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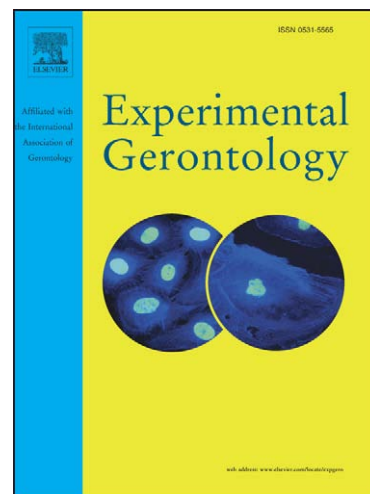
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**The 4977 bp deletion of mitochondrial DNA in human skeletal muscle, heart and different areas of the brain: a useful biomarker or more?**

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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  $5 \times 10^{-3}$  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.

## 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 mitochondrion 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 is located between the origins of replication  $O_H$  and  $O_L$  at np 5760 and np 190 of mtDNA (Bank et al., 2000). There is growing

experimental evidence, especially from single cell studies that the overwhelming majority of large scale deletions is 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 ten 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.

## **Material and methods**

### **2.1 Sample material**

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 iliopsoas muscle of a total of 92 individuals. Tissue samples were immediately frozen in liquid nitrogen and kept at  $-70^{\circ}\text{C}$  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  $\mu$ l reaction mixture containing 50 mM KCl, 1.5 mM MgCl<sub>2</sub> and 10 mM Tris-HCl (pH 8.4), 200  $\mu$ M of each of the dNTP's including dUTP, 0.5  $\mu$ M each of the primers, 0.5 U AmpErase UNG, 1 U of Platinum<sup>TM</sup> 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 sec and 72°C for 15 s were performed. Forward primers were labeled 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'-CCCTTCGCTGACGCCATA-3' (forward) and int2R, 5'-AGTAGAAGAGCGATGGTGAGAGC-3' (reverse) was generated. Another pair of primers del1F 5'-CACCAT-AATTACCCCCATACTCCTTA-3' and del2R (5'-GAGGAAAGG-TATTCCTGC-TAATGC-3') was used to amplify a fluorescent labeled 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- $\mu$ l aliquot of each PCR reaction and 0.5  $\mu$ l of the internal size standard (GS350, Applied Biosystems, Weiterstadt, Germany) labelled with the fluorescent dye ROX (Applied Biosystems, Weiterstadt, Germany) were denatured 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-reaction 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 28-32 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 of samples where the 4977 bp deletion was not detectable, a set of arbitrary abundance values between 0.00001% and 0.00009% was generated by the random number generation system of Microsoft

Excel 11.0. The mean correlation coefficient  $r$  was obtained by taking the average of  $r$  values from the regression analyses based on 10 independent sets of randomly generated values below the detection limit of 0.00009%.

## Results

Applying a quantitative PCR approach we calculated the amount of the 4977 bp deletion in nine different areas of postmitotic tissues of 92 different individuals (table 1). All of the tissues revealed a positive correlation between deletion amount and advancing age of the subjects, but there was a strong variability even between individuals of same age decade. In human brain the deletion was even detectable in individuals as young as 10 or 12 years (table 1). The different areas of the brain displayed striking differences in the level of the common deletion. The basal ganglia exhibit the highest deletion amounts with values up to 2.93% in substantia nigra of a 68-year-old individual and up to 2.14% in caudate nucleus of a 76-year-old person. We observed differences in deletion levels between early adolescence and older ages of up to  $2.6 \times 10^3$  in caudate nucleus. The frequency of the common deletion was nearly the same in substantia nigra, putamen and caudate nucleus, but values in frontal lobe brain were on average an order of magnitude lower. The lowest values of the deletion were observed in cerebellar tissue where the amount was on average only  $5 \times 10^{-3}$  of the basal ganglia. In a single case the common deletion was not detectable in cerebellum, in spite of an amount of 1.12% in substantia nigra of the same 93-year-old individual. In frontal lobe the deletion was detectable at the age of 12 with an amount of 0.0016% and reached values between 0.039% and 0.18% by the end of the sixth decade and 102 years, respectively (table 1). In skeletal muscle, the amount of the 4977 bp deletion was roughly half of that detected in frontal lobe. In general, the deletion started to appear in iliopsoas muscle in the early thirties with

