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Mitochondrial DNA Deletions in Human Cardiac Tissue Show a Gross Mosaic Distribution

Chunfang Zhang,*[,1] Amelia Lee,* Vincent W. S. Liu,*[,2] Salvatore Pepe,† Franklin Rosenfeldt,† and Phillip Nagley*

**Department of Biochemistry and Molecular Biology, Monash University, Clayton, Victoria 3168, Australia; and †Cardiac Surgical Research Unit, Baker Medical Research Institute and Alfred Hospital, Prahran, Victoria 3181, Australia*

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The variability of mitochondrial DNA (mtDNA) deletional patterns has been investigated in adjacent slices of human heart atrium. Using quantitative PCR we found differential abundances of one particular mtDNA deletion, that of 4977 bp (mtDNA^{4977}), among sets of adjacent slices of right atrial trabeculae pectinatae from 10 subjects. Some subjects had relatively constant abundance of mtDNA^{4977} among the tissue slices, while others covered a wide range. A qualitative PCR procedure was used to visualize the patterns of multiple deletions within an 8.64-kb segment of the mtDNA genome, in the same set of atrial trabeculae samples. Some subjects showed completely different multiple deletional patterns in each of the trabeculae slices analyzed. There was no correlation between the variation of the abundance of mtDNA^{4977} and that of the multiple deletions. The results are consistent with the notion that the occurrence of mtDNA deletions during aging is a random process, involving their production throughout the lifetime of an individual. In this view, the patterns of new deletions are superimposed on those already accumulated by propagation and segregation of mutations formed earlier in life.

Each human cell contains up to several thousand copies of mitochondrial DNA (mtDNA) whose genes are concerned exclusively with bioenergy production (1). The aging of humans is accompanied by decline in oxidative phosphorylation functions (reviewed in Refs. 2–5) in many tissues, particularly post-mitotic tissues characterized by high energy demand and low rate of cell division, such as skeletal muscle and cardiac muscle. The age-associated occurrence and accumulation of mtDNA mutations in human tissues has been well documented in the last 10 years, including deletions, point mutations and duplications (reviewed in Ref. 6). Among them, deletions have been the most extensively studied and a 4977-bp deletion (mtDNA^{4977}) has been the focus of many investigations. This deletion is generated between a pair of 13-bp direct repeats, located at nucleotide (nt) positions 8470–8482 and nt 13447–13459, respectively (7–9). Furthermore, many other deletions also occur in human tissues during aging (8–14). When different mtDNA deletions are encountered in the same tissue extract, these are known as "multiple deletions" (6, 8).

In most studies to date on tissues of aging human subjects, mtDNA deletions have been analyzed by PCR using total cellular DNA extracted from homogenates of single pieces of biopsy or post-mortem tissues. These studies have, therefore, examined the prevalence of a particular deletion or multiple deletions averaged over a large number of cells. In this total tissue homogenate approach, the distribution and localization of such deletions within tissues cannot be addressed. To gain deeper understanding of the occurrence and segregation of such mtDNA deletions, the study of their distribution and localization in different portions of the same tissue is important. We recently showed that adjacent parts of the same skeletal muscle sample from a single human subject contained distinct patterns of multiple mtDNA deletions (15). This indicated that deletions may not be evenly distributed among cells in skeletal muscle, a phenomenon we denoted as a "gross mosaic pattern" of deletions (15).

To test if a gross mosaic pattern of mtDNA deletions occurs in other postmitotic tissues, we have undertaken a systematic analysis of cardiac tissues from 10 human subjects by analyzing mtDNA deletions in adjacent slices of each tissue, using PCR procedures. Among adjacent slices of tissues from individual subjects, we found a high degree of variability in both the abundance of mtDNA^{4977} and the patterns of multiple

[1] To whom correspondence should be addressed. Fax: +61-3-9905 4699. E-mail: chunfang.zhang@med.monash.edu.au.
[2] Present address: Department of Obstetrics and Gynaecology, Queen Mary Hospital, University of Hong Kong.

