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The 4977 bp deletion of mitochondrial DNA in human skeletal muscle, heart and different areas of the brain: A useful biomarker or more?

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ABSTRACT

It has been suggested that deletions of mitochondrial DNA (mtDNA) are important players with regard to the ageing process. Since the early 1990s, the 4977 bp deletion has been studied in various tissues, especially in postmitotic tissues with high energy demand. Unfortunately, some of these studies included less than 10 subjects, so the aim of our study was to quantify reliably the deletion amount in nine different regions of human brain, heart and skeletal muscle in a cohort of 92 individuals. The basal ganglia contain the highest deletion amounts with values up to 2.93% and differences in deletion levels between early adolescence and older ages were up to three orders of magnitude. Values in frontal lobe were on average an order of magnitude lower, but lowest in cerebellar tissue where the amount was on average only 5×10^{-3} of the basal ganglia. The deletion started to accumulate in iliopsoas muscle early in the fourth decade of life with values between 0.00019% and 0.0035% and was highest in a 102-year-old woman with 0.14%. In comparison to skeletal muscle, the overall abundance in heart muscle of the left ventricle was only one-third. The best linear logarithmic correlation between amount of the deletion and age was found in substantia nigra with $r = 0.87$ ($p < 0.0005$) followed by anterior wall of the left ventricle ($r = 0.82$; $p < 0.0005$). With regard to mitochondrial DNA damage, we propose to use the 4977 bp deletion as an ideal biomarker to discriminate between physiological ageing and accelerated ageing. The biological meaning of mitochondrial deletions in the process of ageing is under discussion, but there is experimental evidence that large-scale deletions impair the oxidative phosphorylation in single cells and sensitize these cells to undergo apoptosis.

1. Introduction

It has been postulated that alterations of mitochondrial DNA (mtDNA) are involved in the progression of the ageing process. MtDNA is a double-stranded DNA ring, packed into a nucleo-protein complex, called the nucleoid (Chen and Butow, 2005; Holt et al., 2007), lacks any introns and encodes for 13 proteins necessary for the assembly of the respiratory chain, 22 tRNAs and two ribosomal RNAs (Graeber et al., 1998). The general thinking for the past three decades has been that the ageing phenotype is a consequence of oxidative damage of proteins, lipids and DNA inflicted by free radicals which in turn are generated as normal byproducts of the oxidative phosphorylation (OXPHOS) (Miquel et al., 1980; Harman, 1981; Ames, 1989). The mtDNA is at higher risk, because it is located in close proximity of the respiratory chain, and displays reduced repair capacity relative to nuclear DNA. Oxidative damage of mtDNA can be detected on the molecular level as base adducts like 8-hydroxy-2′-deoxyguanosine (8-OHdG). These modified bases are especially prone to mispairing of repetitive elements and are correlated with deletions (Lezza et al., 1999). This observation has led to the proposal that deletions are induced via mispairing and slippage during replication (Shoffner et al., 1989). Nowadays it is quite clear that large-scale deletions may be attributed to polymerase slippage, homologous recombination, double strand breaks, inefficient repair mechanisms, or mutations in the helicase twinkle (Zeviani et al., 2003; Phadnis et al., 2005; Srivastava and Moraes, 2005). Furthermore, deletion events may be inflicted by tissue-specific nuclear genes, interactions between the mitochondrium and the nucleus or endonucleases that could shift mtDNA haplotypes (Ballard and Dean, 2001; Srivastava and Moraes, 2005).

The vast majority of large-scale deletions are located between the origins of replication O_H and O_L at np 5760 and np 190 of

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mtDNA (Bank et al., 2000). There is growing experimental evidence, especially from single cell studies that the overwhelming majority of large-scale deletions are generated by clonal expansion of a single stochastic mutational event in childhood or early adolescence (Elson et al., 2001; Bodyak et al., 2001; Storm et al., 2002; Khrapko et al., 2003; Gokey et al., 2004). It seems reasonable that large-scale deletions may be involved in an age-dependent decline of cell or tissue function, because essential parts of coding sequences are removed. The loss of the corresponding gene products may lead to faulty assembly of the respiratory chain. The resulting impaired function of OXPHOS, especially in postmitotic tissues with high energy demand, can explain the decline of cognitive function and memory, cardiovascular pathologies or loss of muscular strength. Additionally, mitochondria play a key role in the initiation of the apoptotic cascade, which may also play a role in the pathology of the ageing phenotype (Kujoth et al., 2007).