values between 0.00019% and 0.0035%, but interestingly there were three individuals aged 0.5, 1 and 8 years with a relatively high level of up to 0.00159%. The highest abundance was detected in a 102-year-old woman with 0.14%. This means roughly a 1000-fold increase from the early thirties up to very old age. In human heart the 4977 bp deletion was detected around thirty years of age, too, but it was even observed in the anterior wall of the left ventricle and the right ventricle of a one year old child and even in the right ventricle of a half year old child with an abundance of 0.00475%. The highest amount (0.0929%) was observed in the posterior wall of a 31-year old individual. At around age 85 the abundance of the 4977 bp deletion varied between 0.0109% and 0.0282% (table 1). Interestingly the total amounts in the anterior and posterior wall of the left ventricle were very similar in the majority of the subjects, but varied up to fivefold in some individuals. In comparison to skeletal muscle, total deletion abundance in heart muscle of the left ventricle was only one third. The amount of the 4977 bp deletion displayed the greatest diversity with regard to age when the right ventricle was analysed. The lowest detectable value was 0.00015% in a normal adult 31 years old, the highest 0.17% in an 8-year-old individual. Comparing the nine different tissues examined, our data clearly show the overall abundance of the common deletion varies with tissue type, in the following descending order: substantia nigra > caudate nucleus > putamen > right ventricle > frontal lobe brain > skeletal muscle > left ventricle (anterior and posterior wall) > cerebellum.

The best linear logarithmic correlation between amount of the deletion and age was achieved in substantia nigra with  $r = 0.87$  ( $p < 0.0005$ ), followed by anterior wall of the left ventricle ( $r = 0.82$ ;  $p < 0.0005$ ), caudate nucleus ( $r = 0.80$ ;  $p < 0.0005$ ), putamen ( $r = 0.79$ ;  $p < 0.0005$ ), frontal lobe brain ( $r = 0.79$ ;  $p < 0.0005$ ), iliopsoas muscle, ( $r = 0.70$ ;  $p < 0.0005$ ), posterior wall of the left ventricle ( $r = 0.67$ ;  $p > 0.0005$ ), cerebellum ( $r = 0.66$ ;

$p < 0.0005$ ) and right ventricle ( $r = 0.57$ ;  $p < 0.0005$ ). The data clearly indicate an age-dependent accumulation of the 4977 bp deletion in the nine different regions of postmitotic tissues (Fig. 1A – Fig. 1I).

## Discussion

This study demonstrates, that there are striking differences in the amount of the common deletion. The abundance of the 4977 deletion is highest in the basal ganglia with values of more than three orders of magnitude higher in comparison to cerebellar tissue. It has been speculated, that the higher amount of the 4977 bp deletion may be due to tissue properties. The basal ganglia are regions of high iron levels, because these regions have low or no transferrin-binding sites, whereas grey matter regions with low levels of iron have a high density of transferrin-binding sites (Griffiths et al., 1999). Additionally, in corpus striatum and substantia nigra brain dopamine levels are high in comparison to other regions. Auto-oxidation and deamination of dopamine by monoamine oxidase B, which level increases in an age dependent manner (Goldstein and Lieberman, 1992), leads to the generation of  $H_2O_2$ , which can react with iron via the Fenton reaction forming the highly reactive hydroxyl radical (Soong et al., 1992). Interestingly, when the number of mtDNA molecules carrying the deletion approached more than 1.5% of all mtDNAs in the basal ganglia, a plateau in the logarithmic scale was observed. It is suggested, that in case the amount of rearranged mtDNAs within the cell reaches a certain threshold, the oxidative stress becomes too high and the cell and its mutant mtDNAs are eliminated via the opening of the mitochondrial transition pore and subsequent apoptosis. A very interesting observation is the low abundance of the common deletion in the grey matter of the cerebellum. In alignment with our findings, earlier

studies from other groups showed that the mtDNA of cerebellar cells has about 0.5% to 1% of the deletion amount of the basal ganglia (Soong et al., 1992; Corral-Debrinski et al., 1992a; Meissner et al., 2000b). Therefore, the cerebellum seems to be protected by tissue properties like anti-oxidant enzyme protection or status of repair systems (Gershon, 1999). The remarkable quantitative differences in the deletion between the tissues seen here, indicate that the accumulation is not universal, but tissue specific. Because tissues exhibiting the lowest deletion load (cerebellum) and tissues with the highest deletion load (basal ganglia) are made up of neurons and glial cells, tissue properties like dopaminergic connectivity must be more important than cell properties. It should be stressed, that mitochondrial deletions do not accumulate in postmitotic neurons only, but also (and at a similar rate) in dividing cells like astrocytes (Storm et al., 2002). In the same manner, deletion loads between iliopsoas muscle and heart varied up to threefold on average, despite both skeletal muscle and heart muscle consisting of striate myocytes. The differences are even more striking, when all mitochondrial deletions are analysed. In comparison to skeletal muscle, heart muscle tissue exhibits a deletion pattern that is uniform across individuals, indicating that tissue properties play the key role in accumulation of mitochondrial deletions (Meissner et al., 2006).

