

Assessment of mitochondrial DNA copy number variation relative to nuclear DNA quantity between different tissues

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ABSTRACT

Mitochondrial DNA is a widely tested genetic marker in various fields of research and diagnostics. Nonetheless, there is still little understanding on its abundance and quality within different tissues.

Aiming to obtain deeper knowledge about the content and quality of mtDNA, we investigated nine tissues including blood, bone, brain, hair (root and shaft), cardiac muscle, liver, lung, skeletal muscle, and buccal mucosa of 32 deceased individuals using two real-time quantitative PCR-based assays with differently sized mtDNA and nDNA targets.

The results revealed that the quantity of nDNA is a weak surrogate to estimate mtDNA quantities among tissues of an individual, as well as tissues across individuals. Especially hair showed extreme variation, depicting a range of multiple magnitudes of mtDNA molecules per hair fragment. Furthermore, degradation can lead to fewer fragments being available for PCR. The results call for parallel determination of the quantity and quality of mtDNA prior to downstream genotyping assays.

1. Introduction

The human mitochondrial genome (mitogenome) has been subject to diverse scientific investigations in the past four decades. After the first draft sequence (CRS, (Anderson et al., 1981)) was published, this molecule has extensively been studied in various fields of research, including but not restricted to population genetics (e.g., Giles et al., 1980), archaeology (e.g., Pääbo, 1985), medicine (e.g., Brown et al., 1992) and forensic genetics (e.g., Higuchi et al., 1988). Mitochondrial DNA (mtDNA) is maternally inherited and (much) more abundant in the cell than nuclear DNA (nDNA). Particularly mtDNA copy number has been investigated with respect to its relevance in research and practical applications. For example, a decline in mtDNA copy number was associated with increasing age (e.g., Lee and Wei, 2005) and it was shown that both, the relative proportion as well as the absolute amount of mutated mtDNA are determinants in mitochondrial disease and cancer (e.g., Filograna et al., 2021; Xu et al., 2018). In addition to DNA located within cells, so-called circulating cell-free DNA (cf-DNA) is also present in the bloodstream as well as other body fluids and compartments,

deriving from both mtDNA and nDNA from various origins (Aucamp et al., 2018; Hui et al., 2015; Mehra et al., 2007). Therefore, cf-mtDNA has become the focus of studies investigating diagnostic and prognostic relevance in various diseases and syndrome (Behnke et al., 2023; Borah et al., 2023; Ingelsson et al., 2018; Mehra et al., 2007; Pérez-Treviño et al., 2020; Trumpff et al., 2022). This list could be detailed and expanded, which shows that the determination of the amount of mtDNA present in a cell/tissue is relevant to many aspects of research.

In forensic genetics, mtDNA is typically tested when – among other factors – nDNA is not present in sufficient quantity or quality to generate standard STR profiles. This is particularly frequent in environmentally challenged samples such as solid tissue remains for human identification or in hair shaft samples that contain nDNA, but at too small fragment sizes to be analyzed with conventional STR markers (e.g., Strobl et al., 2018). Therefore, in these specimens mtDNA analysis is the method of choice (Budowle et al., 2003; Parson et al., 2014). However, other tissues that would contain enough nDNA for STR profiling also undergo mtDNA analysis for different reasons, including e.g., reference samples to which mtDNA haplotypes from unknown samples are compared to or

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tissues to establish a maternal pedigree (e.g., Coble et al., 2009; Parson et al., 2015).

Despite the many applications that would benefit from the knowledge of the amount of mtDNA in a given tissue, the amount of mtDNA is often estimated from the nDNA amount determined by real-time qPCR or the total DNA amount in a cell by e.g. fluorometric or photometric analysis. However, the determination of DNA using fluorometric or photometric methods includes also the microbial DNA, often present especially in saliva and old samples. Only few commercial mtDNA-specific quantitation methods (ScienCell, Absolute Human Telomere Length and Mitochondrial DNA Copy Number Dual Quantitation qPCR Assay Kit; Sigma-Aldrich, NovaQUANT™ Human Mitochondrial to Nuclear DNA Ratio Kit, Takara Bio Inc, Human Mitochondrial DNA (mtDNA) Monitoring Primer Set) and academic tools (e.g., Gallimore et al., 2018, Xavier et al., 2019 (and references therein)) are available.