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TABLE 1

Analysis of mtDNA Deletions in Adjacent Slices of Right Atrial Appendage from 10 Human Subjects

Subject	Age (years)	Number of slices	mtDNA^{4977} Abundance in each slice (% total mtDNA)	mtDNA^{4977} Range	mtDNA^{4977} Mean	mtDNA^{4977} Standard deviation (SD)	mtDNA^{4977} Coefficient of variation (SD/Mean)	Banding pattern grade for multiple deletions
GS	43	10	0.01, 0.01, 0.02, 0.01, 0.01, 0.0002, 0.001, 0.02, 0.01, 0.01	0.0002–0.02	0.01	0.0065	0.65	1
DGJ	50	8	0.005, 0.005, 0.001, 0.005, 0.005, 0.001, 0.001, 0.001	0.001–0.005	0.003	0.0021	0.7	3
RIF	56	10	0.005, 0.005, 0.005, 0.002, 0.003, 0.003, 0.01, 0.005, 0.01, 0.01	0.002–0.01	0.0058	0.0031	0.53	2
HAE	62	10	0.006, 0.006, 0.003, 0.002, 0.003, 0.001, 0.003, 0.001, 0.001, 0.002	0.001–0.006	0.0028	0.0019	0.68	3
JP	63	9	0.005, 0.01, 0.01, 0.01, 0.005, 0.03, 0.06, 0.005, 0.03	0.005–0.06	0.018	0.019	1.06	6
KI	67	12	0.001, 0.003, 0.001, 0.003, 0.01, 0.005, 0.001, 0.003, 0.01, 0.01, 0.001, 0.001	0.001–0.01	0.0044	0.0038	0.86	5
KC	68	8	0.001, 0.001, 0.002, 0.001, 0.005, 0.005, 0.005, 0.002	0.001–0.005	0.0028	0.0019	0.68	1
API	69	10	0.05, 0.007, 0.01, 0.002, 0.005, 0.01, 0.01, 0.01, 0.01, 0.003	0.003–0.05	0.012	0.014	1.17	5
HM	70	10	0.002, 0.02, 0.002, 0.02, 0.003, 0.001, 0.003, 0.005, 0.002, 0.05	0.001–0.05	0.011	0.016	1.45	1
TP	74	5	0.001, 0.0007, 0.001, 0.001, 0.001	0.0007–0.001	0.00094	0.00013	0.14	2

Note. Subjects are listed in order of increasing age. Banding pattern grades are evaluated on the basis of data in Fig. 2.

mtDNA deletions, indicative of a gross mosaic pattern in most subjects analyzed.

MATERIALS AND METHODS

Human subjects and tissues. The tip of the right atrial appendage was obtained from 10 male patients, aged between 43 and 74 years, undergoing surgery for coronary artery bypass grafts or aortic valve replacement. No patients had symptoms of overt mitochondrial diseases. The myocardium was dissected free of visible fat and connective tissue. Depending on the initial size of the tissue available, trabeculae were cut into 5–12 slices of 5–10 mg each, prior to isolation of DNA. The study was approved by Monash University Standing Committee on Ethics in Research on Human and the Alfred Hospital Ethics Committee.

DNA isolation and polymerase chain reaction (PCR). Total cellular DNA was extracted and PCR carried out using previously described procedures (8). Synthesis of oligonucleotide primers and their nomenclature were as described (16). The PCR products were size-fractionated by electrophoresis on a 1% agarose gel and visualized under UV light after staining with ethidium bromide.