All of the nine areas of postmitotic tissues exhibit an increase of the 4977 bp deletion in an age-dependent manner, which is in accordance with other studies (Soong et al., 1992; Corral-Debrinski et al., 1992a; Zhang et al., 1998; Liu et al., 1998). Our data give the best correlation between age and In mtDNA% for the substantia nigra (Fig. 1A), while the correlation for the right ventricle is much weaker (Fig. 1I). This may be due to tissue sampling artefacts, because samples of the right ventricle sometimes include the cell layers beneath the endocardium, which are especially prone to ischemic/hypoxic conditions, and therefore would explain increased amounts of the

deletion (Merril et al., 1996). The relatively high linear correlation of  $\ln(\text{dmtDNA } \%)$  with age in all the investigated brain tissues indicates that there is a basal rate in the accumulation of deletions which may be modulated by internal or external stressors. The 4977 bp deletion and other deletion events are induced by external factors like UV radiation (Berneburg et al., 2004), ionising radiation (Prithivirajsingh et al., 2004) deuterium oxide (Berneburg et al., 1999) or by pathologic conditions like chronic hypoxia/reperfusion (Merril et al., 1996; Bagenholm et al., 1998), ischemia (Corral-Debrinski et al., 1992b) or end-stage renal disease (Lim et al., 2000). All of these factors are associated with an increased generation of free radicals. Mitochondrial deletions are somatically acquired during human lifetime in a tissue. The first mutagenic event appears to be stochastic oxidative damage of single base pairs of mtDNA (Lezza et al., 1999), inflicted by free radicals or other oxidative stressors. It is important to mention that damage of mtDNA is largely confined to the mitochondria themselves, because free radicals cannot diffuse very far. On the contrary, the amount of free radicals has been overestimated for a long time and is probably not more than 0.2% of mitochondrially consumed oxygen (St-Pierre et al., 2002). Additionally, there is a lot of experimental evidence that the base excision repair (BER) pathway in mitochondria is efficient and oxidative lesions like 8-OHdG are removed from the genome (Stevnsner et al., 2002). Therefore local repair mechanisms may be sufficient to hold most of the oxidative mtDNA damage in check, of which there might be not as much as had been assumed in the past. In case of oxidative damage of single base pairs, this mutagenic lesion favours the generation of large scale deletions, which are clonally expanded over the passage of time in a single cell as has been shown in an elegant experimental study (Khrapko et al., 2003), that confirmed a previous computer simulation approach (Elson et al., 2001). But there must be a selective force at work against mitochondrial deletions. The half

life of a neuronal mitochondrion lies between 18 and 24 days (Korr et al., 1998). This means that mitochondria in the brain of an 80-year-old individual have undergone anywhere between 1200 to 1600 duplications. Given that the half life of mitochondria is the same as the half life of mtDNA (Gross et al., 1969) and that turnover of mitochondrial genomes alone would determine the accumulation rate, one would calculate a  $10^{18}$ -fold increase of a single initial mtDNA deletion during the human lifespan of eighty years in a tissue (Simonetti et al., 1992). Our study clearly demonstrates that the common deletion in normal human caudate nucleus or iliopsoas muscle increases from young adulthood to old age merely by a factor of  $10^3$ , indicating a selective force against the accumulation of mitochondrial deletions, one of which may be apoptosis.

It has been shown in several studies that mitochondrial deletions can serve as an excellent biomarker of photoageing in human skin (Koch et al., 2001; Berneburg et al., 2004; Eshaghian et al., 2006). The results of the present study lead us to suggest in agreement with others (Ross et al., 2002) to use the 4977 bp deletion as a biomarker of biological ageing in general, because age seems to reveal the strongest correlation with its abundance. In case of human oocytes it has been confirmed that age exhibits the only significant correlation with the presence of the 4977 bp deletion, while other parameters like smoking or early follicular phase FSH did not reveal any correlation (Chan et al., 2005). In recent years, a number of carefully conducted studies reported, that the level of the 4977 bp deletion is not increased in different tumour tissues (Dani et al., 2004; Dai et al., 2006; Ye et al., 2007) and in conditions associated with chronic inflammation like ulcerative colitis (Fukushima and Fiocchi, 2004). Though our data display a great variability even in individuals of the same age group, the large pool of data may help to discriminate between physiological ageing and pathological conditions associated with accelerated ageing. Interestingly an