In the majority of applications, it seems common practice that the amount of mtDNA is estimated from the measured quantity of nDNA for which multiple commercial products do exist and which are implemented into the laboratory workflow. Obviously, this approach is biased by the cell-based mtDNA/nDNA ratio and cf-DNA, at hand and may result in substantial over- or underestimation of the mtDNA copy number. This in turn has been described as critical input value for mtDNA sequencing applications, particularly with respect to the interpretation of genuine signal, heteroplasmy, nuclear elements of mtDNA (NUMTs), artifacts and others (see review Marshall and Parson, 2021).

In this systematic study, we determined mtDNA and nDNA quantities from a total of nine tissue types in 32 individuals to better understand absolute and relative amounts between and across tissues and their impact on downstream genotyping approaches.

2. Material and methods

2.1. Sample collection

Nine different tissue types from the same 32 individuals (blood, bone, brain, hair, cardiac muscle, liver, lung, and skeletal muscle, as well as a swab from the buccal mucosa surface) were selected from available material collected during a previous study (Naue et al., 2015). Samples for this study were selected based on the following available criteria: (i) age and (ii) sex balance and (iii) availability of all tissues needed from each individual. This study was approved by the ethics committee of the University of Freiburg (264/10).

2.2. DNA extraction

Enough DNA extract of the tissues (stored at -80°C) was available for most samples from the previous study. For the tissues of ID101 and lung of ID021, additional DNA extractions were performed following the previously described protocol (Naue et al., 2015). New DNA extractions were also performed for all hair samples using the following protocol: the hair samples were examined under a microscope for hair condition, presence of root and possible contamination (e.g., blood), and three hairs per individual were chosen. Three hair samples (ID009, ID070 und ID101) with possible contamination were washed with 0.5 % SDS and air-dried. Subsequently, the hair fragment was cut into two parts, the proximal 0.5 cm of the hair shaft including the root (referred to as 'hair root sample') and the adjacent 1 cm shaft (referred to as 'hair shaft sample'). Root and shaft of three hairs per individual were used for separate DNA extractions with the DNA Micro Kit (Qiagen, Hilden, Germany) following the manufacturer's protocol.

2.3. Nuclear and mitochondrial DNA quantification

Initially, the quantity and degradation state of nuclear DNA (nDNA) was determined for all samples except for the hair shafts with the real-time quantitative PCR (qPCR) PowerQuant System (Promega,

Madison, WI, USA) following the manufacturer's protocol. Samples were normalized to 1 ng nDNA and aliquots were quantified again in triplicates using the real-time qPCR SD quant system ('Sensitive detection tetraplex-system') (Xavier et al., 2019). In brief, the SD quant assay integrates two differently sized mtDNA targets (69 bp (mtND1) and 143 bp (mt143bp), respectively), as well as a 70 bp nDNA (RNU) target, which are amplified in a 20 μL reaction volume containing 10 μL 2x TaqMan Fast Universal PCR Master Mix (Thermo Fisher Scientific, Waltham, MA, USA), 100 nM IPC primer, 100 nM IPC probe, 900 nM RNU primer, 500 nM RNU probe, 300 nM mtND1 primer, 100 nM mtND1 probe, 300 nM mt143bp primer, 100 nM mt143bp probe, and 2.5 μg BSA. As standard curve a 5-fold dilution series of G1521 genomic control DNA (Promega, Madison, WI, USA) from 62.5 ng to 0.0008 ng nDNA was used, containing 5,413,110 to 69 copies of mtDNA. QPCR was run under the following conditions: 2 min at 50°C , 20 sec at 95°C , 40 cycles of 3 sec at 95°C and 30 sec at 60°C . All real-time-quantitative PCRs were run on an ABI Prism 7500 instrument (Thermo Fisher Scientific). This allowed the comparison of mtDNA and nDNA quantities in a sample with additional consideration of the mtDNA degradation state. The experiments were performed in accordance with the MIQE guidelines (Bustin et al., 2009).

2.4. Data analysis & statistics

Data analysis and statistical evaluation was done using Python 3.9.7 with the following packages: pandas version 1.2.4 (data analysis), pingouin version 0.3.11 (statistics), scipy version 1.6.3 (statistics) and seaborn version 0.11.1 (visualization). Triplicates of each sample were analyzed individually and the results were combined for data interpretation. For non-detectable nDNA (but measurable mtDNA) the corresponding nDNA amount at Ct 40 was used to avoid division by zero when normalizing mtDNA to nDNA content. The 143 bp mtDNA target copy number was set to one copy (corresponding to the copy number at Ct 40) when the shorter 69 bp mtDNA fragment was present but no 143 bp mtDNA detected (this affected measurements of three hair roots and nine hair shaft samples). Detected nDNA and mtDNA below the dynamic range and therefore possible stochastic effects were set to the stable limit of detection (according to the lowest standard in the SD quant and 0.5 pg in the PowerQuant assay).