Quantification of mtDNA^{4977}. The abundance of mtDNA^{4977} was quantified following the procedure of Zhang *et al.* (17). This involved the use, as external reference in PCR, of the recombinant plasmid pCZ21 which contains a 773-bp segment of the mtDNA (nt 7901 to 13650) bearing the breakpoint of mtDNA^{4977}. A 468 bp region common to both full length mtDNA molecules and mtDNA^{4977} molecules was amplified using primers L7901[20] and H8368[25], whereas a 392-bp region spanning the deletion breakpoint was amplified using primers L8282[24] and H13650[20]. The percentage of mtDNA^{4977} in the tissue DNA (expressed in relation to total mtDNA) was calculated from the relative amounts of mtDNA^{4977} molecules and total mtDNA molecules.

Analysis of multiple deletions by PCR. The widely spaced primers L7901[20] and H16540[27] were used for PCR under conditions described (8). For evaluation of the similarities or differences between banding patterns of PCR products of multiple deletions in tissue slices from individual subjects, the following arbitrary grading system was adopted: grade 1, multiple common bands in >50% tissue slices; grade 2, one common band in >50% tissue slices; grade 3, multiple common bands in <50% tissue slices; grade 4, one common band in <50% tissue slices; grade 5, one common band in <20% tissue slices; grade 6, no common bands/completely distinct.

Statistical analyses. The Microsoft-Excel statistical package was used to compute statistical parameters and to carry out statistical tests including correlation analyses.

RESULTS

Variations in the abundance of mtDNA^{4977} in adjacent slices of human right atrial trabeculae. The abundance of mtDNA^{4977} (expressed as percentage of total mtDNA) was determined in the series of slices of each of the 10 cardiac tissue samples (Table 1). The extent of variation in the abundance of mtDNA^{4977} between adjacent slices of cardiac tissue was different for each human subject. For example, the difference between the highest and lowest abundances in different slices of atrium from subject TP was only 1.4-fold, whereas the highest abundance in one slice of tissue from subject GS was 100 times greater than the lowest abundance in another slice of that subject's atrial tissue. The results are presented in a consolidated form by means of a histogram (Fig. 1) showing the mean

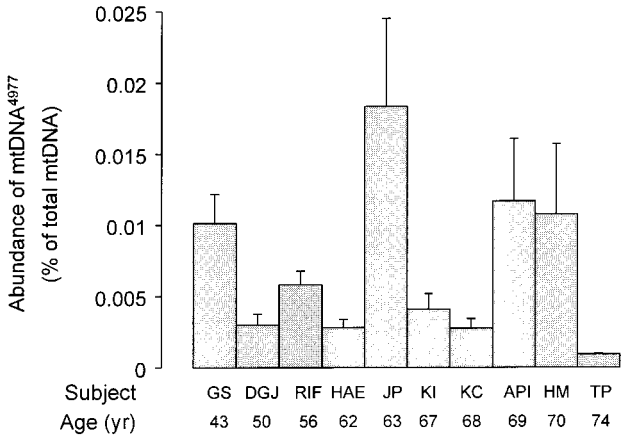

FIG. 1. Abundance of mtDNA[4977] in human right atrial trabeculae. This histogram shows the mean abundance (column height) and the standard deviation (bars) in adjacent slices of tissues from 10 subjects. The individual subjects and their ages are indicated under each column (data compiled from Table 1).

abundance ± standard deviation for each subject. It can be seen that some subjects (especially DGJ, KC and TP) had a relatively low standard deviation, whereas others (exemplified by JP, API, and HM) had a much higher standard deviation.

