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Mitochondrial DNA copy number and telomere length in peripheral blood mononuclear cells in comparison with whole blood in three different age groups

Egija Zole[a,b,*], Renāte Ranka[a,c]

[a] Latvian Biomedical Research and Study Centre, Ratsupites iela 1, Riga LV-1067, Latvia
[b] Faculty of Biology, University of Latvia, Jelgavas Street 1, Riga LV-1004, Latvia
[c] Riga Stradins University, Dzirciema iela 16, Riga LV-1007, Latvia

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ABSTRACT

There are more and more studies on telomere length (TL) and mitochondrial DNA (mtDNA), and it has been proven that these factors play a significant role in the aging of the immune system thereby it is important to understand how it varies in different cell types for more accurate conclusions. The aim of this study was to look into dynamics of mtDNA amount in conjunction with TL in peripheral blood mononuclear cells (PBMC) during aging in comparison with whole blood (WB) cells.

Overall, 53 samples were divided into three age groups: 20–39 year age group, 40–59 year age group and 60–79 year age group. MtDNA amount was determined by qPCR TaqMan, and TL was measured by Southern blotting of terminal restriction fragments (TRFs).

PBMC had much higher mtDNA copy number (CN) amount than WB samples. Furthermore, with age, it increased in PBMC, while in WB mtDNA CN count did not change. TL in the elderly group was shorter in PBMC fraction than in WB cells. It also looked like that in PBMC TL shortened faster than in WB.

In conclusions, it appears that during the aging process both mtDNA CN and TL were more stable in WB than in PBMC fraction where changes were more drastically pronounced, but more studies using larger sample cohorts should be performed to confirm this observation.

1. Introduction

Peripheral blood is the primary source of lymphoid cells widely used in aging research. Such studies are usually performed using whole blood (WB) leukocytes or samples of purified peripheral blood mononuclear cells (PBMC). Peripheral white blood cells are classified into two cell types: (1) PBMC fraction consists of cells having a round nucleus including T cells and B cells (plasma cells) belonging to the adaptive immune system, dendritic cells (messengers between the adaptive and the innate immune systems), natural killer (NK) cells, monocytes (immature macrophages) and immature mast cells belonging to innate immune system; (2) cells with segmented nuclei, also known as granulocytes or polymorphonuclear leukocytes (neutrophil, eosinophil, basophil), which belong to the innate immune system (Banchereau & Steinman, 1998; Delves, Martin, Burton, & Roitt, 2011). At present, both telomere length (TL) and mitochondrial DNA (mtDNA) content are accepted as reflective biomarkers of aging.

Mitochondria and mtDNA are major players in the immune response in both the innate and adaptive immune systems (reviewed in Fang, Wei, & Wei, 2016; West, Shadel, & Ghosh, 2011; West et al., 2015). In the innate immune system, mitochondria are essential as a part of the signaling transduction, and mtDNA is released into cytosol during infection or inflammation (Nakahira et al., 2011; Shimada et al., 2012). The mtDNA amount differs among different blood cell types, in some studies the highest of which is in pellets, then granulocytes, and twice as much is in lymphocytes compared to the especially small amount found in monocytes and B cells (Shen et al., 2008). Pyle and colleagues showed the opposite results in which platelets and granulocytes had the lowest mtDNA content, but monocytes and lymphocytes had the highest (Pyle et al., 2010). Similar observations were made by Maianski and collaborators; they showed that neutrophils had lower mtDNA amounts than PBMC, and there were comparable results in another work where WB had slightly lower mtDNA counts than the lymphocytes (Chan, Chevalier, Aprikian, & Chen, 2013; Maianski et al., 2004).

* Corresponding author at: Latvian Biomedical Research and Study Centre, Ratsupites iela 1, Riga LV-1067, Latvia.
E-mail address: egija.zole@gmail.com (E. Zole).