The integrity of the DNA samples was analyzed by the two real-time qPCR assays: Nuclear DNA degradation was evaluated via the ratio between the two autosomal amplicons of the Power Quant assay (84 bp and 294 bp) and mtDNA degradation was evaluated via the ratio between the two mtDNA fragments of the SD quant assay (69 bp and 143 bp). Statistical analysis of mtDNA degradation differences between tissues was performed via Kruskal-Wallis (overall difference) and paired Wilcoxon test taking into consideration the false discovery rate according to Benjamini-Hochberg.

SD quant results for nDNA and the 69 bp mtDNA fragment were used for calculating the mtDNA copy number per diploid cell (except for hair). These calculations were performed with the following formula:

$$\text{number of mtDNA copies per diploid cell} = 0.007 \text{ ng} * \text{measured mtDNA copy number [mtDNA GE}/\mu\text{L}]/\text{measured nuclear DNA concentration [ng}/\mu\text{L}].$$

In hair, the mtDNA content was based on the total amount in the 45 μL eluate, which corresponds to the mtDNA content in the analyzed 0.5 cm of hair root (including adjacent region) and 1 cm hair shaft, respectively. Statistical analysis of differences in the mtDNA copy number per diploid cell between tissues was performed with Kruskal-Wallis (overall difference) and paired Wilcoxon test (all tissues were tested against blood as reference material apart from hair samples). Correlation of mtDNA copy number per diploid cell to age was tested by Spearman correlation with false discovery rate according to Benjamini-Hochberg. Dependence of mtDNA copy number per diploid cell to sex was tested with the Mann-Whitney-U-Test and with false discovery rate according to Benjamini-Hochberg to consider multiple testing of

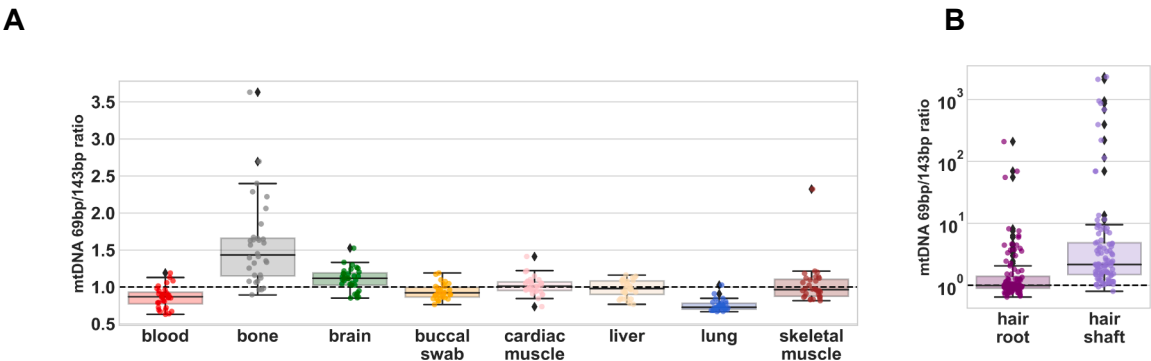


Fig. 1. Mitochondrial DNA degradation per tissue type. Visualization of mtDNA degradation per sample based on the ratio of shorter over longer amplicons. Values exceeding 1 suggest degradation. Dots represent mean values of the technical triplicates; dashed line: value of 1. The mtDNA degradation estimate of the buccal swab from ID088 yielded a distant outlier (value of 255.6). The value was therefore excluded for better visualization (cf. Fig. 2a, Supplementary material S2). Undetected mtDNA copy numbers for the 143 bp amplicon were substituted by 1 copy to avoid underestimation of DNA degradation due to missing data. A: mtDNA degradation in internal tissues (without hair) B: mtDNA degradation in hair samples (3 per individual) presented using Y-axis log10 scale.

multiple tissues.

3. Results and discussion

The knowledge on the functional number of mtDNA templates in a DNA extract is important for various applications investigating mtDNA to avoid missing, poor, artificial and error-prone results. Furthermore, the nDNA to mtDNA ratio in a cell is tissue-dependent and thus, can diverge to a large degree between samples. Therefore, we examined the mtDNA/nDNA content as well as the mtDNA degradation in nine

different tissue types of 32 deceased individuals (Suppl. Material S1). Tissues included blood, bone, brain, buccal mucosa, cardiac muscle, hair, liver, lung, skeletal muscle as well as hair roots and shafts.