increase in deletion amounts is observed in association with smoking (Ballinger et al., 1996), ethanol consumption (Mansouri et al., 1997) or sunlight exposure (Ray et al., 2000), all of which seem to accelerate the ageing process. There are at least three aspects that render the deletion an excellent biomarker. First, the high sensitivity of any PCR based assay reveals this deletion even in minute amounts in lymphocytes or other blood cells (Mohamed et al., 2004). The detection limit of 0.00009% as was observed in our study, nearly equals the detection of a single deleted molecule in one million molecules and is sensitive enough to show early signs of biological ageing. Second, the common deletion is observable in all tissues, so a comparison of different tissues in the same individual is easy to achieve. Third, there seems to be a basal rate for the accumulation of the deletion with a high correlation to age only, which is not modulated by chronic inflammation, tumour development or progression. As outlined above the abundance of the 4977 bp deletion is only determined by conditions associated with a increased generation of free radicals. In spite of the use of the common deletion as an ideal biomarker, with regard to damage of mtDNA the measurement of a panel of different deletions may be more useful to monitor biological ageing.

The role of the common deletion and other deletion events regarding the ageing process has been elucidated recently in single cell studies and cybrid experiments. It is obvious, that large scale deletions remove coding sequences of proteins and tRNAs, that are necessary for correct assembly of the ETC. This should impair the respiratory capacity of the affected mitochondrion where the ratio of deleted to wild type mitochondrial DNA molecules determines severity. The 4977 bp deletion encompasses the genes of four subunits of NADH dehydrogenase (Complex I), subunit 3 of cytochrome oxidase (COX; Complex IV) and for subunits 6 and 8 of ATPase (Complex V). The loss of these proteins is associated with severe

mitochondrial diseases like Pearson's syndrome, Kearns-Sayre syndrome or chronic progressive external ophthalmoplegia (Peng et al., 2006). In these diseases the deletion amount exceeds usually 50%-55% in a given tissue and therefore is more than one to three orders of magnitude higher than in normal ageing tissues. Therefore it is difficult to imagine that the 4977 bp deletion may account for the age related changes in a tissue. On the cellular level there is a lot of evidence that mtDNA deletions do have functional consequences, as is reflected by the correlations demonstrated between deletions and biochemical parameters. The fate of single cells carrying high amounts of the common deletion has been shown recently. In a lot of studies it was shown that single COX-negative fibres of skeletal muscle or COX-negative neurons contain a heterogeneous array of different deletions (Kopsidas et al., 2002, Gokey et al., 2004; Bua et al., 2006; Bender et al., 2006; Kraysberg et al., 2006). In all studies a strong correlation between deletions, ETC abnormalities and even sarcopenia or Parkinsonism was observed. Thus, on the single-cell level there appears to be a causal link between the age-related rise in mitochondrial deletions and the reported reductions in respiratory chain enzyme activities. From this data it seems evident that the amount of intact mtDNA correlates with unimpaired function of the respiratory chain. However, even in single cells the impact of mitochondrial deletions is still being questioned, because in human heart and skeletal muscle the observed abundance of all deletion species per cell is in the order of 100 mtDNA molecules or less (Kajander et al., 2000), which should not impair the metabolism of the cell. Additionally, it is yet to be proven, whether mtDNA or ETC abnormalities considerably compromise metabolic function in muscle bundles or even in complete muscles, because in muscle biopsies of aged human individuals no change in ETC activity was observed (Rasmussen et al., 2003; Chabi et al., 2005). Conversely, one has to keep in mind that skeletal muscle is organized in a segmental manner and the

force of the muscle bundle depends on the weakest segment where COX-negative fibres are present in a significant number of cells (Bua et al., 2006). It has been estimated, that 6% - 31% of fibres with ETC abnormalities may account for fibre breakage and, ultimately, fibre loss (Bua et al., 2006). As mentioned above mtDNA deletions eliminate tRNAs essential for mitochondrial translation. Translation should not be possible in mitochondria containing only deleted mitochondrial genomes (Mita et al., 1989). In a heteroplasmic state, the amount of wild type mtDNA determines the amount of synthesized proteins. The inhibition of protein synthesis could induce the autophagic transcript ATG12, which is involved in an early step of the mammalian autophagy pathway, probably as a cytoprotective mechanism (Prigione and Cortopassi, 2007).