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Telomeres are specialized chromosomal DNA-protein structures that cap and protect the terminal regions of eukaryotic chromosomes. The sequence of telomeric DNA in humans is $(TTAGGG)_n$, and its length ranges from 5 to 15 kb. Loss of TL in immune cells with age is an important factor of immunosenescence (Kaszubowska, 2008; Takubo et al., 2010). In different T-cell subtypes, TL is very dynamic and age dependent, especially in naïve CD4+ T cells, which also have the highest TL among different types of CD4+ and CD8+ cells. Herpesvirus reactivation can lead to a boost in the memory T-cell frequency and to a dramatic increase of TL, which could be important for the perseverance of long-lived T-cell memory (O'Bryan, Woda, Co, Mathew, & Rothman, 2013). Among different T and B cells, CD8 + CD28− T cells have the shortest telomeres, and naïve B cells have the longest telomeres and highest telomerase activity, although they have the fastest telomere ablation. Monocytes have a similar TL as T cells (Lin et al., 2010, 2015, 2016; Weng, Granger, & Hodes, 1997). Previous studies have also reported that the TL in PBMC was longer than in WB cells (9.4% longer) and in bone marrow cells (2.7% longer) (Kimura et al., 2010; Sakoff et al., 2002). In studies in chronic idiopathic neutropenia patients the TL was longer (~6442 bp) in PBMC of healthy individuals while it was shorter in granulocytes (~4874 bp), and a correlation with age was more pronounced in PBMC than in granulocytes (Pavlaki et al., 2012). Activity of telomerase can influence TL in these cells. Glucose level and IL-6 (Interleukin-6) level in plasma can negatively influence TL as obesity is connected with shorter telomeres in adults and IL-6 cytokine is associated with cell aging. IL-15 maintains TL of memory CD8+ T cells by inducing telomerase activation through the Jak3 and PI3K/AKT pathways. Telomerase is also involved in IL-7-mediated differential survival of naïve and memory CD4+ T cells (Li, Zhi, Wareski, & Weng, 2005; Lin et al., 2015; Yang, An, & Weng, 2008; Zannolli et al., 2008). Both IL-15 and IL-7 prolong telomeres, possibly by affecting TERT gene expression in cord blood cell cultures (Brazvan et al., 2016).

As telomere shortening in aged immune cells is an important factor of immunosenescence and mtDNA has been proven as a significant part of immune response, it is essential to understand how mtDNA content of the white blood cells changes with age in conjunction with TL. The aim of this study was to look into dynamics of mtDNA and TL in PBMC during aging in comparison with WB samples.

2. Methods

2.1. Study participants

Peripheral whole blood samples were collected from healthy volunteers 20–78 years old. The exclusion criterion was the presence of Alzheimer's disease, Parkinson or any type of cancer that could be associated with TL or mtDNA alterations. Written informed consent was obtained from all participants for the use of their phenotypic and genetic data that were voluntarily provided *via* anonymous health and heredity questionnaires approved by the Central Medical Ethics Committee of Latvia. In total, 53 blood samples were collected from individuals divided into three age groups (Table 1).

2.2. Acquisition of peripheral blood samples

10 mL of peripheral EDTA-anticoagulated whole blood samples were obtained from all participants and immediately divided into two 5 mL fractions. PBMC were obtained from 5 mL blood by centrifugation using ACCUSPIN System-HISTOPAQUE-1077 tubes (Sigma–Aldrich, Germany) based on the manufacturer's protocol. Briefly, five ml of fresh whole blood was poured into the upper chamber of each pre-filled tube, and then centrifuged for 50 min, 1000 × *g* at room temperature. After centrifugation, the plasma layer was carefully aspirated with a pipette to within 0.5 cm of the opaque interface containing the mononuclear cells. The mononuclear band was carefully transferred with a pipet into a clean centrifuge tube and was washed by adding 10 ml of isotonic PBS. The cells were resuspended by gentle aspiration with a pipette and were then centrifuged for 10 min 250 × *g*, at room temperature to remove platelet fraction.

Table 1
Description of the samples.