3.1. Applicability of the SD quant assay

All quantitation experiments using the short amplicons (below 100 bp) yielded PCR efficiencies between 90 and 105 %, which is in concordance with MIQE guidelines. The 143 bp mtDNA fragment of the SD quant assay showed slightly lower efficiency values between 77.4

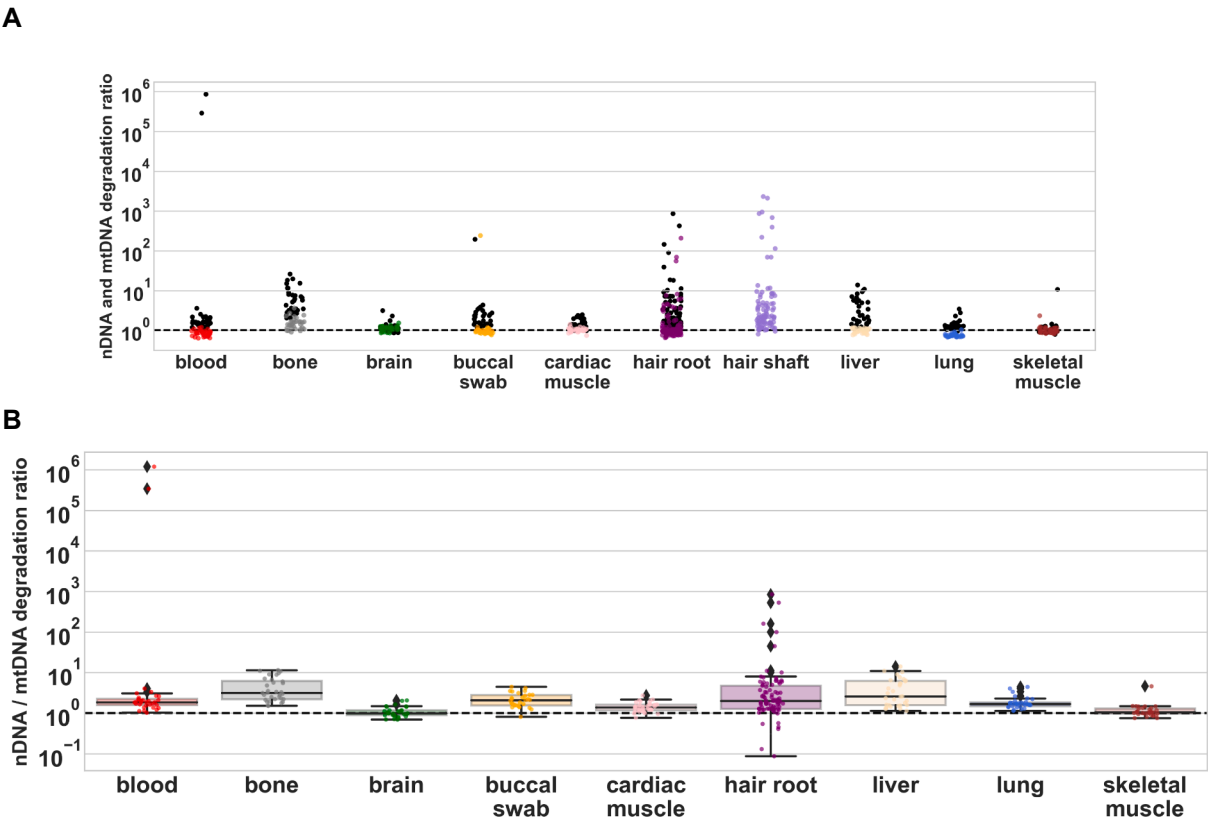


Fig. 2. Comparison of nuclear and mitochondrial DNA degradation per tissue type. A: Visualization of nDNA (black dots) as well as mtDNA degradation (colored dots) per sample based on the ratio of the shorter over the longer amplicons. Values exceeding 1 indicate degradation (black dashed line represents 1 degradation threshold) B: Ratio between nDNA and mtDNA degradation based on the ratio of the nDNA and the mtDNA degradation indexes (from A, hair shafts excluded). Values exceeding 1 indicate stronger nDNA degradation than mtDNA degradation. Dots represent mean values of the technical triplicates. Y-axis: log10 scale; dashed line: value of 1.

and 90.5 %. This fragment was only used for the calculation of the mtDNA degradation state within the study.

