROS, Nested-PCR, mtDNA.

1. INTRODUCTION

Mitochondria are the hub of cellular metabolism in which fatty acid, NADH and FADH are converted into ATP via Oxidative Phosphorylation (OXPHOS). Respiratory function of mitochondria in human tissues such as muscle fibers, brain cells, liver and skin, decrease with age [1, 2]. The age-related decrease in the respiratory enzymes activities was also reported in fly, rats, dogs and monkeys [3, 4]. Because of the crucial role of mtDNA in the OXPHOS, accumulation of mtDNA mutations may contribute to aging phenotype [5, 6]. Furthermore, the age-associated decrease in mitochondrial energy production can lead to higher Reactive Oxygen Species (ROS) formation and mtDNA mutations in aged tissues, creating a vicious cycle of mutagenesis that amplifies the production of free radicals [2, 7, 8]. Mutant mtDNA was reported to accumulate with time in cultured cell [9]. mtDNA mutations progressively accumulate with age [10] in a variety of human and mice cells [11], and show a tissue-specific variation [12]. In differentiated tissues with active oxidative metabolism, such as skeletal muscle, heart, and

brain, a higher level of mutant mtDNA accumulate during the aging process [13-15]. In contrast, in rapidly dividing cells the mtDNA mutation level gradually decreases with time. For example, cancerous tissues present a lower frequency in mtDNA deletion than adjacent non-cancerous tissue [16] as well as in progenitor cells [17]. The mitochondrial common deletion (Δ mtDNA⁴⁹⁷⁷) is a large-scale 4997 base pair deletion spanning ATPase 8 to ND5 [18]. The Δ mtDNA⁴⁹⁷⁷ causes loss of nearly one-third of mtDNA genome [19]. The clinical features associated with Δ mtDNA⁴⁹⁷⁷ has been reported in several sporadic diseases including Alzheimer's disease, Pearson's syndrome, skin photo-aging, Kearns-Sayre syndrome and chronic progressive external ophthalmoplegia [16]. Alterations in mitochondrial function due to Δ mtDNA⁴⁹⁷⁷ accumulate in tissues during aging and have been used as an indicator of mtDNA oxidative damage and ROS production [20]. Furthermore, ROS production is considered a probable mechanism in the pathogenesis and development of many diseases, including obesity and metabolic syndrome [21]. Obese people display elevated levels of systemic oxidative stress and adipose tissue represents an important source of ROS [22]. In this study, we compared the Δ mtDNA⁴⁹⁷⁷ level in overweight cases to normal controls, taking into account age, gender, LDL and HDL. We hypothesized that age and BMI level are two ROS

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producing factors, which have additive effects on mtDNA deletion level. Therefore, subjects with higher BMI and more chronological age would show a higher level of Δ mtDNA⁴⁹⁷⁷.

2. MATERIALS AND METHODS

2.1. Participants

In this case-control study, we selected 172 subjects from Golestan Province, Iran. Participants were between 10 and 80 years old. Samples younger or older than this age range were excluded. The participants had no apparent mitochondrial diseases. The Ethics Committee approved the study protocol and informed consent was obtained from participants before blood sample collection. We recorded personal information including age, sex and BMI for all participants.

2.2. Anthropometric Measurements

Using a digital scale, the participants' weight was measured with a precision of 0.1 kg. Height was measured in centimeters with an accuracy of a decimal point. The subjects were divided into three categories according to Body Mass Index (BMI): Normal ($18.5 < \text{BMI} \leq 24.9$), overweight ($25 < \text{BMI} \leq 30$) and obese ($\text{BMI} > 30$). LDL and HDL levels were measured and 2.5 ml of blood was stored in a refrigerator at 4°C for DNA extraction.