Age group	Females, amount of samples – age, yo	Males, amount of samples – age, yo
Young, 20–39 yo, *n* = 23, mean age 28 years	*n* = 17, 1–20, 21, 22, 24, 27, 30, 33, 34, 38 yo 2–26 yo 2–36 yo 4–29 yo	*n* = 6, 1–20, 21, 22, 26, 27, 31 yo
Middle, 40–59 yo, *n* = 10, mean age 52 years	*n* = 6, 1–58 yo 2–43 yo 3–59 yo	*n* = 4, 1–42, 43, 56, 59 yo
Elderly, 60–79 yo, *n* = 20, mean age 66 years	*n* = 15, 1–63, 72, 73, 78 yo 2–64 yo 3–60, 62, 67 yo	*n* = 5, 1–62, 64, 70, 71, 74 yo

yo, years old; *n*, sample amount.

The peripheral white blood cell (WB cells) samples were obtained from the second 5 mL whole blood fraction by the standard method (Sambrook, Fritsch, & Maniatis, 1989). Briefly, blood samples were centrifuged for 15 min, 2000 × *g*. Plasma layer was removed, and the rest of content was transferred in a clean 15 tube. 10 mL of red blood cell (RBC) lysis buffer was added and samples were incubated for 15 min at 4 °C followed by centrifugation for 15 min, 2000 × *g* at 4 °C. Supernatant was removed, and cells were washed by resuspension in RBC Lysis buffer and centrifugation for 15 min, 2000 × *g* at 4 °C. All PBMC and WB samples were stored at −20 °C until processed.

2.3. Extraction of genomic DNA

Genomic DNA was extracted simultaneously from the PBMC fraction and WB samples using the standard phenol–chloroform method as previously described (Sambrook et al., 1989).

2.4. Southern blots of terminal restriction fragments (TRFs)

The Southern blot of terminal restriction fragments (TRFs) method described in Kimura et al., was used, with some modifications, to determine TL in blood samples (Kimura et al., 2010). A TeloTAGGG Telomere Length Assay kit (Roche, UK) was used. Briefly, concentrated DNA (~1 μg) was digested with restriction endonucleases *Hinf* I (10 U) and *Rsa* I (10 U). Digested DNA samples, a DNA size marker (GeneRuler 1 Kb DNA ladder, Thermo Scientific, USA), and the DIG Molecular weight marker (Roche, UK) were loaded into a 0.8% agarose gel and run for 23 h (19 V and 25 mA) to resolve fragment sizes. The DNA in the gel was then depurinated in 0.25 M HCl for 10 min followed by denaturation procedure in 0.5 L of 0.5 M NaOH and 1.5 M NaCl by two 20-min washes. The gel were neutralized in 1 L of 0.5 M Tris-OH containing 3 M NaCl (pH 7.5) for two 20-min washes. The DNA was transferred from the gel to a positively charged nylon membrane (Amersham Hybond™-N^+, GE Healthcare Life Sciences, UK) for 2 h using a vacuum blotter (VacuGene Pump, Pharmacia Biotech, Sweden) with a 20× SSC transfer buffer solution that contained 0.3 M sodium citrate and 3 M NaCl (pH 7.0). DNA was fixed to a membrane using a 30-s UV exposure, and the membrane was briefly washed in 2× SSC solution. The subsequent steps were performed using the manufacturer's protocol for the TeloTAGGG Telomere Length Assay kit (Roche, UK). The membrane was visualized on a high performance chemiluminescence film (GE Healthcare Life Sciences, UK). The film was

scanned, and the TRF signals were detected and analysed. DNA migration distances were measured using the Kodak Digital Science D1 program (Kodak, US); the DIG ladder was used as a molecular size reference. The optical density of the DNA fragments was measured using the ImageJ software (Schneider, Rasband, & Eliceiri, 2012). TL was calculated using the following equation: mean TRF length = Σ(ODi)/Σ(ODi/Li), where ODi = optical density at position i and Li = TRF length at position i. For each group of samples, the mean, SEM (standard error of the mean) and SD (standard deviation) of TL was calculated. For entire samples: PBMC mean = 6415; SEM = 174.2, SD = 1268; WB mean = 6562, SEM = 136.7, SD = 995.