The nDNA component of the SD quant assay of the hair shaft samples was also used to investigate possible remaining surface DNA contamination. The quant values were lower than 7 pg/ μ L nDNA in 93 of the 96 samples. The other three hair shaft samples still contained between 8 and 21 pg/ μ L nDNA after cleaning, however that could relate to residual nDNA in hair shafts (Brandhagen et al., 2018; Grisedale et al., 2018) or DNA drop-in effects (Gill et al., 2012), particularly, since the analyzed hair shafts were in close proximity to the hair root. However, residual contamination cannot be completely excluded, but does not impact the overall results. Generally, when no nDNA was detected by the SD quant, the corresponding quantity of 0.01 pg/ μ L at Ct 40 was used for normalizing mtDNA content (cf. 2.4).

3.2. DNA degradation

This study focused on the specific determination of the mtDNA content per cell to obtain a better estimate of mtDNA input into downstream sequencing, for example. The decision on the selected sequencing method also depends on the degradation state of the DNA (Strobl et al., 2018), which was calculated from the quantitation values of the differently sized PCR fragments. A value above 1 indicates a higher amount of short DNA fragments and suggests DNA degradation, which would require shorter PCR amplicons or capture-based methods for successful sequencing (Eduardoff et al., 2017).

3.2.1. MtDNA is mostly stable but tissue dependent as well as inter-individual dependent

A median mtDNA degradation index around 1 (indicating no degradation) was observed for most sample types except for bones and hair shafts with significantly (Kruskal-Wallis $p < 0.001$) higher median values of 1.43 and 2.16, respectively (Fig. 1a and b). The pairwise analysis between tissues (Wilcoxon test with corrected p-value for multiple testing) revealed statistically significant differences, however, relevant higher inter-individual differences and more extreme outliers were observed particularly in hair shafts and roots and to a lesser extent in bone. Also intra-individually, these differences were still dependent on the tissue and without an indication on an impact due to the individual examined (Suppl. Material S2a and b). Although only individuals with a short time since death were included, a larger intra-individual impact on mtDNA degradation in all tissues cannot be excluded.

As expected, hair roots and shafts showed increased variability, both among and within individuals, with a general trend of higher mtDNA degradation in hair shafts compared to the root portion (e.g., ID005, ID021, ID043). Surprisingly, substantially increased mean degradation in hair root mtDNA compared to hair shaft mtDNA was measured in two

individuals (ID014, ID039) and equal or slightly increased mtDNA degradation in the root in four individuals (ID008, ID029, ID038 and ID079) (Suppl. Material S2b). Comparing mtDNA degradation and mtDNA content, also the mtDNA content in the root was comparable to the hair shaft and not higher as expected. One reason might be that the obtained hairs came from varying hair cycle phases and therefore variable hair root (including attached cells) quality.

It has to be considered, that the interpretation of the amount of degradation is restricted as the difference between the short (69 bp) and long fragment (143 bp) is only 74 bp. However, the chosen sizes of these two amplicons are an important indicator for downstream analysis (especially for MPS assays).

3.2.2. The degree of nDNA versus mtDNA degradation within a sample is tissue-dependent

The degradation state of a biological sample or its DNA is typically determined by nDNA degradation as the PowerQuant System (Ewing et al., 2016). In this study, we investigated whether or not differences between nDNA and mtDNA degradation for individual samples have to be considered (Fig. 2). Within the study, tissue samples as well as DNA extracts were kept at -80°C between sampling, DNA extraction and analysis. However, small variation due to storage cannot be completely excluded, despite no systematic differences were observed between older and newer DNA extractions. General tissue-dependent differences in nDNA and mtDNA degradation can be expected as it is known from previous studies that the speed of postmortem autolysis as well as the amount of enzymes (i.e. DNases) is dependent on the tissue type (Alaeddini et al., 2010; Tomita et al., 2004).

Median ratios (\pm standard deviation; sd) between nDNA and mtDNA degradation ratios revealed varying degradation between tissues. Differences were generally smaller for brain (ratio 0.98 ± 0.34), skeletal muscle (1.04 ± 0.65), cardiac muscle (1.37 ± 0.42), lung (1.68 ± 0.75) and blood (1.85 ± 217031). Slightly elevated differences occurred in buccal swabs (2.06 ± 0.9), hair roots (2.0 ± 108.73) and liver (2.57 ± 3.35). The highest discrepancies between nDNA and mtDNA degradation ratios were observed for bone (3.145 ± 3.11). The elevated standard deviation in blood was caused by single outliers (Fig. 2b) as the calculated median of 1.85 suggests a generally balanced nDNA/mtDNA stability. However, in hair roots, higher discrepancies were observed in more cases. Even if the median nDNA/mtDNA degradation ratios were well balanced, differences occurred and have to be considered for further downstream mtDNA genotyping.

