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Variability of mitochondrial DNA mutagenesis in human blood

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Abstract. The 4977 bp deletion of mitochondrial DNA (mtDNA) is known to accumulate with age in postmitotic tissue. Meanwhile, this mutation can also be detected in tissues with a fast turnover: blood or skin. However, in those tissues there seem to be several factors influencing mtDNA mutagenesis additionally to the age of an individual, e.g. specific drugs, cigarette smoke or alcohol. From a forensic point of view the question arose whether mt mutagenesis in blood might be used as an indicator to estimate the age of an individual. We investigated mtDNA mutagenesis in blood from 10 persons (22–60 years) over a time period of 6 months. Specific habits of the participants such as smoking, alcohol consumption or intake of medicine were also monitored. Every 2 weeks a blood sample was taken and the following parameters were analysed: (1) amount of total mtDNA/cell and (2) the occurrence of 4977 bp deleted mtDNA (dmtDNA). Real time PCR results showed values between 1003 and 3275 mtDNA copies/cell with a very strong variation within one individual from time point to time point. Using Duplex-PCR, the occurrence of dmtDNA also varied considerably, showing dmtDNA/mtDNA ratios from 0 to 1.7 in the same individual only at different days with no correlation to age or gender. Taken together, the 4977 bp deletion of mtDNA in blood is not suited in a forensic approach to estimate the age of an individual. © 2005 Published by Elsevier B.V.

Keywords: Mitochondrial DNA; 4977 bp deletion; Blood; Real time PCR

1. Introduction

Mitochondrial DNA can be influenced by many intrinsic and extrinsic factors. Well known is the age dependence of specific alterations such as the 4977 bp deletion in postmitotic tissues, e.g. skeletal muscle and brain.

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Even in fast replicating tissues, like skin [1] or blood [2], specific deletions could be detected, whereas an age dependency in differentiated tissues like skeletal muscle [3,4] or brain [5] has not yet been confirmed. Recently, alterations of mtDNA were also found in blood cells from alcoholic patients [6], indicating that alcohol as an exogenous stressor can also effect mtDNA in other tissues that are not directly involved in ethanol decomposition. There might be many other factors that could influence mtDNA maintenance in blood or skin.

The aims of this study were the following:

Does the total amount of mitochondrial DNA (mtDNA) in blood accumulate with age?
Does the specific 4977 bp deletion of mtDNA in blood accumulate with age?
Are there other factors that influence mitochondrial mutagenesis more than age?
Is the amount of deleted mtDNA in blood an indicator for the age of an individual?

2. Material and methods

Ten persons (five male, five female) volunteered for the project, aged 23–60 years. For 6 months, every 2 weeks a blood sample was taken (14 samples/person) and immediately subjected to DNA extraction using the Nucleo Spin Blood Quick pure kit (Macherey-Nagel, Germany).

Quantification of total DNA was done using real time PCR as described in [7]. Mitochondrial DNA was also quantified in a real time PCR according to [4]. Relative quantification of 4977 bp deleted mtDNA was performed in a Duplex-PCR, amplifying a 260 bp for total and a 238 bp fragment specific for deleted mtDNA with subsequent fragment analysis on an ABIPrism 310. The area under the curve of the specific fragments (=relative fluorescence units, rfu) was obtained using the Gene scan 3.1.2 software. For quantification, the quotient of the peak areas of the amplification products specific for deleted and total mtDNA was determined. The calculation was only done when the signals were between 15,000 and 60,000 rfu to ensure that the signals were still in a quantifiable range according to the manufacturers.

3. Results and discussion

3.1. DNA amount in human blood and mitochondrial copy number

DNA amounts were between 15 and 172 ng with no correlation between DNA amount and the age or gender of the individual. High inter- and intraindividual variation of the DNA content could be observed.

Between 1004 and 3276 mitochondrial genomes (average 2127, median 2316) per cell were detected according to real time PCR and standard amplifications. High intraindividual variations from time point to time point were found in all persons with no correlation to age or gender. Only one person showed significantly lower mtDNA amounts over the whole observation period. This woman was pregnant during the study.

3.2. Detection and relative quantification of 4977 bp deleted mtDNA

dmtDNA/mtDNA ratios ranged between 0 and 1.226 after calculating the average of all measurements with considerable intraindividual variation. No correlation between dmtDNA and the

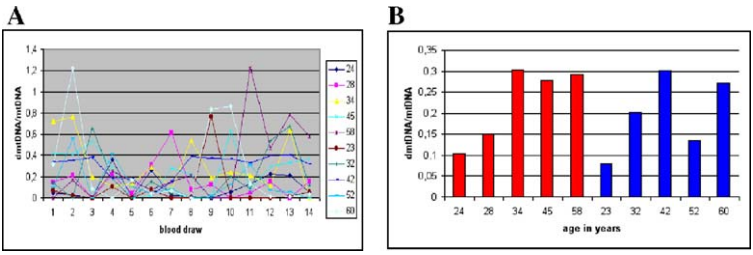

Fig. 1. Relative quantification of 4977 bp deletion in human blood; (A) shows the 14 single dmtDNA/mtDNA ratios per individual over a time period of 6 months; (B) demonstrates the average per individual, separated in female (light: age 24, 28, 34, 45, 58) and male probands (dark: age 23, 32, 42, 52, 60).

age of an individual or the gender was observed (Fig. 1A and B). The younger persons (23–32 years) showed significantly lower dmtDNA levels compared to the older persons (42–69 years). The pregnant woman showed the highest amount of dmtDNA which could be explained by the apparent loss of total mtDNA. Every person showed sporadic samples in which no dmtDNA could be detected, showing the mitochondrial genome as very flexible indicator with high potential for repair and restoration. Regarding those results, it will be interesting to elucidate possible relations between mtDNA mutagenesis and the monitored possible mtDNA stressors, e.g. alcohol [8], nicotine, some medication, etc.

MtDNA mutagenesis is a very flexible process; the total amount and also the occurrence of specific deletions can vary considerably.

Thus, mtDNA is not suited to estimate the age of an individual for a forensic approach. For clinical purposes the experimental design is crucial: either the number of samples or individuals has to be very high to eliminate single outliers due to other stressors than the biological age or several analyses per individual should be conducted.

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