2.5. Relative qPCR TaqMan mtDNA copy number quantification assay

The relative mtDNA copy number (CN) was measured using qPCR with the Maxima Probe/ROX qPCR Master Mix (2X) (Thermo Scientific, USA). MtDNA CN amount was normalized by simultaneous measurement of the nuclear gene *Gapdh* and the mitochondrial D-loop. Forward and reverse primers (1250 nM each) for *Gapdh* gene were GapdhF 5′-GAAGGTGAAGGTCGGAGT-3′ and GapdhR 5′-GAAGATGGTGATGG GATTTC-3′, respectively, and TaqMan probe (250 nM per one reaction) GapdhTqM 5′-CAAGCTTCCCGTTCTCAGCC-3′. Forward and reverse primers (50 nM each) for the mitochondrial D-loop were FmtMinArc 5′-CTAAATAGCCCACACGTTCCC-3′ and RmtMinArc 5′-AGAGCTCCCG TGAGTGGTTA-3′, respectively and TaqMan probe PmtMinArc (250 nM) – 5′-CATCACGATGGATCACAGGT-3′ (Kim, Kim, Ko, Bang, & Lee, 2013;Phillips, Sprouse, & Roby, 2014). Concentration of DNA samples was 10 ng/μl per 15 μl reaction volume. After a denaturation step at 95 °C for 10 min, DNA samples were incubated 40 cycles at 95 °C for 15 s, 57 °C for 30 s, and 72 °C for 30 s. Each DNA sample was run in triplicate and twice in two separate runs ($P < 0.0001$, $r^2 = 0.8766$). A no-template control and two the same calibrator DNA samples were used in all runs to allow comparison of the results across the runs. A relative mtDNA CN was calculated using threshold cycle values and the following equation: relative units (ru) = $2^{\Delta Ct}$($\Delta Ct = Ct_{Gapdh} - Ct_{D\text{-}loop}$). For each group of samples, the mean, SEM and SD of a relative mtDNA CN was calculated. For entire samples: PBMC mean = 4.505; SEM = 0.2201, SD = 1.59; WB mean = 0.9438, SEM = 0.03714, SD = 0.27.

2.6. Statistical analysis

Unpaired two-tailed *t*-test and linear correlation was performed using GraphPad Prism version 5 for Windows, GraphPad Software (La Jolla, CA, USA, www.graphpad.com). Data were expressed as means ± SEM and differences of $P < 0.05$ were considered significant.

3. Results

3.1. Mitochondrial DNA copy number in peripheral blood cells in different age groups

In this study, mtDNA CN were analysed in PBMC and WB samples in individuals in three age groups. The obtained results showed a clear significant difference of mtDNA CN for PBMC and WB samples in all age groups (Fig. 1, Supplementary Figure 1). In the 20–39 year age group the mean relative mtDNA CN in PBMC samples was 3.68 ru, whereas in WB samples of the same individuals it was only 1.03 ru; this difference was statistically significant ($P < 0.0001$). In the 40–59 year age group the mean relative mtDNA CN in PBMC was 4.96 ru, but in WB samples it was 0.86 ru, and this difference was statistically significant ($P < 0.0001$). Similarly, in the 60–79 year age group the mean relative mtDNA CN in PBMC was 5.25 ru but in WB samples it was 0.89 ru and this difference was statistically significant ($P < 0.0001$). Interestingly, the mean relative mtDNA CN in PBMC samples was higher in the two older age groups in comparison to the youngest age group by 34.8% (middle) and 42.5% (oldest) ($P = 0.0142$ and $P = 0.0003$, respectively). In contrast, in the WB samples it was declined with age by 17.0% (middle) and 13.5% (oldest) in comparison to the youngest age group, although not statistically significant in this sample cohort ($P = 0.1077$ and $P = 0.1198$, respectively).

