BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS **233,** 56–60 (1997)
ARTICLE NO. RC976400

Gross Mosaic Pattern of Mitochondrial DNA Deletions in Skeletal Muscle Tissues of an Individual Adult Human Subject

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Received February 19, 1997

Using polymerase chain reaction techniques, the patterns of mitochondrial DNA deletions (which characteristically occur at low abundance during ageing) were compared in different skeletal muscle samples of an adult human subject. In one particular section of the biceps muscle, an unusual pattern of mitochondrial DNA deletions was detected; the common 4977 bp deletion was absent, but other deletions were observed. In pectoralis (chest) muscle, deletions commonly seen in normal adults were readily detected, including the 4977 bp deletion. Significantly, different patterns of mtDNA deletions were found in two adjacent parts of the same biceps muscle sample: one was the unusual pattern mentioned above, but the other part clearly contained the 4977 bp deletion. The results therefore demonstrate a gross mosaic pattern of mtDNA deletions in the skeletal muscle tissues of an individual human subject.

Human mitochondrial DNA (mtDNA) is a 16.6 kb double-strand circular molecule represented in several thousand copies per cell. It has been recognised in the past 10 years that mutations in mtDNA are associated with various degenerative diseases and the ageing process (1-4). Large deletions in mtDNA were first reported to occur in tissues of patients with mitochondrial myopathies (5,6) in which the mutant subgenomic mtDNA molecules can represent up to 95% of cellular mtDNA (7). Low levels (generally less than 1%) of such subgenomic mtDNA molecules that bear similar deletions occur and accumulate in normal ageing (2,8-18).

The age-associated mtDNA deletions include particularly the "common" 4977 bp deletion and this is also prevalent in certain mitochondrial myopathies. It is generated between a pair of 13 bp direct repeats, located at nucleotide (nt) positions 8470-8482 and nt 13447-13459, respectively. Other frequently detected age-associated deletions include a 7436 bp deletion which occurs between a pair of 12 bp direct repeats at nt 8637-8648 and nt 16073-16084 (2,10,13,18) and a family of heterogeneous 8.04 kb deletions generated between a pair of 5 bp direct repeats, located at nt 8031-8034 and nt 16071-16075 (10,16).

It was proposed that the segregation of somatic mtDNA mutations would result in a tissue bioenergy mosaic where different cells would have different bioenergetic capacities (1,2). Such a mosaic pattern has been proved at the level of enzyme activity, in particular cytochrome *c* oxidase, by histochemical procedures (19-21). We have earlier shown that different tissues of the same individual could contain different mtDNA deletions and skeletal muscle in humans generally accumulate more deletions than do other tissues (10). In the present study, we compared the same tissue type (skeletal muscle) from two different regions of the body of an adult human and found distinctly different patterns of mtDNA deletions. Furthermore, we found different mtDNA deletion patterns between two adjacent parts of the same skeletal muscle sample.

MATERIALS AND METHODS

Human subjects and tissues. The two human subjects have been described previously (10,22). A biopsy from the left biceps brachii was taken from AW, a male patient with chronic fatigue syndrome at the age of 54 (22). For a comparison between skeletal muscle of two different regions of the body, a biopsy was obtained from the chest (pectoralis muscle) during surgery for gynaecomastia at the age of 55. Furthermore, the biceps muscle was subsequently divided into two portions. A skeletal muscle sample was obtained at autopsy from KK, a female patient who died at the age of 69 from complications of primary carcinoma of the colon (10,22).

DNA isolation, polymerase chain reaction (PCR) and DNA sequencing. Total cellular DNA was extracted and PCR carried out using previously described procedures (10). Synthesis of oligonucleotide primers and the revised nomenclature are as described (23). Each primer is designated by the letter L or H that refers to the light or

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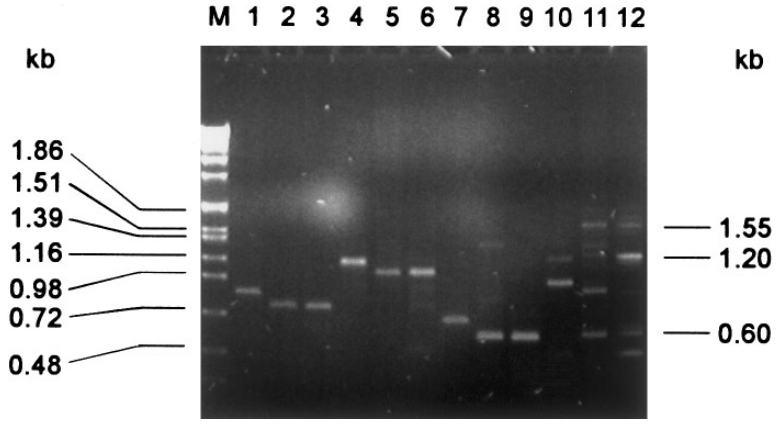

