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Mitochondrial DNA Deletions Are Associated With Ischemia and Aging in Balb/c Mouse Brain

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Abstract Deletions in the mitochondrial DNA (mtDNA) of Balb/c mouse cerebrums, resembling deletions found in elderly humans or in patients with certain disorders, were detected by PCR. Analysis was carried out on mice of various ages and on mice in which the bilateral common carotid arteries had been incompletely ligated to reconstruct cerebral ischemia. A 3,867 bp mtDNA deletion was present only in old or ischemic mouse groups. Among the non-ischemic groups, it was found in 0 of 12 weaning, 0 of 12 young, and four of eight old mice. Among the ischemic groups, it was found in 12 of 17 young and 11 of 11 old mice. Moreover, the percentage of total mtDNA containing deletions was 22% for the old non-ischemic group, 37% for the young ischemic group, and 69% for the old ischemic group. In addition, PCR analysis detected two other deletions of 3,726 bp and 4,236 bp in 4 of the 11 old ischemic cerebrums. The results indicate that mtDNA deletions are associated with aging, that ischemia increases the incidence of mtDNA deletions, and that mtDNA deletions resulting from ischemia are more likely to occur in old mice than in young mice. J. Cell. Biochem. 73:545–553, 1999.

Key words: mitochondrial DNA; deletion; ischemia; aging; mutation; mouse cerebrum; PCR; carotid artery; stroke

Brain cells, especially neurons, are very sensitive to injuries, including ischemia, hypoxia, infection, and trauma. This vulnerability of neurons often makes it difficult to treat patients suffering from injuries to the central nervous system [Abe et al., 1995]. The cause of neuronal damage is not fully understood, although many mechanisms have been proposed. It is now clear that oxygen-free radicals or oxidants play a major role in the development of many neurological disorders and brain dysfunctions [Chan, 1996]. Recent data suggest that mutations in mitochondrial DNA (mtDNA) and reduction of mitochondrial enzyme activity are also associated with ischemia, hypoxia, and tissue aging [Corral-Debrinski et al., 1992]. Therefore, it has been proposed that the accumulation of somatic gene mutations in mtDNA caused by oxygen-free radicals or oxidants is an important contributor to aging and some diseases [Zhang et al., 1992].

Mammalian mtDNA is a closed circular molecule of approximately 16 kb. Several thousand mtDNA molecules are present in the average somatic cell. The mtDNA encodes many critical components of the oxidative phosphorylation pathway which is responsible for generating ATP for the cell. Oxidative phosphorylation is carried out by a chain of five enzyme complexes, assembled from polypeptides encoded by 13 mtDNA genes, as well as more than 50 nuclear DNA genes [Shoffner and Wallace, 1990]. Diseases involving oxidative phosphorylation are frequently caused by heteroplasmic mtDNA mutations in which both mutant and wild-type mtDNAs are present within each cell. Both cell division and mitochondrial proliferation result in a process called replicative segregation, where cells accumulate variable proportions of mutant and wild-type mtDNA [Wallace, 1986]. The impact of an mtDNA mutation on a tissue depends on the reliance of the component cells

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on mitochondrial ATP production [Wallace, 1986]. Each cell or tissue requires a minimum threshold level of mitochondrial ATP production to maintain normal function. Thus, when mtDNA mutations cause a decline in ATP production, cellular degeneration, and death can occur if the ATP level falls below the threshold for a particular tissue. Organ systems with the highest ATP requirements, such as the central nervous system, will have the most frequent manifestation of problems related to mtDNA mutations.

Deletions comprise one class of mtDNA mutation which has recently been shown to be associated with disease [Wallace, 1992]. These deletions seem to occur frequently between direct repeated sequences of 13–15 bp [Schon et al., 1989; Shoffner et al., 1989]. We used a polymerase chain reaction (PCR) approach to investigate whether deletions in the mtDNA of the central nervous system might be associated with the aging process and whether the same deletions might occur as part of the damage resulting from cerebral ischemia. Here we report on the incidence of mtDNA deletions in Balb/c mouse cerebrums of different ages in the absence or presence of ischemia.