Varied patterns of multiple mtDNA deletions in adjacent slices of human right atrial trabeculae. The oligonucleotide primer pair L7901[20] and H16540[27], which spans an 8.64 kb region of human mtDNA, was used to analyze by PCR the patterns of multiple deletions arising within this sector of the mtDNA genome. The full length product of 8.64 kb is normally too long to be amplified under the PCR conditions used; the shorter products, when sequenced, are routinely found to represent mtDNA deletions (8, 9, 15, 17, 18). When this pair of primers was used, multiple PCR products were amplified from most slices of the cardiac tissue samples from the 10 subjects (Fig. 2). In general, the band patterns clearly differed between slices of the same tissue. No individual had the same patterns of deletions in all slices. Some individuals had one or two common products which appeared in most slices. For example, a 0.89-kb product can be seen in every slice of subject GS and 0.64 kb product was apparent in at least 8 out of the 10 slices analyzed. A 1.25-kb product was amplified from 7 of 9 slices of KC. Two products of 0.95 and 0.64 kb in size are present in 9 out of 10 slices of subject HM. The 0.64-kb band in both GS and HM is presumed to arise from a deletion (or set of deletions) of close to 8.04 kb, and are likely to represent that set of closely related deletions characterized in detail by Baumer *et al.* (9). The 1.25-kb product amplified from KC possibly represents the 7.4 kb deletion, which is frequently detected in elderly subjects (8, 9) and was initially suggested to be specific to human cardiac tissues (19), although this is now known not to be the case (8, 9).

On the other hand, some individuals, such as JP and API, do not seem to have common products between any two slices. In these subjects, the variation between adjacent slices can be as great as the variation between tissue samples from different subjects.

Absence of correlation between the variation of the abundance of mtDNA[4977] and that of multiple deletion. We checked whether in subjects with a relatively high variation of the abundance of mtDNA[4977] there was a correspondingly extensive disparity in the patterns of multiple deletions amongst the tissue slices. To approach this question statistically, the banding pattern of the multiple deletions of each subject was assigned a number on a scale of gradation (see Materials and Methods), based on the degree of similarity or difference between the varying bands of the multiple deletions in the set of slices analyzed from that subject (Fig. 2). Thus, an individual with multiple common bands (based on size) in more than half of the tissue slices was assigned grade 1, whereas a set of slices exhibiting completely distinct bands in each slice was assigned grade 6 (Table 1). It was found that there was a weak, positively significant, correlation between the standard deviation of the abundance of mtDNA[4977] and the grades of the multiple deletions ($r = 0.41$, $P < 0.05$) (Fig. 3A).

Considering the wide differences between the mean abundances of mtDNA[4977] of individuals, the standard deviation may not be the most appropriate description of the variation. A subject having a high abundance of mtDNA[4977] would have a correspondingly amplified standard deviation (such as JP, see Fig. 1 and Table 1), and one having a low abundance of mtDNA[4977] would have a relatively small standard deviation (such as TP). Therefore, the coefficient of variation (standard deviation/mean abundance) was tentatively considered a more useful parameter to describe the extent of variation. Nevertheless, there was no significant correlation between the coefficient of variation of the abundance of mtDNA[4977] in each tissue and the grades of the corresponding multiple deletion sets ($r = 0.30$, $P > 0.1$) (Fig. 3B). These results suggest that there is no strong relationship between the propensity of a given individual to display a large variance in the abundance of mtDNA[4977] and an extensively divergent mtDNA mosaic assessed by multiple deletion patterns.

DISCUSSION

We have previously shown that different mtDNA deletions may occur in different tissues of the same individual (8, 9), and these accumulate with age (10, 11, 17). We have further shown that the same type of tissue (namely, skeletal muscle) from different loca-