These results confirm the expected fact that nDNA generally seems to be more prone to degradation than mtDNA (Allentoft et al., 2012; Schwarz et al., 2009). Nevertheless, the need to use two different PCR systems with different purposes (SD quant assay and PowerQuant) to address this question, led to a comparison of degradation based on

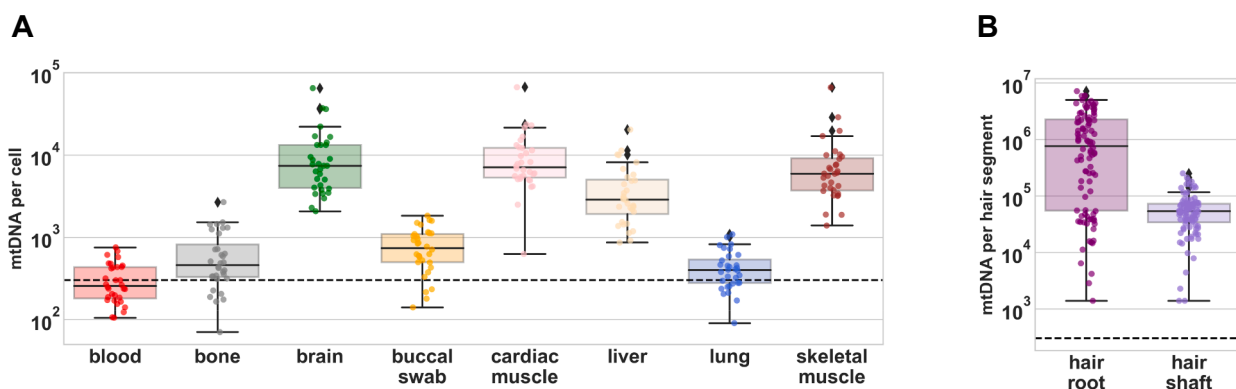


Fig. 3. MtDNA content per cell, and hair segment respectively. A: mtDNA content per cell and tissue. B: mtDNA content per hair segment (0.5 cm hair including root and 1 cm hair shaft, respectively). The dots represent the mean value of the technical replicates. Y-axis: log10 scale; black dashed line: value of 300 copies, as representative value for the mean mtDNA content in blood.

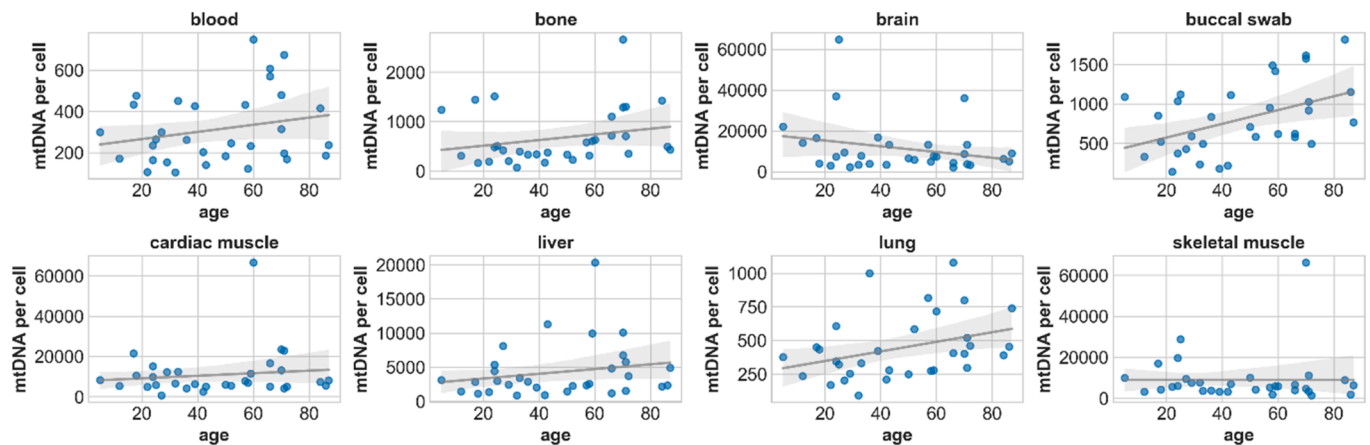


Fig. 4. Mtdna content per cell relative to age. The dots represent the mean mtDNA content per cell of the technical triplicates in dependence on age; line and grey area: linear correlation line with 95% confidence interval for visualization.