MATERIALS AND METHODS

Animals

Sixty healthy, pure-bred Balb/c mice were obtained from the Department of Experimental Animals, Beijing Medical University, Beijing. They were divided into three age groups: weaning (4 weeks old), young (8–12 months old), and old (28–32 months old). Males and females were equally represented in each group. Mice were given free access to food and water prior to surgery.

Surgery

A model of cerebral ischemia can be reconstructed by an operation in which the bilateral common carotid arteries accompanied with the vagus are incompletely ligated [Xu et al., 1991]. This operation was performed on one-half of the young and old mice. The other mice were only pseudo-operated. General anesthesia was induced with 2% halothane and maintained with 0.5% halothane by means of an open-face mask. For each mouse, the common carotid arteries with vagus were exposed in the neck. A 4-0 silk thread was placed under each artery and vagus and a 0.3 mm stainless steel wire was placed alongside each artery. The thread was tied around each bundle and then each wire was removed. The neck was closed and the mice were allowed to recover from anesthesia.

The extent of ischemic damage in the mouse cerebrums after ligation was estimated according to the score on the modified stroke index [Ohno et al., 1984], presented in Table I. Experimental mice reached their maximum score 12–48 h after ligation. They were sacrificed by decapitation 36–72 h later (48–96 h after ligation). Control mice were sacrificed on the same time scale. The cerebrums were separated and DNA was extracted immediately. Pathological sections of cortex were examined by hematoxylin and eosin stain [Bancroft and Stevens, 1977] to determine whether ischemia had occurred in the mouse cerebrums.

PCR Primers

Three 20-base primers were designed by us based on the mtDNA sequence of Bibb et al. [1981], and synthesized by Radon Laboratory (Frederick, MD). The sequences of the primers and their corresponding positions in the mtDNA sequence [Bibb et al., 1981] were as follows:

L1 8858 5′TCTATTCATCGTCTCGGAAG3′ 8877

L2 12883 5′TACCATTCCTAACAGGGTTC3′ 12902

H 13354 5′TTTATGGGTGTAATGCGGTG3′ 13335 (reverse orientation).

The primer pair L1/H was expected to amplify deleted mtDNA fragments, resulting in PCR products of 0.63 kb, 0.77 kb, or 0.26 kb, depending on the size of the deletion. The primer

TABLE I. Modified Stroke Index[a]

Hair roughed up to tremor	1
Obtunded or paucity of movement	1
Hypersthesia of ear	1
Head cocked	3
Eye fixed open	3
Ptosis	1
Splayed out hind limb	3
Circling	3
Seizures or abrupt explosive movement	3
Extreme weakness	6
Total	25

[a]Reprinted from Brain Research, Vol. 297, Ohno et al., "Regional cerebral blood flow and stroke index after left carotid artery ligation in the conscious gerbil," pages 151–157, 1984, with permission from Elsevier Science.

pair L2/H would amplify a 0.47 kb fragment from undeleted wild-type mtDNA and would also serve as an internal control for quantitation (Fig. 1).

MtDNA Isolation and PCR Conditions

MtDNA was extracted from individual mouse brains [Bulpitt and Piko, 1984; Wiesner et al., 1991] immediately after sacrifice and quantitated by UV absorbence spectrophotometry. PCR amplification was performed in a 30 ul reaction mixture containing 1 × Promega Taq DNA polymerase buffer, 1.5 mM $MgCl_2$, 0.3 uM mixed primers (0.3 uM each of L1, L2, and H), 200 uM dNTPs, and 40 ng target mtDNA. After denaturation for 5 min at 94°C, 1.5 units of Taq DNA polymerase (Promega, Madison, WI) were added. Cycling conditions for amplification of the 0.63 kb and 0.47 kb products were 30 cycles of 30 sec at 94°C, 30 sec at 60°C, and 1 min at 72°C, with a final 7 min extension at 72°C. The 0.77 and 0.26 kb fragments were amplified by 40 cycles of 40 sec at 94°C, 45 sec at 40°C, and 1.5 min at 72°C, with a final 7 min extension at 72°C.