There are observations leading to the conclusion that mitochondrial deletions play a role in apoptosis of single cells. Cybrids with amounts of the 4977 bp deletion ranging between 16% and 80% of all mitochondrial genomes exhibit a significantly enhanced ROS production. Treatment of these cells with H<sub>2</sub>O<sub>2</sub> induced the morphological signs of apoptosis like membrane blebbing or nuclear condensation (Peng et al., 2006). Other cybrid experiments indicated that the generation of mitochondrial ROS is increased depending on the deletion amount, but displayed no threshold effect because elevated mitochondrial ROS production was even detectable in cells with a relatively low amount of 16% of the common deletion (Peng et al., 2006, Indo et al., 2007). UV-induced programmed cell death was increased in cybrids harbouring amounts of the 4977 bp deletion up to 80% leading to the suggestion that large scale deletion sensitize a cell to undergo apoptosis (Schoeler et al., 2006; Liu et al., 2007). One has to keep in mind that not the common deletion alone, but up to 200 different types of deleted mitochondrial genomes have been detected in bulk tissues of skeletal muscle, human heart and brain (Hayakawa et al., 1996; Kowalenko et al.,

1997; Meissner et al., 2006). Therefore it seems conceivable that any enhancement of oxidative stress by external or internal conditions will initiate the apoptotic cascade in deletion bearing cells. An ongoing apoptosis may exhaust stem cell renewal capacities, leading to the ageing phenotype with features such as hair greying, cardiac enlargement or sarcopenia (Kujoth et al., 2005; Kujoth et al., 2007). Therefore the total of all mitochondrial deletions can promote a deterioration of tissue function through the loss of irreplaceable cells by programmed cell death (Meissner, 2007).

### **Conclusion**

The age-related increase of the common deletion is not universal, but clearly tissue-specific. It has been demonstrated in our study and others that different organs or even different areas of the same organ accumulate the 4977 bp deletion at different rates, with differences up to more than three orders of magnitude. This makes this deletion an ideal biomarker to determine age related changes in a given tissue. With regard to a causal link between the amount of the common deletion and a decline of tissue function with advancing age, it is not conceivable that one single mitochondrial deletion can be the sole cause of the ageing phenotype. On the other hand, recent single cell studies and cybrid experiments have shown conclusively that the common deletion predisposes for an increased sensitivity to oxidative stress and therefore triggers apoptosis. It is well known that up to 200 different types of deleted mitochondrial genomes are detectable in postmitotic tissues. The clonal amplification of any deletion in a single cell will heighten the risk of this cell to undergo programmed cell death. An increased apoptosis in itself may lead to an exhaustion of stem cell renewal capacity that may then contribute to the ageing phenotype with a deterioration of function in tissues with high energy demand.

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Fig.1. Accumulation of the 4977 bp mitochondrial deletion during human ageing in substantia nigra (A), putamen (B), caudate nucleus (C), frontal lobe (D), cerebellum (E), iliopsoas muscle (F), posterior wall (pw) of the left ventricle (G), anterior wall (aw) of the left ventricle (H) and right ventricle (I). The natural logarithm of the abundance of the 4977 bp deleted mtDNA in percentage of total mtDNA ( $\ln[\text{abundance dmtDNA} \ %]$ ) was plotted as a function of age of the individuals. Black circles in each panel represent quantified values for the 4977 bp deletion, white circles in each panel represent values having been generated randomly.