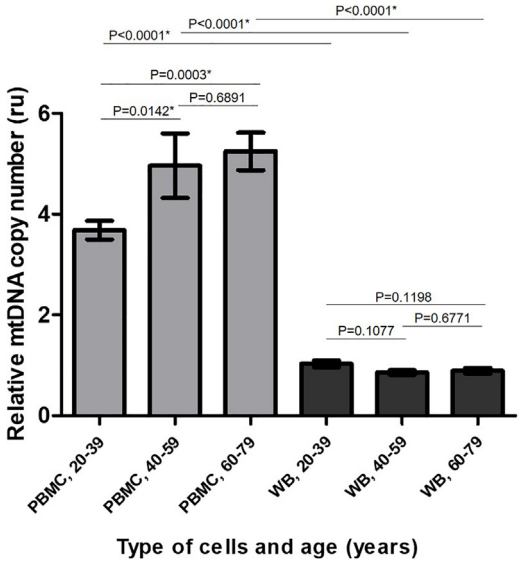

Fig. 1. MtDNA copy number in PBMC and WB. MtDNA copy number amount was much greater For PBMC than for WB in the all age groups. PBMC, peripheral blood mononuclear cells; WB, whole blood white cells; ru, relative units. Data were expressed as mean ± SEM, *Statistically significant.

When a possible correlation between the relative mtDNA CN in the different cell types was evaluated, the results showed that correlation between mtDNA CN relative values in the PBMC and WB samples of the study participants was not statistically significant (Pearson $r = 0.03873$, $P = 0.7830$) (Supplementary Figure 2). When the relative mtDNA CN was compared between females and males, statistically significant difference was not observed neither for PBMC samples nor WB samples ($P = 0.2478$ and $P = 0.9130$, respectively) (Supplementary Figure 3).

3.2. Telomere length in peripheral blood cells in different age groups

TL was evaluated in PBMC and WB samples for all the participants of the study. The results showed that there was a small difference between mean TL of PBMC and WB samples of the study participants within the same age group. In the 20–39 year age group the mean TL of PBMC and WB samples was 7213 bp and 6671 bp, respectively ($P = 0.0983$) (Fig. 2). Similarly, in the 40–59 year age group TL in PBMC and WB samples did not differ significantly (5898 bp and 6690 bp, respectively) ($P = 0.0886$). But in the 60–59 year age group TL in PBMC was significantly shorter than in WB samples (5754 bp and 6373 bp, respectively) ($P = 0.0165$).

When the mean TL was compared between the three age groups, a statistically significant decline in TL in the PBMC samples was observed for individuals 40–59 and 60–79 years old in comparison to the 20–39 year age group ($P = 0.0038$ and $P < 0.0001$, respectively); this decline was as high as 18.2% and 20.2% (Fig. 2, Supplementary Figure 4). By contrast, in WB samples of same individuals the mean TL was similar in all three age groups.

Similarly, to the mtDNA CN variations, statistically significant correlation between mean TL values in the PBMC and WB samples of the study participants was not observed (Pearson $r = 0.08669$, $P = 0.5371$) (Supplementary Figure 5). To evaluate the possible gender-dependent difference, the mean TL was compared between females and males for