Identification of PCR Products

PCR products were analyzed on 1.2–1.5% agarose gels run in Tris-borate buffer and saturated with ethidium bromide. Size of fragments was determined by comparison to PCR markers (Sino-American Biotechnology Company, Beijing). Laser scanning and IOD (Integral Optical Densitometry) of the gels were carried out on an IBAS Interactive Image Analysis System 2000. Rank sum test analysis of the results was carried out according to standard statistical methods on the same computer system.

Restriction Enzyme Analysis of PCR Products

The amplification products from deleted mtDNA (0.63 kb) and from wild-type mtDNA (0.47 kb) were digested with the restriction endonuclease Hinf I, which recognizes the sequence GANTC. The products of digestion were separated by electrophoresis in the presence of ethidium bromide.

Southern Blot

Total DNA [Sambrook et al., 1989], nuclear DNA [Blobel and Potter, 1966], and mtDNA [Bulpitt and Piko, 1984; Wiesner et al., 1991] were prepared, digested with EcoR I, separated by electrophoresis and transferred to a nitrocellulose membrane according to standard protocols [Sambrook et al., 1989]. The products from deleted mtDNA and wild-type mtDNA were labeled with ^{32}P-ATP by PCR incorporation and used as probes for Southern hybridization according to standard protocols. Blots were analyzed by autoradiography.

RESULTS

Animal Model of Ischemia

We wanted to discover whether deletions in mtDNA accumulate with age in Balb/c mice and then to determine whether the same deletions might occur as a result of cerebral ischemia. The mice were divided according to age into weaning, young, and old groups and were further subdivided into ischemic and non-ischemic groups. All mice in the ischemic groups

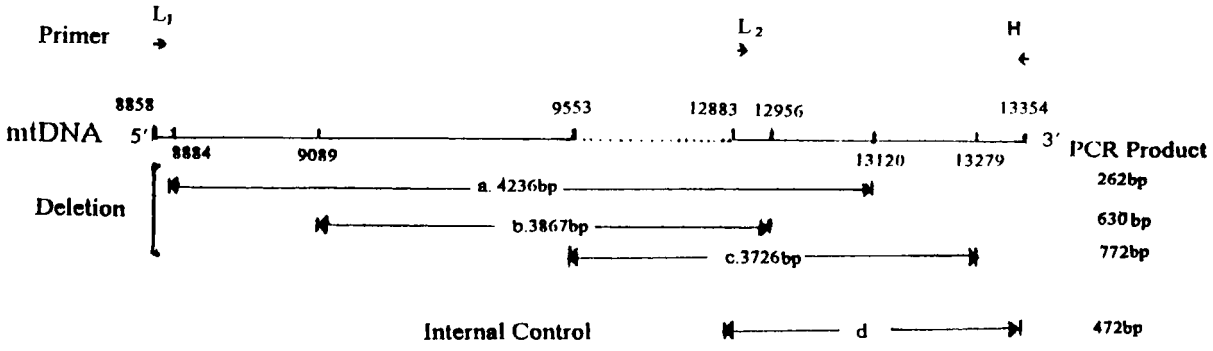

Fig. 1. Map of the anticipated deletions in mtDNA. Small arrows indicate the location of the three primers: L1 (8858–8877), L2 (12885–12904), and H (13354–13335, opposite strand). **a–c**: Refer to the sizes of the PCR products obtained if the corresponding sequences were deleted. Exact locations of the deletions are provided in Table III. **d**: The 472 bp PCR product from undeleted mtDNA which served as the internal control for quantitation.