Fig.1

Fig 1

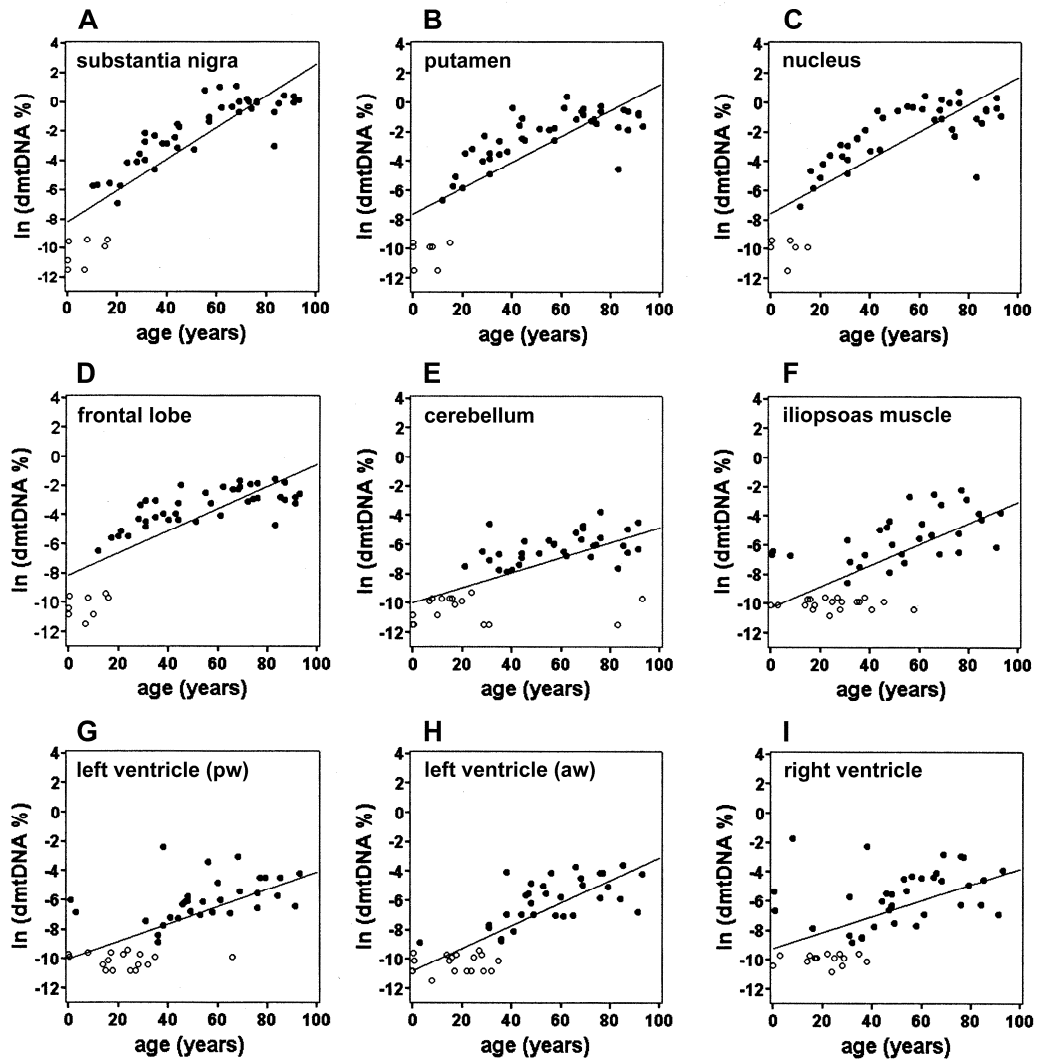


Table 1. Amount of the 4977 bp deletion (in %) in nine different regions of postmitotic tissues