FIG. 1. PCR analysis of two separate skeletal muscle samples of AW and one from KK. DNA from the biceps muscle (Lanes 1, 4, 7 and 10) and the pectoralis muscle (Lanes 2, 5, 8 and 11) of AW and skeletal muscle of KK (Lanes 3, 6, 9 and 12) was amplified using the primer pairs L7901[20] + H13650[20] (Lanes 1,2 and 3), L7901[20] + H13928[24] (Lanes 4, 5 and 6), L8282[24] + H13851[20] (Lanes 7, 8 and 9) and L7901[20] + H16540[27] (Lanes 10, 11 and 12). Lane M contains SPPl DNA digested with *Eco*RI as DNA size markers and the sizes of some fragments are shown at the left. The positions of the 1.55 kb, 1.20 kb and 0.60 kb bands common to Lanes 11 and 12 are indicated at the right.

heavy strand containing the primer sequence, followed by a number that represents the nucleotide position of the 5′-end of the primer, with another number in square brackets that indicates the length of the primer in nucleotides. The PCR products were size-fractionated by electrophoresis on a 1% agarose gel and visualised under UV light after staining with ethidium bromide. Sequencing of PCR products was carried out using an Applied Biosystems 373A DNA Sequencer and the ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit (Perkin Elmer), following the manufacturer's instructions.

RESULTS

Different patterns of mtDNA deletions between the biceps muscle and the pectoralis muscle of AW. PCR analysis was carried out to compare the pattern of mtDNA deletions in DNA samples extracted from the biceps muscle and the pectoralis muscle of subject AW, with DNA from skeletal muscle of KK serving as a reference. The portion of the biceps tissue used in the immediately following section is that already described by Zhang et al. (22). When the conventional oligonucleotide primer pair L7901[20] + H13650[20] (8,10,12,24) was used, the major single PCR product from the biceps muscle of AW was 0.87 kb (Fig. 1, Lane 1) representing a 4881 bp deletion (22). In contrast, the major product from the pectoralis muscle of AW was 0.77 kb (Fig. 1, Lane 2). A similarly sized product of 0.77 kb (Fig. 1, Lane 3) was amplified from subgenomic mtDNA molecules carrying the common 4977 bp deletion in skeletal muscle of KK (10). Using the primer pair L7901[20] + H13928[24] (10) which should amplify a segment of mtDNA 278 bp larger than that from the primer pair L7901[20] + H13650[20], the product from the biceps muscle of AW increased 0.28 kb to 1.15 kb (Fig. 1, Lane 4) representing the 4881 bp deletion (22) whereas those from the pectoralis muscle of AW (Fig. 1, Lane 5) and skeletal muscle of KK (Fig. 1, Lane 6) increased 0.28 kb to 1.05 kb. The primer pair L8282[24] + H13851[20] (18) was also used and a 0.69 kb product from the biceps muscle of AW (Fig. 1, Lane 7), known to contain the 4881 bp deletion (22), was obtained. A 0.59 kb product was instead amplified from the pectoralis muscle of AW (Fig. 1, Lane 8), similar in size to that carrying the breakpoint of the 4977 bp deletion amplified from KK (Fig. 1, Lane 9).

It is apparent from the data above that the 4881 bp deletion was not detectable from the pectoralis muscle of AW, but this sample always gave a PCR product corresponding in size to that of the 4977 bp deletion. To confirm the presence of the 4977 bp deletion in the pectoralis muscle of AW, the 0.77 bp PCR product (Fig. 1, Lane 2) was sequenced using the primer L8282[24]. It was found that this product contained precisely the breakpoint of the common 4977 bp deletion.

A comparison was also made between the initial biceps muscle sample and the pectoralis muscle sample of AW using the primer pair L7901[20] + H16540[27], which had been successfully used for the detection of multiple mtDNA deletions in tissues of adults (2,10,16). Multiple PCR products were amplified from all three samples (including the reference KK), but were different in each case (Fig. 1, Lanes 10-12). The two prominent bands of 1.18 kb and 0.96 kb from the biceps muscle of AW (Fig. 1, Lane 10) represented, respectively, a 7459 bp deletion between nt 8613 and nt 16071 and a 7683 bp deletion between nt 8388 to nt 16070 (22).