2.3. Genotyping

Genomic DNA was extracted from the whole blood using phenol-chloroform method. To detect low levels of the 4977 bp deletion, nested PCR analysis was performed. Two pairs of nested primers for detection of the 4977 bp deletion were; 1F: AACCACAGTTTCATGCCCATC; 1R: TGTTAG-TAAGGGTGGGGAAGC for first and 2F: ACCCTATTGCACCCCTCTAC; and 2R: CTTGTCAGG G AGGTAGCGATG for second PCR rounds. PCR carried out in 25 μ l reaction volume containing each primer 1 μ M, 2.5 μ M en-

zyme buffer, 2 μ M Mgcl₂, 1 μ M dNTP mix and, 0.2 μ M Tag enzyme. Then, the extracted DNA was added to 1 μ M in 25 μ l total volume (all materials from TaKaRa, Seoul, Korea). The PCR condition for each round was set as initial denaturation at 94°C for 5 min; then 30 cycles at 94°C for 30 s, 55°C for 45 s and 72°C for 60 s; and a final extension at 72°C for 10 min. PCR products were electrophoresed on a 2% agarose gel and verified by sequencing.

2.4. Statistical Analysis

SAS JMP (JMP[®], Version 11. SAS Institute Inc., Cary, NC, 1989-2007.) was used for data analysis. Logistic regression and contingency table were used to evaluate the relationship between Δ mtDNA⁴⁹⁷⁷ with Age, Gender, BMI, LDL and HDL. Matrix of interaction plots was generated to test possible interaction effects among age, gender, BMI and Δ mtDNA⁴⁹⁷⁷ in the proposed model. A p-value of 0.05 was considered as the threshold of significance level for each test.

3. RESULTS

One hundred and seventy-two samples were studied. The presence of deletion was indicated by the appearance of a 474 bp band after the first round of PCR and 358 bp band after the second round of PCR. Wild-type mtDNA did not yield any PCR product because of the large flanking region (>5-kb) (Fig. 1).

Tables 1 shows patient's characteristics. Totally, 93 (54.06%) samples showed Δ mtDNA⁴⁹⁷⁷. The deletion was more abundant in female than male (p-value <0.0001). Δ mtDNA⁴⁹⁷⁷ was observed in 19 normal, 45 overweight and 29 obese individuals. Although the rate of deletion increases with BMI, there is no significant correlation between deletion level and BMI score (Table 1). There was no significant correlation between Δ mtDNA⁴⁹⁷⁷ and serum lipoproteins. However, age showed a significant relationship with deletion (Table 1).