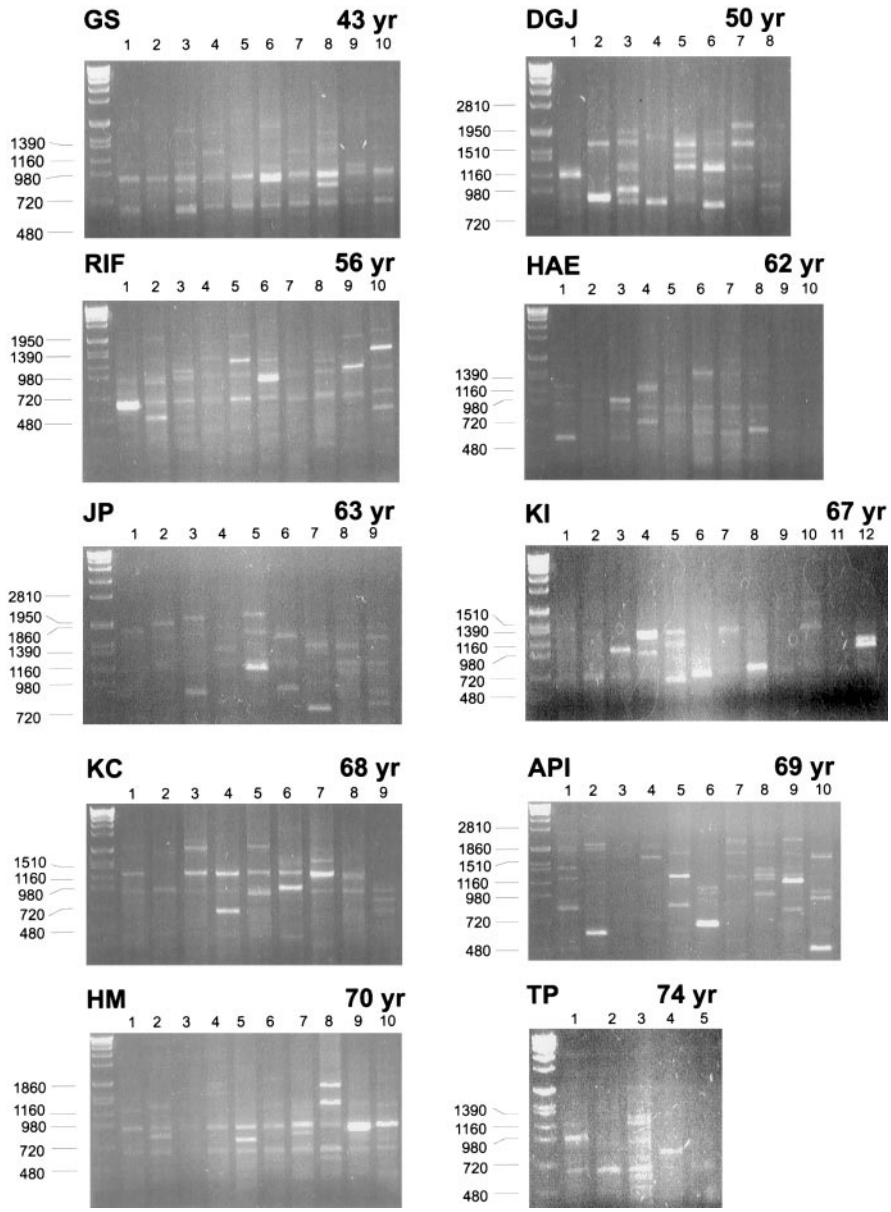

FIG. 2. Multiple mtDNA deletions in adjacent slices of right atrial trabeculae of 10 human subjects. Each panel represents the multiple deletion patterns (obtained using widely spaced PCR primers) arising from slices of tissues from a single subject. The initials and ages of the subjects are indicated above each panel. The lanes are labeled by the serial numbers of adjacent slices of each tissue. The lane at the left of each panel contains plasmid SPP1 DNA digested with *Eco*RI as size markers; the sizes of some bands (bp) are indicated.

tions of the body can harbor different deletions (15). Furthermore, two adjacent slices of the same skeletal muscle specimen were found to contain distinct patterns of multiple deletions (15). Different regions of the human brain have also been found to carry different levels of a particular deletion, mtDNA^{4977} (20, 21). However, in those studies, the tissue portions were not necessarily recovered from adjacent regions of the same human brain specimen.