different amplicon lengths especially for the longer fragment (143 bp vs 294 bp). A slight overestimation of the nDNA degradation value cannot be excluded. However, the amount of tissue- and inter-individual variation is not affected by that. The determination of the degradation status based on nDNA quantification and calculation of the degradation index cannot be directly transferred to the mtDNA degradation level for all tissues based on the obtained results. Furthermore, some tissues do show high intra-individual variation, suggesting that constant variables to translate mtDNA degradation status from nDNA are not generally applicable. In general, the determination of DNA quantity as well as degradation is always based on the used quantification strategy and therefore amplicon size(s) used. Therefore, quantification results as well as observed degradation values can vary between studies.

3.3. MtDNA copy number per cell/hair fragment

3.3.1. The amount of mtDNA is tissue-dependent

The mtDNA content per cell was experimentally determined and normalized to the nDNA amount present in one cell. It has to be considered, that the obtained results present the mean mtDNA content per cell over the whole tissue analyzed, including cf-DNA as well as different cell types. The mtDNA content can still vary between the cell types present. E.g., in blood, the mtDNA content per nucleated cell is rather overestimated due to the additional presence of platelets containing mtDNA but no nDNA (Picard, 2021). However, in hair, the mtDNA content was determined relative to the whole DNA eluate volume corresponding to 0.5 cm of hair root and 1 cm of hair shaft, respectively. Generally, two classes of tissues were identified with respect to normalized mtDNA content. Based on an assumed 300 copy number of mtDNA in blood, as determined in this study, and comparable to other studies (Anderson et al., 2021; Kelly et al., 2012; Picard, 2021; Rausser et al., 2021; Wachsmuth et al., 2016), our samples from, bone, buccal tissue and lung showed normalized mtDNA content within an order of magnitude, while brain, cardiac muscle, liver and skeletal muscle yielded substantially higher mtDNA ratios between one and two orders of magnitude (Fig. 3a). These findings demonstrate that an estimation of the mtDNA amount by conversion from nDNA quantitation values would lead to wrong estimates in some tissues. Besides various mtDNA copy numbers in the cells, the cf-DNA can further increase the risk of overestimation or underestimation of the mtDNA copy number. Physiologically, the higher mtDNA content in liver, brain, heart, and skeletal muscle also stands in accordance with high resting metabolic rates of these organs (Wang et al., 2010).

This observation is even more significantly pronounced in hair, where the mtDNA ratios converted from the 300 copy number threshold resided between 1 and 6 orders of magnitude (Fig. 3b). This has

particular implications for research investigating hair, such as in forensic science.

Furthermore, inter-individual differences were observed that render a general conversion of the mtDNA content through nDNA quantitation of one tissue to the other impossible (Suppl. Material 3).

3.3.2. Mitochondrial DNA copy number is highly variable in hair samples and can lead to larger technical variation

As we expected higher variation of mtDNA copy number in hair samples, three hairs per individual were analyzed using technical triplicates (Fig. 3b and Suppl. Material 4), which resulted in a heterogeneous picture: in most cases, the technical replicates showed more congruent quantities than the biological replicates from different hairs. However, it has to be considered that five replicates with no detected mtDNA are not presented in the diagrams. Also, the mean mtDNA quantity of the technical replicates was expectedly higher in the hair roots than in the corresponding hair shaft fragments. However, the variation was in total very high with a mean difference of $1,397,768 \pm 1,699,636$ more copies of mtDNA in the eluate of hair root versus hair shaft. In 21 out of 96 hairs, a higher amount of mtDNA was detected in the shaft compared to the root. These 21 hairs originated from eleven individuals with a single hair being affected in four individuals, two hairs in other four individuals and all three hairs in three individuals. The data suggest that hairs with increased mtDNA content in the shaft compared to the root are not randomly observed but in an individual-specific-manner. However, more hairs from the same individuals as well as more individuals have to be analyzed to confirm that trend, which was not the scope of our study. Our results support previous studies finding high inter- and intra-individual differences as well as differences along the hair sample (Desmyter et al., 2016).