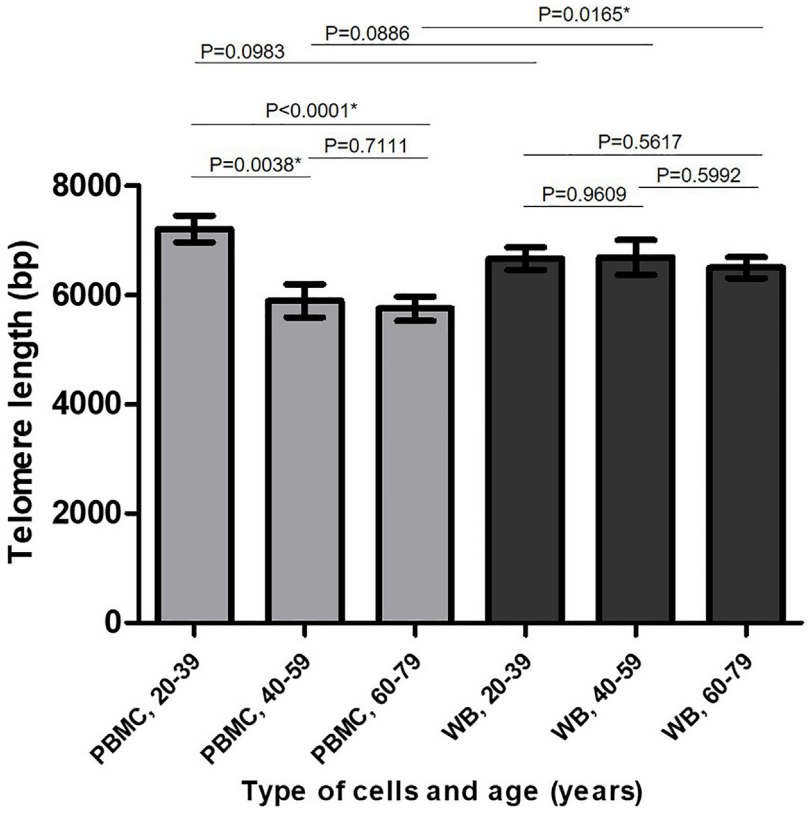

Fig. 2. Telomere length in PBMC and WB. In the middle and elderly groups telomeres are shorter in PBMC than WB cells. PBMC, peripheral blood mononuclear cells; WB, whole blood white cells; bp, base pairs. Data were expressed as mean ± SEM, *Statistically significant.

both sample types. In this analysis, statistically significant difference was not observed neither for PBMC samples nor WB samples ($P = 0.8455$ and $P = 0.1797$, respectively) (Supplementary Figure 6).

3.3. Association between mtDNA copy number and telomere length in PBMC and WB cells

Further, a possible correlation between the relative mtDNA CN and the mean TL in different cell types was evaluated. The results showed that correlation between mtDNA CN and the TL for PBMC samples had statistically significant inverse relationship (Pearson $r = -0.4506$, $P = 0.0008$) (Fig. 3A). This finding was true for the middle (40–59 yo; Pearson $r = -0.5978$, $P = 0.08916$) and the oldest (60–79 yo; Pearson $r = -0.3862$, $P = 0.0926$) age groups but not for the youngest group (20–39 yo; Pearson $r = 0.08705$, $P = 0.6929$) although P values were not statistically significant (Supplementary Figure 7). By contrast, for the WB samples mtDNA CN and the TL correlation was not statistically significant in our cohort (Pearson $r = -0.01075$, $P = 0.9391$) (Fig. 3B).

4. Discussion

Our results indicated that mtDNA content in PBMC samples was significantly higher than in WB samples in the all age groups analysed. As mentioned above the mtDNA amount differs between different blood cell types (Pyle et al., 2010; Shen et al., 2008). Studies show that the blood cell count influences mtDNA CN measured in healthy individuals. The average mtDNA amount increases with platelet count and is inversely associated with white blood cells, particularly due to segmented neutrophils, monocytes and lymphocytes, but eosinophils and basophils do not influence it (Hurtado-Roca et al., 2016; Knez et al., 2015). As the results are controversial as described above, it is not clearly understood why there are differences among cell types. These differences could also occur due to different assay and calculation approaches, which makes it hard to determine the correct results; conflicting results indicate that significantly more research is needed. For our samples one possible

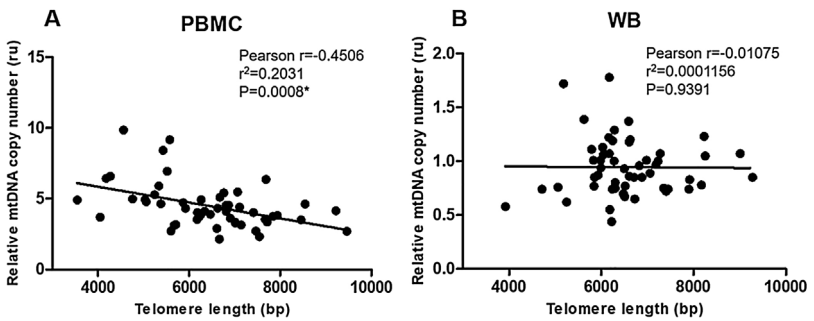

Fig. 3. Connection between mtDNA copy number and TL. (A) PBMC had an inverse relationship between mtDNA CN and TL. (B) WB had no correlation between the two cell components. PBMC, peripheral blood mononuclear cells; WB, whole blood white cells; bp, base pairs: ru, relative units. *Statistically significant.