| Age (years) | substantia nigra | putamen | caudate nucleus | frontal lobe | cerebellum | iliopsoas muscle | posterior wall of left ventricle | anterior wall of left ventricle | right ventricle |
|-------------|------------------|---------|-----------------|--------------|------------|------------------|----------------------------------|---------------------------------|-----------------|
| 0.2         | 0.00000          | 0.00000 | 0.00000         | 0.00000      | 0.00000    | -                | -                                | -                               | -               |
| 0.3         | 0.00000          | 0.00000 | 0.00000         | 0.00000      | 0.00000    | -                | -                                | -                               | -               |
| 0.3         | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 0.5         | -                | -       | -               | -            | -          | 0.00128          | 0.00000                          | 0.00000                         | 0.00475         |
| 0.5         | 0.00000          | 0.00000 | 0.00000         | 0.00000      | 0.00000    | -                | -                                | -                               | -               |
| 1           | -                | -       | -               | -            | -          | 0.00159          | 0.00242                          | 0.00000                         | 0.00129         |
| 3           | -                | -       | -               | -            | -          | 0.00000          | 0.00104                          | 0.00014                         | 0.00000         |
| 7           | 0.00000          | 0.00000 | 0.00000         | 0.00000      | 0.00000    | -                | -                                | -                               | -               |
| 8           | 0.00000          | 0.00000 | 0.00000         | 0.00000      | 0.00000    | -                | -                                | -                               | -               |
| 8           | -                | -       | -               | -            | -          | 0.00122          | 0.00000                          | 0.00000                         | 0.16938         |
| 10          | 0.00316          | 0.00000 | 0.00000         | 0.00000      | 0.00000    | -                | -                                | -                               | -               |
| 12          | 0.00334          | 0.00123 | 0.00082         | 0.00155      | 0.00000    | -                | -                                | -                               | -               |
| 14          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 15          | 0.00000          | 0.00000 | 0.00000         | 0.00000      | 0.00000    | 0.00000          | 0.00000                          | 0.00004                         | 0.00000         |
| 16          | 0.00000          | 0.00331 | 0.00938         | 0.00000      | 0.00000    | -                | -                                | -                               | -               |
| 16          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00039         |
| 17          | 0.00377          | 0.00612 | 0.00292         | 0.00375      | 0.00000    | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 18          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 20          | 0.00096          | 0.00281 | 0.00582         | 0.00406      | 0.00000    | -                | -                                | -                               | -               |
| 21          | 0.00327          | 0.03179 | 0.01497         | 0.00573      | 0.00056    | -                | -                                | -                               | -               |
| 22          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 24          | 0.01557          | 0.04187 | 0.02814         | 0.00425      | 0.00009    | -                | -                                | -                               | -               |
| 24          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 25          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 27          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 28          | 0.01731          | 0.01784 | 0.05560         | 0.01393      | 0.00155    | -                | -                                | -                               | -               |
| 28          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 29          | 0.02807          | 0.10293 | 0.02650         | 0.03512      | 0.00000    | -                | -                                | -                               | -               |
| 29          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 31          | 0.11920          | 0.00727 | 0.00803         | 0.01153      | 0.00000    | -                | -                                | -                               | -               |
| 31          | 0.01931          | 0.02190 | 0.02069         | 0.00805      | 0.00082    | -                | -                                | -                               | -               |
| 31          | 0.06590          | 0.03201 | 0.05294         | 0.04718      | 0.01015    | -                | -                                | -                               | -               |
| 31          | -                | -       | -               | -            | -          | 0.00019          | 0.00057                          | 0.00046                         | 0.00024         |
| 31          | -                | -       | -               | -            | -          | 0.00353          | 0.00058                          | 0.00039                         | 0.00335         |
| 32          | -                | -       | -               | -            | -          | 0.00080          | 0.00000                          | 0.00000                         | 0.00015         |
| 35          | 0.00969          | 0.07336 | 0.08620         | 0.01523      | 0.00126    | -                | -                                | -                               | -               |
| 35          | 0.10261          | 0.02859 | 0.09278         | 0.04588      | 0.00043    | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 36          | -                | -       | -               | -            | -          | 0.00000          | 0.00014                          | 0.00016                         | 0.00020         |
| 36          | -                | -       | -               | -            | -          | 0.00054          | 0.00023                          | 0.00018                         | 0.00021         |
| 38          | 0.05833          | 0.03556 | 0.15139         | 0.01934      | 0.00038    | -                | -                                | -                               | -               |
| 38          | -                | -       | -               | -            | -          | 0.00000          | 0.00000                          | 0.00000                         | 0.00000         |
| 38          | -                | -       | -               | -            | -          | 0.00126          | 0.09290                          | 0.01673                         | 0.10481         |
| 40          | 0.05790          | 0.66400 | 0.03662         | 0.01284      | 0.00043    | -                | -                                | -                               | -               |
| 41          | -                | -       | -               | -            | -          | 0.00000          | 0.00075                          | 0.00031                         | 0.00044         |
| 43          | 0.08787          | 0.20885 | 0.57348         | 0.01898      | 0.00060    | -                | -                                | -                               | -               |
| 44          | 0.04436          | 0.08328 | 0.04080         | 0.01276      | 0.00133    | -                | -                                | -                               | -               |
| 44          | 0.22048          | 0.32713 | 0.03853         | 0.03851      | 0.00097    | -                | -                                | -                               | -               |
| 44          | -                | -       | -               | -            | -          | 0.00703          | 0.00069                          | 0.00092                         | 0.00244         |
| 45          | 0.18343          | 0.07612 | 0.35704         | 0.14033      | 0.00305    | -                | -                                | -                               | -               |
| 46          | -                | -       | -               | -            | -          | 0.00005          | 0.00184                          | 0.00353                         | 0.00403         |
| 47          | -                | -       | -               | -            | -          | 0.00835          | 0.00217                          | 0.00398                         | 0.00137         |
| 48          | -                | -       | -               | -            | -          | 0.01281          | 0.00313                          | 0.00790                         | 0.00381         |
| 48          | -                | -       | -               | -            | -          | 0.00039          | 0.00222                          | 0.00206                         | 0.00185         |
| 49          | -                | -       | -               | -            | -          | 0.00264          | 0.00112                          | 0.00091                         | 0.00054         |
| 51          | 0.03788          | 0.16684 | 0.55876         | 0.01138      | 0.00133    | -                | -                                | -                               | -               |
| 53          | -                | -       | -               | -            | -          | 0.00133          | 0.00086                          | 0.00666                         | 0.01150         |
| 54          | -                | -       | -               | -            | -          | 0.00072          | 0.00210                          | 0.00387                         | 0.00500         |
| 55          | 2.23447          | 0.15906 | 0.76558         | 0.08272      | 0.00319    | -                | -                                | -                               | -               |
| 56          | -                | -       | -               | -            | -          | 0.06739          | 0.03346                          | 0.01665                         | 0.01367         |
| 57          | 0.26211          | 0.07467 | 0.71718         | 0.04056      | 0.00263    | -                | -                                | -                               | -               |
| 57          | 0.34217          | 0.17025 | 0.73683         | 0.03998      | 0.00246    | -                | -                                | -                               | -               |
| 58          | -                | -       | -               | -            | -          | 0.00000          | 0.00105                          | 0.00089                         | 0.00047         |
| 60          | -                | -       | -               | -            | -          | 0.00392          | 0.00815                          | 0.00299                         | 0.01171         |
| 61          | 2.80975          | 0.66349 | 0.63927         | 0.01729      | 0.00152    | -                | -                                | -                               | -               |
| 61          | -                | -       | -               | -            | -          | 0.01045          | 0.00248                          | 0.00082                         | 0.00098         |