3.3.3. Lack of significant sex- and age-specific mtDNA copy number discrepancies

The observed mtDNA copy numbers were evaluated relative to age and sex in all tissues except for hair that showed high intra-individual variation. We observed a weak tendency towards moderate age correlation (Spearman's rho) in buccal mucosa ($\rho = 0.42$, uncorrected p-value 0.016) and lung tissue ($\rho = 0.4$, uncorrected p-value of 0.025; Fig. 4). However, in both tissues, the p-value adjustment using Benjamini-Hochberg for multiple testing led to a corr. p-value of 0.1. The results stand in accordance with Miller et al. who also did not find a change of copy number with age in skeletal and cardiac muscle (Miller et al., 2003). Other studies, such as Wachsmuth et al. observed differences in skeletal muscle and liver. However, the interpretation is restricted as the decrease in skeletal muscle was especially observed in males and in liver the increase was caused by a subset of individuals

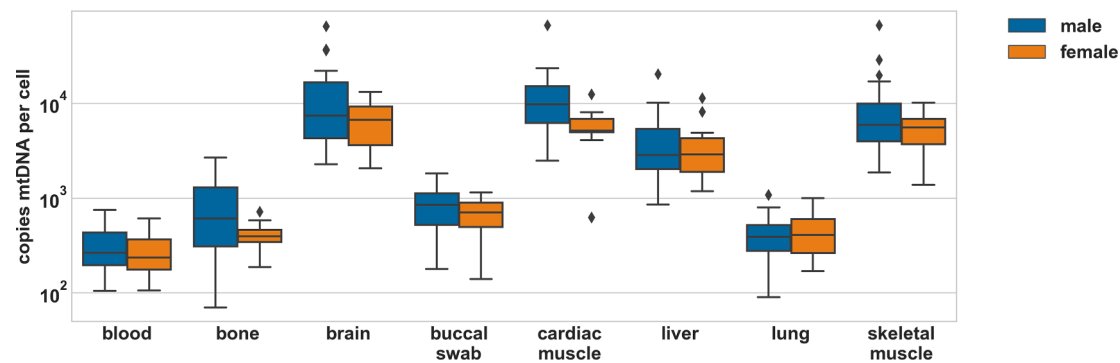


Fig. 5. Mtdna content per cell relative to sex. No statistically significant (considering multiple testing) differences between the mtDNA content and the sex was found, although a tendency for increased mtDNA content in male cardiac muscle was noted. Y-axis: log10 scale.

(Wachsmuth et al., 2016). Generally, observed differences reported in published studies may depend on the methods as well as the targeted cohorts (e. g., disease studies, individuals with different BMI range) (Bordoni et al., 2019; Castellani et al., 2020; Longchamps et al., 2020).

A sex difference in the mtDNA content was observed in cardiac muscle (uncorrected $p = 0.008$; Fig. 5), however, also here, the Benjamini-Hochberg correction failed to provide statistical significance for that observation (corr. p -value 0.08). More samples and a well-balanced dataset would be needed to further investigate a possible sex effect.

4. Conclusions

4.1. The determination of the nuclear DNA content does not reflect the available mtDNA template number

Mitochondrial DNA is the subject of molecular analyses in various research and diagnostic studies, particularly in medical, anthropological and forensic genetics. Typically, mtDNA (mini-) sequencing is performed using mtDNA template amount for PCR or library preparation that is based on nDNA quantities. Here, we demonstrate that the amount of mtDNA copies available is often far higher than one would expect from a standardized conversion from nDNA quantities. Furthermore, tissue/cell-type-dependent differences, cf-mtDNA, and inter-individual variation complicate the inference of mtDNA quantities from nDNA. Strongly deviating mtDNA copy numbers are known to affect sequence quality and thus, may interfere with a correct interpretation of the results. The importance was also recently demonstrated by Obal et al. for aged bones (Obal et al., 2023). We suggest to base downstream genotyping on more specific and stable mtDNA quantities to minimize stochastic effects thus, obtain reliable results.

4.2. DNA degradation can decrease the available DNA template number for PCR

Also, nDNA derived quantitation values provide little information on the degradation state of the mtDNA. We show that in most cases, degradation affected nDNA far more often than mtDNA. The analysis of the mtDNA content with only the 69-bp amplicon already allows a reliable mtDNA quantification for downstream analysis, whereas in case of nDNA a longer amplicon size for quantification is favorable to obtain the amount of intact nDNA available for further analysis. However, especially, in hair and bone samples, mtDNA degradation should be considered in cases of a downstream analysis of a long DNA segment.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.mito.2023.11.006>.

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