In this study, we demonstrate clearly the existence of mtDNA mosaic patterns in adjacent slices of human right atrial tissues, which is manifested at the two levels we have studied here. First, when a particular deletion (mtDNA^{4977}) is quantified, the abundance of this deletion can vary considerably (up to 100-fold difference) between slices of a same tissue. Second, there may be widely distributed patterns of multiple deletions in adjacent slices of a myocardial tissue sample from a single human subject. In cardiac muscle, and most likely also for skeletal muscle, we suggest that the mtDNA mosaic may be evident in much smaller portions of dissected tissues, even down to single fibers. This is known to be the case in mitochondrial diseases. In patients suffering from mitochondrial diseases, dif-

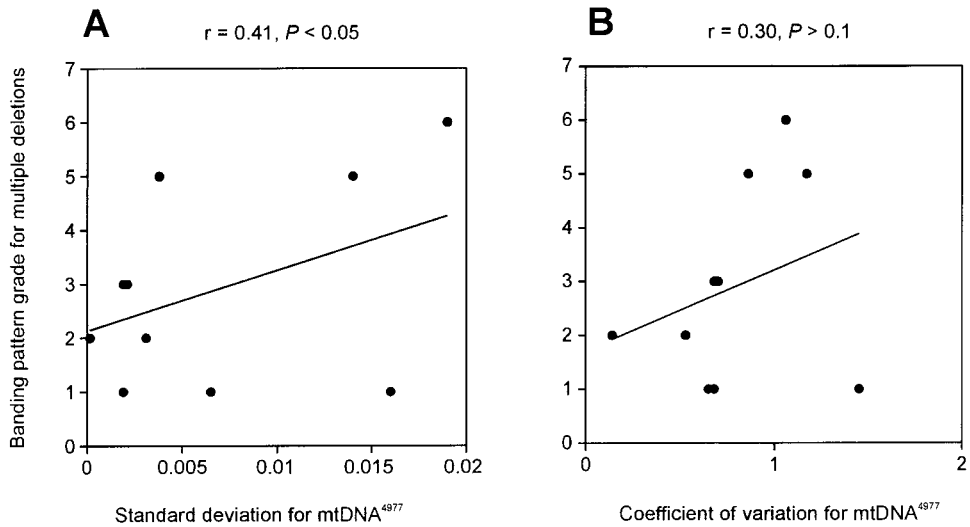

FIG. 3. Correlation between the variation of the abundance of mtDNA^{4977} and that of multiple mtDNA deletions in sets of slices of right atrial trabeculae from particular human subjects. (A) Regression analysis plotting the banding pattern grade for multiple mtDNA deletions in individual tissue sets (Fig. 2, Table 1) against the standard deviation for mtDNA^{4977} (Table 1). (B) Regression analysis plotting the banding pattern grade for multiple mtDNA deletions (as above) against the coefficient of variation for mtDNA^{4977} (Table 1).

ferent percentages of particular mutations have been found in individual fibers of the same skeletal muscle samples. These include mtDNA^{4977} (22), a 4.1-kb mtDNA deletion (23), and the base substitution 3243 A → G (24, 25) as well as other point mutations (26, 27). Such studies have not yet been carried out in normal aging human tissues. However, the uneven distribution of mtDNA^{4977} in different microscopically dissectible portions of a tissue from elderly subjects has been observed by *in situ* PCR techniques in skeletal muscle (28), and using *in situ* hybridization in extraocular muscle fibers (29).

The variation of the abundance of mtDNA^{4977} and that of the pattern of multiple deletions are evidently not related. This may indicate that there is not a unitary mechanism governing the occurrence, segregation and accumulation of mtDNA deletions in general. Our data are consistent with the view that the occurrence of such deletions may well be random, involving the continuous production of deletions throughout the lifetime of an individual and their amplification through mtDNA replication, cellular proliferation and segregation of mutated mtDNA molecules into daughter cells. If two different deletions arise in adjacent cells of a developing tissue, they would be independently propagated, and would contribute to the gross mosaic visualized here. Moreover, deletions arising later in life would be added to the totality of the mtDNA mutant population that has already been laid down in a given tissue region. Should these late-arising deletions become relatively abundant, the patterns of multiple deletions will vary from one region of a tissue to another, again contributing to the observed gross mtDNA mosaic.

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