|     |         |         |         |         |         |         |         |         |         |
|-----|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| 62  | 0.65900 | 1.43546 | 1.54104 | 0.12212 | 0.00114 | -       | -       | -       | -       |
| 65  | -       | -       | -       | -       | -       | 0.00501 | 0.00101 | 0.00090 | 0.01260 |
| 66  | 0.69877 | 0.31988 | 0.31596 | 0.10453 | 0.00563 | -       | -       | -       | -       |
| 66  | -       | -       | -       | -       | -       | 0.08023 | 0.00000 | 0.02439 | 0.01706 |
| 68  | 2.93260 | 0.52520 | 0.60886 | 0.10034 | 0.00343 | 0.00135 | 0.04655 | 0.01153 | 0.01002 |
| 69  | -       | -       | -       | -       | -       | 0.03949 | 0.00435 | 0.00711 | 0.05913 |
| 69  | 1.04154 | 0.65909 | 1.25138 | 0.12064 | 0.00880 | -       | -       | -       | -       |
| 69  | 0.49375 | 0.41551 | 0.32594 | 0.18316 | 0.00735 | -       | -       | -       | -       |
| 72  | 1.22888 | 0.28128 | 0.99297 | 0.04324 | 0.00107 | -       | -       | -       | -       |
| 72  | -       | -       | -       | -       | -       | -       | -       | -       | -       |
| 73  | 1.01947 | 0.31959 | 0.16215 | 0.14146 | 0.00227 | -       | -       | -       | -       |
| 74  | 0.62762 | 0.23092 | 0.10377 | 0.05250 | 0.00235 | -       | -       | -       | -       |
| 76  | 1.03917 | 0.77372 | 2.14265 | 0.15326 | 0.00387 | -       | -       | -       | -       |
| 76  | 0.94818 | 0.54062 | 0.99899 | 0.05790 | 0.02379 | -       | -       | -       | -       |
| 76  | -       | -       | -       | -       | -       | 0.00154 | 0.00398 | 0.01666 | 0.05361 |
| 76  | -       | -       | -       | -       | -       | 0.00541 | 0.00140 | 0.00293 | 0.00191 |
| 77  | -       | -       | -       | -       | -       | 0.10783 | 0.01105 | 0.01597 | 0.05080 |
| 79  | -       | -       | -       | -       | -       | 0.05798 | 0.01161 | 0.00630 | 0.00764 |
| 83  | 0.04885 | 0.01100 | 0.00636 | 0.00889 | 0.00000 | -       | -       | -       | -       |
| 83  | 0.50538 | 0.17953 | 0.33266 | 0.20600 | 0.00049 | -       | -       | -       | -       |
| 84  | -       | -       | -       | -       | -       | 0.02166 | 0.00325 | 0.00270 | 0.00191 |
| 85  | 0.89096 | 0.61358 | 0.24221 | 0.06026 | 0.00234 | 0.01418 | 0.01121 | 0.02816 | 0.01092 |
| 87  | 1.50599 | 0.15673 | 0.55849 | 0.05050 | 0.00145 | -       | -       | -       | -       |
| 87  | 1.55016 | 0.53381 | 0.62518 | 0.16687 | 0.00687 | -       | -       | -       | -       |
| 91  | 1.45059 | 0.43071 | 1.36403 | 0.03911 | 0.00182 | -       | -       | -       | -       |
| 91  | 0.95200 | 0.48032 | 0.68750 | 0.05857 | 0.01158 | 0.00218 | 0.00164 | 0.00115 | 0.00098 |
| 93  | 1.12614 | 0.19640 | 0.40864 | 0.07361 | 0.00000 | 0.02337 | 0.01484 | 0.01531 | 0.02105 |
| 102 | 1.19788 | 0.15698 | 0.45744 | 0.07448 | 0.01298 | 0.14073 | 0.00474 | 0.02164 | 0.00455 |