The pectoralis muscle of AW (Fig. 1, Lane 11) had more PCR products than the biceps muscle (Fig. 1, Lane 10). Moreover, none were identical to those in Lane 10. Strikingly, the 1.18 kb and 0.96 kb products

seen in the biceps muscle (Lane 10) could not be amplified from pectoralis muscle (Lane 11). Three products of the pectoralis muscle of AW were the same in size as those amplified from KK (Lane 12) of sizes 1.55 kb, 1.20 kb and 0.60 kb, but others were different. The PCR products from KK (Fig. 1, Lane 12), described by Zhang et al. (10), included a prominent product of 1.2 kb representing the 7436 bp deletion excised at a pair of 12 bp direct repeats, and a 0.6 kb product carrying the breakpoint of an 8.04 kb deletion generated between a pair of 5 bp direct repeats.

It would be useful to corroborate the identity of the deletions represented by the PCR products of sizes 1.2 kb and 0.6 kb common to the pectoralis muscle of AW and skeletal muscle of KK, but because the 1.2 kb product from the pectoralis muscle of AW (Fig. 1, Lane 11) was not very prominent, only the 0.6 kb product in the pectoralis muscle was sequenced. This 0.6 kb product was confirmed using L7901[20] as the sequencing primer to contain the breakpoint of the 8.04 kb deletion generated between a pair of 5 bp direct repeats (CCCAT) located at nt 8030-8034 and 16071-16075, respectively. The data from automated sequencing showed unambiguous peaks until nt 8030 and then double peaks at each position. This is because the 8.04 kb deletion consists of a heterogenous family of closely related deletions generated at the same repeats, each differing by a single base pair (16). By analysing the two peaks at each base of the sequence, it was found that the 0.6 kb PCR product contained both 8043 bp and 8044 bp deletions, with exactly the same breakpoints as those reported by Baumer et al. (16). The identities of the remaining two visible PCR products of the pectoralis muscle of AW (Lane 11), 1.55 kb and 0.90 kb in size, have not been determined.

Different patterns of mtDNA deletions between adjacent parts of the biceps muscle of AW. Twenty-five mg of the biceps muscle from AW was initially obtained and cut into two equal parts. One part was used to isolate total cellular DNA for PCR analysis in the study carried out by Zhang et al. (22), the same part being used as the biceps sample above in data of Fig. 1. The other part of the biopsy was later homogenised and DNA isolated. This DNA sample was compared with that of the first part of the biopsy by PCR using three primer pairs: L7901[20] + H13650[20], L8282[24] + H13851[20] and L7901[20] + H16540[27] (Fig. 2). When the pair L7901[20] + H13650[20] was used, a 0.87 kb product was amplified from the first DNA sample (Lane 1) representing the 4881 bp deletion (22). The same pair of primers generated a 0.77 kb product from the second DNA sample (Lane 2) suggesting the 4977 bp deletion.

With L8282[24] + H13851[20] as primers for PCR, a 0.69 kb product was obtained from the first DNA sample (Lane 3) corresponding to the 4881 bp deletion,

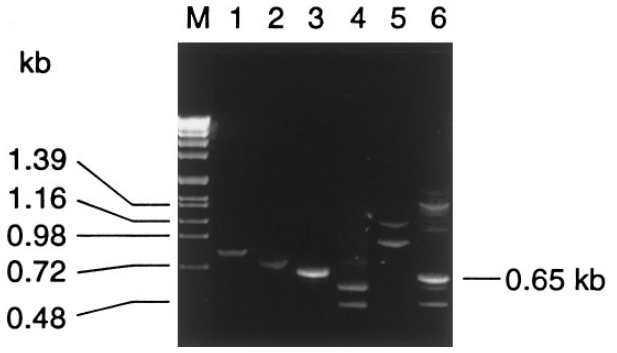

FIG. 2. Comparison of deletion patterns between two adjacent parts of the biceps muscle of AW by PCR analysis. The first DNA sample (Lanes 1, 3 and 5) and second DNA sample (Lanes 2, 4 and 6) from the biceps muscle of AW were amplified using the primer pairs L7901[20] + H13650[20] (Lanes 1 and 2), L8282[24] + H13851[20] (Lanes 3 and 4) and L7901[20] + H16540[27] (Lanes 5 and 6). Lane M contains SPPl DNA digested with *Eco*RI as DNA size markers and the sizes of some fragments are shown at the left. The position of the 0.65 kb band (characterised by sequencing) of Lane 6 is indicated at the right.

whereas a 0.59 kb product was amplified from the second DNA sample (Lane 4), again indicative of the 4977 bp deletion. To confirm the presence of the 4977 bp deletion in the second DNA sample, the 0.59 kb product was sequenced using both L8282[24] and H13650[20] as the sequencing primers. It was found that this PCR product contained precisely the breakpoint of the 4977 bp deletion. The second part of the biceps also generated a 0.46 kb product (Lane 4), but after sequencing with both L8282[24] and H13851[20], the 0.46 kb product was identified as being not a mtDNA origin, but represented the product of spurious priming on some of other template.