explanation to our observations could be the platelet contamination during the white cell-isolation procedures. Platelets have no nucleus but have mitochondria and therefore the presence of platelets in the sample may cause an overestimation of mtDNA content (Knez et al., 2015). Platelet contamination causes great variation as well as overestimation of mtDNA content in PBMC (Urata, Koga-Wada, Kayamori, & Kang, 2008). There is a threshold of which platelets might influence the results, and it is when platelets are present in concentrations 5- and 25-fold higher than PBMC concentrations (Timmermans et al., 2006). On the other hand, in our study, the WB samples rather than PBMC samples may contain platelets because of the nature of the cell fraction's isolation procedures used. In the case of PBMC samples' significant contamination with platelets more similar results between different cell fractions would be expected. However, significant difference between mtDNA CN in PBMC and WB was observed indicating that the contamination was minimal if not negligible and did not influence the results. Also, no correlation for mtDNA CN between PBMC and WB fractions was observed in this study, thus indicating on strong inter-individual variation.

Other explanations could be that neutrophils, which are present in the WB fraction, as they contain very little mitochondria and adenosine triphosphate (ATP) is not produced in large amounts (Maianski et al., 2004). Neutrophils are the second most abundant white cell type in WB samples and in that way, neutrophils might reduce the relative mtDNA CN in WB fraction. Also, the type of energetic metabolism in each cell type is important to look into. Neutrophils are mostly entirely glycolytic, while lymphocytes are almost entirely oxidative by using oxidative phosphorylation which means that they need to have more mitochondria to provide a sufficient amount of oxidative phosphorylation chain proteins (reviewed in Kramer, Ravi, Chacko, Johnson, & Darley-Usmar, 2014). One more factor that might affect the mtDNA amount is the response to infection. Cells which are enrolled in the innate immunity use mtDNA stress as a priming step for antiviral response by release mtDNA into cytosol where it is degraded afterwards (Nakahira et al., 2011; West et al., 2015); this process might decrease mtDNA CN in those cells.

Surprisingly, in our study for PBMC samples the relative mtDNA CN were higher in the older age group when compared to the younger group; for WB fraction mtDNA amount was similar in the age groups studied. In general, it has been shown that mtDNA amount decreases with age but at the same time higher mtDNA amount at older age correlates with better health outcomes and survival (Mengel-From et al., 2014; Takahashi et al., 2018; Zole, Zadinane, Pliss, & Ranka, 2018). In the current study the samples do not represent people who are nonagenarians or centenarians which does not allow to see the tendency for mtDNA to be higher for people who live longer than average for WB samples, and also samples size is relatively small which might not allow to show the tendencies fully. Previously it was reported that neutrophils count increases, but platelets and lymphocytes count decreases with age (Biino et al., 2013; Wikby, Johansson, Ferguson, & Olsson, 1994). Further, during the aging, the number of naïve T cells in peripheral blood decreases, but the number of memory T cells stays approximately at the same level (Yan et al., 2010). It could be proposed that these processes may affect the mtDNA CN in human cells thus explaining the results observed in this study. Further, in mice, the memory T cells have a greater mitochondrial mass and mtDNA amount than naïve T cells (van der Windt et al., 2013). For T and B cell development, the important processes are mitophagy and autophagy, which are directed by the mitochondria (Miller et al., 2008; Pua, Guo, Komatsu, & He, 2009) and the cellular autophagy and mitophagy decline with age that subsequently leads to mtDNA accumulation in aged cells (reviewed in Cuervo & Macian, 2014; Palikaras, Lionaki, & Tavernarakis, 2015). In addition, high mtDNA CN amount can work as a compensatory mechanism due to higher mutation stress in mtDNA with age. Mao and colleagues have described this hypothesis in their work with Rhesus monkeys (Mao et al., 2012). This might explain the increase of mtDNA CN in PBMC samples in the older age group, but it is not known if there are any differences in autophagy or mitophagy between mononuclear cells and granulocytes. Also, Phadwal et al., have shown that in T cells autophagy decreases drastically with age, and in healthy young individuals T cells have much higher autophagy in comparison with B cells thus indicating that indeed the level of autophagy may vary in different cell types (Phadwal et al., 2012). It could be assumed that variations in the aging process could have an impact on the mtDNA content; further studies are required to address this question for each particular cell type.