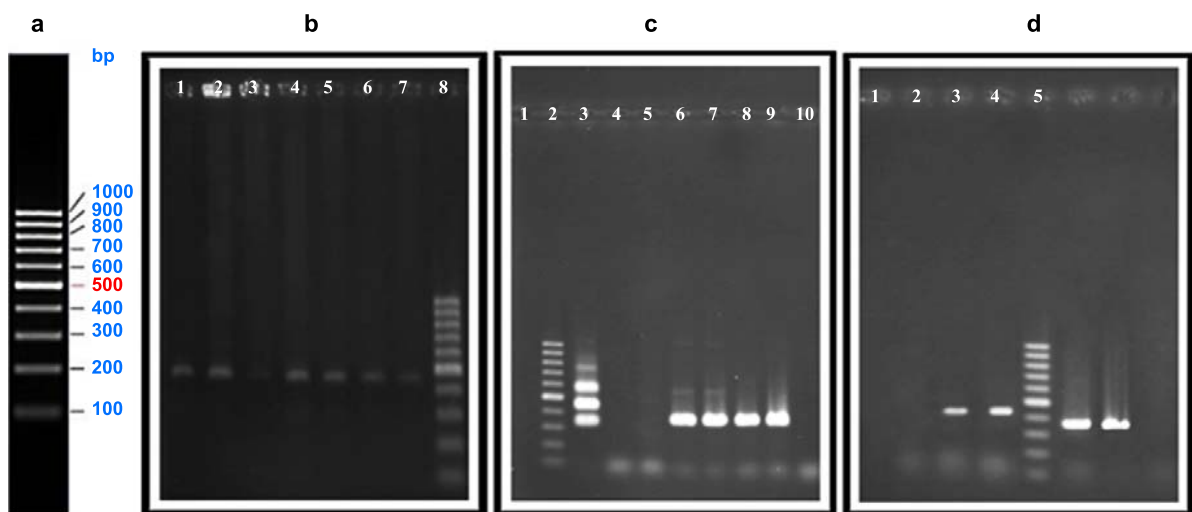


Fig. (1). Agarose gel electrophoresis of the PCR products. (a) The 1 kb DNA Ladder consists of 10 bands spanning 100 bp to 1,000 bp. (b) PCR analysis of Δ mtDNA⁴⁹⁷⁷ in the first round Nested PCR (474 bp band). (c) PCR analysis of 4977 bp deletion in the second round Nested PCR (358 bp band). (d) Comparison between the results of PCR screening of the first and second rounds on one gel, lanes 1-4 contain PCR products amplified in the first round of PCR and lanes 6-8 contain PCR products amplified in the second round of PCR. Lanes 2 and 8, 3 and 7, 4 and 6 contain PCR products of the same subjects respectively that shows subject 2 is lack of deletion.

Table 1. The genotype of Δ mtDNA⁴⁹⁷⁷ and patient characteristics.

		Genotype		OR (95% CI)	p value
		Deletion	No Deletion		
Cases, n (%)		93 (54.06)	79 (45.94)	-	-
Gender	Male, n (%)	32 (37.2)	54 (62.79)	0.1622	<0.0001
	Female, n (%)	67 (77.9)	19 (22.09)		
BMI	Normal, n (%)	19 (20.43)	19 (24.05)	1.01	0.5303
	Overweight, n (%)	45 (48.39)	37 (46.84)		
	Obese, n (%)	29 (31.18)	23 (29.11)		
LDL (Mean±SD)		107.56±26.17	100.08±31.94	0.9772	0.166
HDL (Mean±SD)		37.57±10.59	39.99±13.02	1.018	0.2723
Age (Mean±SD)		43.22±14.62	48.66±13.51	1.027	0.0225

OR, Odds Ratio; CI, Confidence Interval.

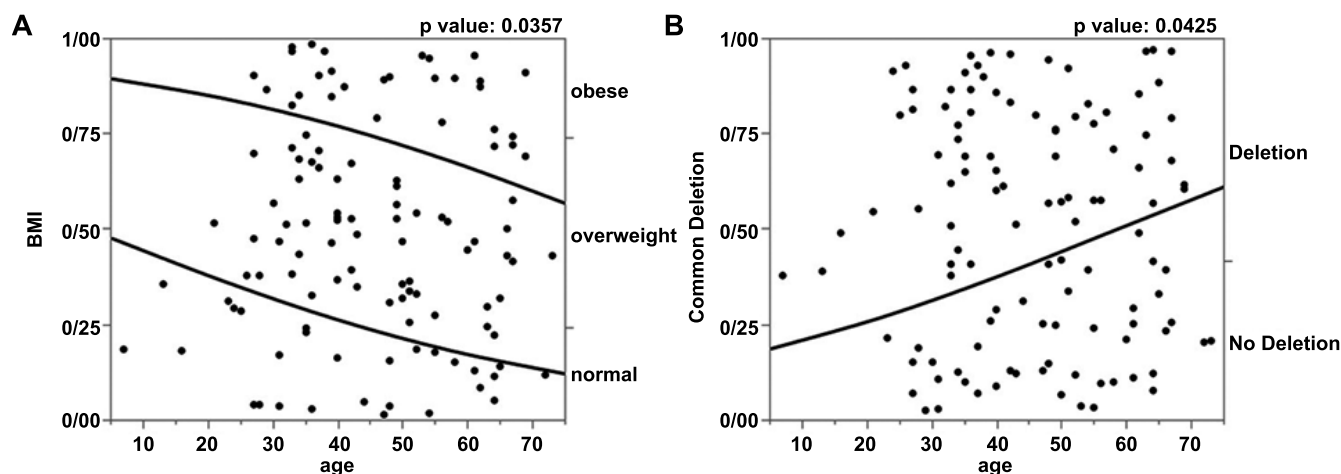


Fig. (2). Regression analysis of Age, mtDNA deletion and BMI. **A)** BMI decreases with age. In obese, overweight and normal groups, BMI level decreases with chronological age (p -value: 0.0357). **B)** Δ mtDNA⁴⁹⁷⁷ decreases with age. Mutation level decreased in older participants (p -value: 0.045).