When the primer pair L7901[20] + H16540[27] was used, totally different multiple products were generated from the two samples. From the first DNA sample (Lane 5), the two prominent bands of 1.18 kb and 0.96 kb represent the 7459 bp and 7683 bp deletions (22). Neither of these two products was detectable in the second DNA sample, but other bands can be seen (Lane 6). Therefore, adjacent parts of the same muscle sample contained different patterns of deletions. A prominent 0.65 kb product (Lane 6) was sequenced and found to carry the breakpoint of a new 7988 deletion. This deletion was generated between a pair of 5 bp direct repeats (CCCCA) located at nt 8081-8084 and 16070-16074, respectively. Six Cs were left at the breakpoint after the deletion. The other products visible in Lane 6, 1.20 kb and 0.48 kb in size, have not been characterised. Nevertheless, the display of PCR products from the second biceps sample (Lane 6) is different from both that of the pectoralis sample (Fig. 1, Lane 11) and skeletal muscle of KK (Fig. 1, Lane 12) using the same primers.

DISCUSSION

In this study, we have demonstrated at two levels a strikingly mosaic pattern of mtDNA deletions in tissues of a single human subject. First, skeletal muscle samples taken from different regions of the body contained different deletions. Second, two immediately adjacent parts of the same skeletal muscle biopsy sample showed a distinctly different pattern of deletions. These conclusions were not only reached on the basis of different PCR products being amplified, in different tissues, using the same pair of primers, but also on the basis of DNA sequencing of PCR products. For instance, the 4881 bp, 7459 bp, 7683 bp and 7988 bp deletions, detectable in one of two adjacent parts of the biceps muscle, were not detectable in the pectoralis muscle which contained predominantly the 4977 bp common deletion. Furthermore, the 4881 bp, 7459 bp and 7683 bp deletions were detected only from one part of the biceps muscle, whereas the 4977 bp and 7988 bp deletions were detected only in the other part.

These data suggest the independent occurrence of mtDNA deletions in different tissues and even different parts of the same tissue, which could occur by two mechanisms. First, these deletions could be somatic in origin, the different deletions then arising in different body parts and even in different cells of the same tissue. Second, the deletions detected in this study could have come from the oocyte; in this case, segregation of mtDNA during very early cell differentiation would have occurred. Note that significant but low levels of mtDNA deletions, in particular the common 4977 bp deletion, have been detected in some oocytes (25). However, irrespective of the mechanism for the occurrence of the deletions, observed in this study, their mosaic pattern was clearly demonstrated in the human tissues studied, skeletal muscle.

We have used the term "gross mosaic pattern" for the mtDNA deletions described here. This term refers to the appearance of distinct patterns of deletions in skeletal muscle samples of the same individual in different anatomical locations or in macroscopically separate sections of the same tissue biopsy. Mosaic patterns have previously been observed at the microscopic level in muscle, at the histochemical level by indicators of cytochrome c oxidase activity (20). Parallel studies of mtDNA heterogeneity at the microscopic level for age-associated somatic mutations have not been reported in detail. This would require analysis at the level of single cells (or single muscle fibres) as has been carried out for mitochondrial disease biopsies (26); in situ hybridisation (27,28) or in situ PCR would also be a potent tool. In a case of severe mitochondrial disease characterised by multiple mtDNA deletions, there was a gross mosaic pattern in various tissues (29). This report for the first time describes a gross mosaic pattern for mtDNA deletions at the very low abundance levels (0.0001-0.1% of total mtDNA) (9) characteristic of somatic mutations associated with normal ageing.

A question immediately arises as to whether the gross mosaic pattern is determined by the pathiphysiological conditions of chronic fatigue syndrome with which the subject AW presented. We suggest this is not so, in view of recent studies of 20 different subjects of ages 40-70 at autopsy (none with overt mitochondrial disease) in which mtDNA deletions from three anatomically separate samples of skeletal muscle were analysed. It was found that, using the widely spaced primer pair L7901[20] + H16540[27], each of the 60 skeletal muscle samples analysed showed a unique pattern of PCR products, each different from the other 59 samples (C. Zhang and P. Nagley, unpublished data). This result demonstrates that gross mosaic pattern of mtDNA deletions is characteristic of normal human ageing.

Schwarze et al. (30) recently carried out PCR analysis on DNA extracted from different bundles of fibres of the same skeletal muscle of Rhesus monkeys and reported mosaic patterns of mtDNA deletions in skeletal muscle of individual monkeys. Therefore, such gross mosaic patterns of mtDNA deletions may be a general phenomenon in primates, and perhaps this may also be the case in other mammalian species.

ACKNOWLEDGMENTS

This work was supported by a grant from the National Health and Medical Research Council of Australia. We thank Dr. Ian R. Mackay for useful discussion.

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