The 4977 bp deletion of mtDNA has attracted the attention of the ageing community since its age dependency in several tissues has been reported in the early 1990s (Cortopassi et al., 1992). Since then quantitative data for nearly all tissues (Corral-Debrinski et al., 1992a; Lee et al., 1994; Papiha et al., 1998; Zhang et al., 1999; Meissner et al., 2000a; Koch et al., 2001) or for specific cell types (Kao et al., 1995; Steuerwald et al., 2000; Storm et al., 2002; Mohamed et al., 2004) have been collected. This large pool of data makes the 4977 bp deletion an ideal biomarker for age-related changes in a tissue or a special compartment thereof. Interestingly, various areas of the human brain have been found to harbour substantially different amounts of the 4977 bp deletion (Corral-Debrinski et al., 1992a; Soong et al., 1992). Though there are a number of studies on the 4977 bp deletion in human skeletal muscle, brain and heart, some important data are still missing. Until now there is no study comparing the amount of the 4977 bp deletion in human heart, skeletal muscle and different areas of the brain with the same method or in the same individuals. Another important point to address is the detected differences in the amount of the deletion. Two studies published more than 10 years ago demonstrated a difference of roughly two orders of magnitude in general in different brain regions of 6 and 7 individuals of various ages (Soong et al., 1992; Corral-Debrinski et al., 1992a). In view of recent single cell or muscle fibre studies (Bodyak et al., 2001; Storm et al., 2002; Khrapko et al., 2003; Gokey et al., 2004), where large deletion amounts in single cells have been observed, the sample of less than 10 individuals seems not to be sufficient to obtain reliable data for a region of the brain. So the objective of our study was to collect reliable quantitative data on the common deletion in nine different areas of postmitotic tissues with high energy demand in a cohort ($n = 92$) of individuals.

2. Materials and methods

2.1. Sample material

About 100 mg tissue was collected at autopsy from substantia nigra, caudate nucleus, putamen, frontal cortex, cerebellum, anterior and posterior wall of the left ventricle of the heart, and iliopsas muscle of a total of 92 individuals. Tissue samples were immediately frozen in liquid nitrogen and kept at −70 °C until DNA was extracted. To exclude mitochondrial or degenerative diseases a complete microscopic examination of each tissue sample was performed. Only individuals dying of acute or peracute causes (e.g. stabbing, suffocation, drowning) were included in the study. Fifty subjects were sampled for each of the nine tissues. Care was taken not to exceed an interval of 48 h between death and autopsy.

2.2. DNA extraction and quantitation

Total DNA was extracted from 100 mg tissue using the Super QUICK-GENE SQG1 Kit (Immucor, Roedermark, Germany) following the instructions of the manufacturer. Fluorometric quantification of the extracts was performed by applying the PicoGreen dsDNA Quantitation Kit according to the recommended assay protocol (Molecular Probes, Willow Creek, OR, USA) and a fluorometer (Bio-Rad, Munich, Germany). The PicoGreen quantitation reagent is a sensitive fluorescent stain for quantifying double-stranded DNA without interference of extracted RNA.

2.3. PCR analysis

PCR was performed in 25 μl reaction mixture containing 50 mM KCl, 1.5 mM $MgCl_2$ and 10 mM Tris–HCl (pH 8.4), 200 μM of each of the dNTPs including dUTP, 0.5 μM each of the primers, 0.5 U AmpErase UNG, 1 U of Platinum™ Taq DNA polymerase (Life Technologies, Gaithersburg, MD, USA) and 5 ng of total DNA in the GeneAmp 2400, thermal cycler (Applied Biosystems, Weiterstadt, Germany). Cycling conditions were as follows: Beginning with one initial step with AmpErase UNG incubation at 50 °C for 2 min, 14–18 or 28–32 cycles at 95° C for 15 s, 60 °C for 30 s and 72 °C for 15 s were performed. Forward primers were labelled with 6-FAM by the manufacturer (Applied Biosystems, Weiterstadt, Germany). A fluorescent labelled 113 bp fragment of the undeleted mtDNA resulting from amplification with primers int1F 5′-C CCTTCGCTGACGCCATA-3′ (forward) and int2R, 5′-AGTAGAAGA GCGATGGTGAGAGC-3′ (reverse) was generated. Another pair of primers del1F 5′-CACCATAATTACCCCCATACTCCTTA-3′ and del2R 5′-GAGGAAAGGTATTCCTGCTAATGC-3′ was used to amplify a fluorescent labelled 123 bp fragment specific for mtDNA carrying the 4977 deletion.

2.4. Quantitation of PCR products

Fragments were detected and quantified by the ABI Prism™ 310 Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany) according to the method described elsewhere (Mohamed et al., 2004). Briefly, a 1-μl aliquot of each PCR and 0.5 μl of the internal size standard (GS350, Applied Biosystems, Weiterstadt, Germany) labelled with the fluorescent dye ROX (Applied Biosystems, Weiterstadt, Germany) were denaturated and separated by capillary electrophoresis (CE) using the ABI Prism™ 310 Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany). When the run was finished, specific fragments were displayed as peaks in an electropherogram using the Gene Scan Analysis Software 3.1. This software determines automatically fragment length and amount of a specific PCR product by calculating the area under the peak. The calculated value of the peak area corresponds directly to the amount of the product. To ensure that the signals derived from wild type molecules were in the exponential phase, the amount of wild type mtDNA was determined applying 14–18 cycles. When the quantitative results of the ABI Prism™ 310 Genetic Analyzer clearly indicate that the PCR of the sample was in the plateau phase, cycle number was reduced and the sample was quantified three times using the optimal number of cycles. The same approach was performed for deleted mtDNA, but numbers varied between 28 and 32 cycles. The frequency of the common deletion was determined by the ratio of the amount of the 123 bp deletion-specific fragment and the 113 bp fragment of intact mtDNA.

2.5. Statistics

Statistical analyses were performed using the SPSS statistical Package 14.0 (SPSS GmbH Software, Munich, Germany). In case