We have reported and discussed the dynamic of TL in PBMC and WB samples during aging in our previous work. It was shown that TL in mononuclear cells was longer than in WB for the individuals 20–40 years old; in contrast, for individuals aged 65–85 years old, TL was shorter in mononuclear cells when compared to WB samples (Zole, Pliss, Ranka, Krumina, & Baumanis, 2013). In this study we have looked at the TL changes in conjunction with mtDNA CN variations in three age groups in different cell fractions. A statistically significant negative relationship between the mean mtDNA CN and the TL for PBMC samples was observed for all the samples together. In contrast, it was not observed for WB samples. After checking this correlation in the three age groups, only middle and elderly age group kept negative but insignificant correlation. Previous studies have reported a positive correlation between mtDNA CN and TL (Kim et al., 2013; Tyrka et al., 2015; Zole et al., 2018); however, WB samples were used in all the studies.

As mitophagy decreases with age and more mitochondria accumulate, more reactive oxygen species (ROS) are produced (Zhou, Yazdi, Menu, & Tschopp, 2011). Further, it has been shown that ROS have a role in TL shortening (Passos et al., 2007). Some studies have reported that ROS amount decreases in the neutrophils of elderly people, although there are controversial results (reviewed in Fulop et al., 2004; Ponnappan & Ponnappan, 2011). The observed shorter TL and increased mtDNA CN in PBMC samples in the older age group may point out on the higher cellular exposure to the ROS in these individuals, which is in agreement with observations that adaptive immune system weakens more drastically with age in the comparison to the innate immune system (rewiewed in McElhaney & Effros, 2009). The study of Rufer et al., has shown that TL in T cells shortens more rapidly than in granulocytes in elderly individuals (Rufer et al., 1999); this finding supports our observations that during the aging TL abbreviates more rapidly in PBMC than in WB cell fraction.

It is a common knowledge that, with age, TL gets shorter in the whole blood leukocytes (Harley, Futcher, & Greider, 1990). While there are many factors exist that affect TL both with mitochondrial interaction and without (reviewed in Blackburn, Epel, & Lin, 2015; Sahin & DePinho, 2012), more studies are required to extend our current knowledge of age-dependent changes in leukocytes and their contribution to aging-related changes in function of the immune system. It seems that during the aging process both mtDNA CN and TL were more stable in WB than in PBMC fraction where changes were more distinct. Which is in agreement with previous works that have shown that telomerase activity decreases with age in lymphoid cells; while mature myeloid cell lines almost do not have active telomerase, have short lifespans and do not undergo further cell division after activation, showing slower decrease rate of TL at older age (*e.g.* reviewed in Kaszubowska, 2008; Weng, 2001). Regarding mtDNA changes in WB and PBMC there is various inconsistent studies and results as described above which askes for more studies in this field to prove the results (Chan et al., 2013; Maianski et al., 2004; Pyle et al., 2010; Shen et al., 2008).

In conclusion, this study explored the dynamics of mtDNA and TL in PBMC and WB samples in different age groups. The results indicated greater stability of both biomarkers in WB fraction during the aging when compared with PBMC samples. Our results highlighted a substantial variation in the aging process for the different cell types.

Variance of these parameters should be considered in future studies of age-related changes in innate and adaptive immune system.

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Conflict of interest

The authors declared no conflict of interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.archger.2019.04.007.

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