BMI score showed a significant association with age, LDL and HDL. Regression analysis (Fig. 2A) showed that BMI level decreases gradually with age in all cases (p value=0.0357). Furthermore, Δ mtDNA⁴⁹⁷⁷ and age have a significant correlation (Fig. 2B) so that, deletion level decreases with age (p value=0.0225). However, interaction profiles did not confirm any direct interactions among age, BMI, gender and Δ mtDNA⁴⁹⁷⁷ level in the participants.

4. DISCUSSION

The free radical theory which proposes that ROS produced by mitochondria produces mtDNA mutation and leads to aging is more than 60 years old [23]. The mtDNA deletion gives replication advantage to defected mitochondria. Therefore, the reduced size of mtDNA increases the rate of replication and the proportion of mutant mitochondria during time. Therefore, we assume that mutation and aging processes strengthen each other, and the rate of Δ mtDNA⁴⁹⁷⁷ increases with age.

Contrary to expectations, our results indicated that the Δ mtDNA⁴⁹⁷⁷ level in blood significantly decreases with age. The accumulation of Δ mtDNA⁴⁹⁷⁷ with age is observed in non-dividing post-mitotic tissues such as brain, heart, muscle and eye [20, 24-26]. The findings with respect to blood as fast replicating tissue are controversial. Several studies have reported that Δ mtDNA⁴⁹⁷⁷ increases with age [27-29] whereas other studies have found that this deletion is not age-dependent [30-34]. The discrepancy might be due to experimental differences related to the other lifestyle and environmental parameters. Lifestyle factors including alcohol consumption, smoking and dietary components have been reported to have harmful or beneficial effects on mtDNA [28, 35, 36]. Lifestyle affects body composition, so BMI may represent the mutual effects of lifestyle factors [37].

Our results showed that although the Δ mtDNA⁴⁹⁷⁷ has no significant relationship with BMI, both factors were concordantly decreased with age. It may suggest that chronological age and BMI have additive effects on mtDNA mutation level

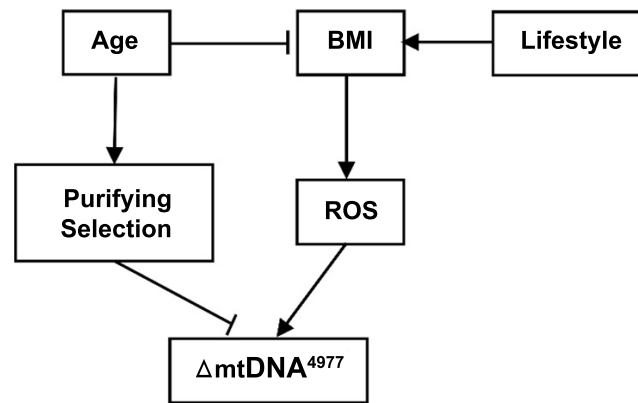


Fig. (3). Proposed model of the additive effects of chronological age and BMI on $\Delta\text{mtDNA}^{4977}$ level in post-mitotic tissues.

in post-mitotic tissues (Fig. 3). According to the proposed model, the chronological age regulates the $\Delta\text{mtDNA}^{4977}$ level in two ways. Firstly, BMI decreases with age, therefore, the amount of ROS and consequently $\Delta\text{mtDNA}^{4977}$ will be reduced. Secondly, blood cells must be continuously replaced throughout life. Hence, cells with reduced levels of mutant mtDNA are selected as a result of purifying selection [38]. Purifying selection is a mitochondrial quality control mechanism which removes damaged mitochondria *via* mitophagy [36, 37, 39, 40]. Therefore, cells with $\Delta\text{mtDNA}^{4977}$ will be replaced by cells without deletion and trigger a decline in mtDNA deletion level during time.

CONCLUSION

Our data suggest that age affects purifying selection and BMI, which may influence the relative level of $\Delta\text{mtDNA}^{4977}$ in blood cells. Future studies are needed to evaluate to what extent the level of $\Delta\text{mtDNA}^{4977}$ is controlled by lifestyle parameters.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by Weill Cornell Medicine Institutional Review Board, Iran.

HUMAN AND ANIMAL RIGHTS

No Animals were used in this study. All human researches were conducted in accordance to international guidelines and approved by Golestan University Bioethics board, Iran.

CONSENT FOR PUBLICATION

Informed consent was obtained from all the patients included in the study.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

Declared none.

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