received an operation in which the bilateral common carotid arteries together with the vagus were incompletely ligated, while the non-ischemic mice received a pseudo-operation. The degree of ischemia was estimated according to the modified stroke index (Table I). Prior to operation, all mice showed no sign of central nervous system injury and had a score of 0. After the operation, all members of the non-ischemic groups were still asymptomatic with scores ranging from 0 to 1 (Table II). However, the ischemic groups presented symptoms of severe central nervous system injury within 12–48 h. All ischemic mice had scores of at least 22 and some reached the maximum score of 25 (Table II). Pathological sections of the post-ischemic cortexes showed medium edema, swelling, and increased acidophilicity of neurons, as shown in Figure 2A. Pathological sections from the non-ischemic mice appeared normal, as shown in Figure 2B. The results suggested that the operation represented a realistic model of ischemia.

PCR Analysis Detects a 3,867 bp Deletion in mtDNA From Old Mice

A PCR strategy was employed to analyze the mouse mtDNA for deletions. Based on the presence of direct repeat sequences, deletions of 4,236 bp, 3,867 bp, or 3,726 bp [Tanhauser and Laipis, 1995] could be predicted to occur in the region which we chose to examine. We designed a pair of primers (L1 and H) which flanked all of the direct repeats. Using these primers we were able to analyze for the presence of any of the three predicted deletions because each deletion would yield a different sized PCR product, as shown in Figure 1.

We also used a second left-hand primer L2, which bound to the template between the direct repeats and inside the potential deletions. Using L2 and H as a primer pair, we could amplify wild-type undeleted sequence from the mtDNA molecules. This served as an internal control which allowed a relative quantitation of deleted and undeleted mtDNA molecules. The locations of the flanking direct repeats, the sizes of the three possible deletions and the sizes of the corresponding PCR products are provided in Table III.

Each mouse cerebrum was examined individually for the presence of the anticipated deletions. We first tested for the 3,867 bp deletion, which was likely to be most abundant due to length and thermodynamic stability of the direct repeats [Tanhauser and Laipis, 1995]. The presence of a deletion was indicated by the appearance of the 0.63 kb PCR product, while intact wild-type mtDNA was indicated by the presence of the 0.47 kb PCR product. Since the full-length PCR product between primers L1 and H would have been 4.5 kb long, it was not produced in detectable quantity with the reaction conditions used here.

When old mice were compared to weaning or young mice in the pseudo-operated group, only the old mice had the 3,867 bp deletion. Figure 3A shows a typical example from each group. The 0.63 kb product was visible in an old mouse (lane 4), but not in a weaning (lane 2) or young (lane 3) mouse. The results for all the young and old non-ischemic mice are summarized in Table 2. The 0.63 kb product was never seen in any weaning or young mouse but was present in four out of eight old mice. This indicated that

TABLE II. Stroke Index Value, Densitometric Data, and Relative Percentage of PCR Product Indicating Deletion in Each Group[a]

Group	Number of mice		Stroke index	IOD + SD		% + SD
	Total	Mutant	(range)	M (0.63 kb)	W (0.47 kb)	M/M + W
YN	12	0	0–1	0	28.8 ± 6.9	0
ON	8	4	0–1	6.9 ± 7.2	25.2 ± 4.4	17 ± 17
YI	17	12	22–25	20.1 ± 14.2	28.0 ± 11.8	36 ± 24
OI	11	11	22–25	36.4 ± 15.0	23.7 ± 8.8	60 ± 10

[a]Y, Young; O, old; N, non-ischemic; I, ischemic; M, mutant mtDNA with deletions; W, wild-type mtDNA without deletions. IOD + SD is the mean integral optical density plus standard deviation within the group for the indicated PCR fragment. % + SD is the mean percentage and standard deviation of the 0.63 kb fragment indicating deletion. This value was determined by calculating M/(M + W) for each individual and then taking the mean for the group. Rank sum test analysis showed that the differences in mean percentage of mutant mtDNA among the four groups were statistically significant ($P < 0.05$).

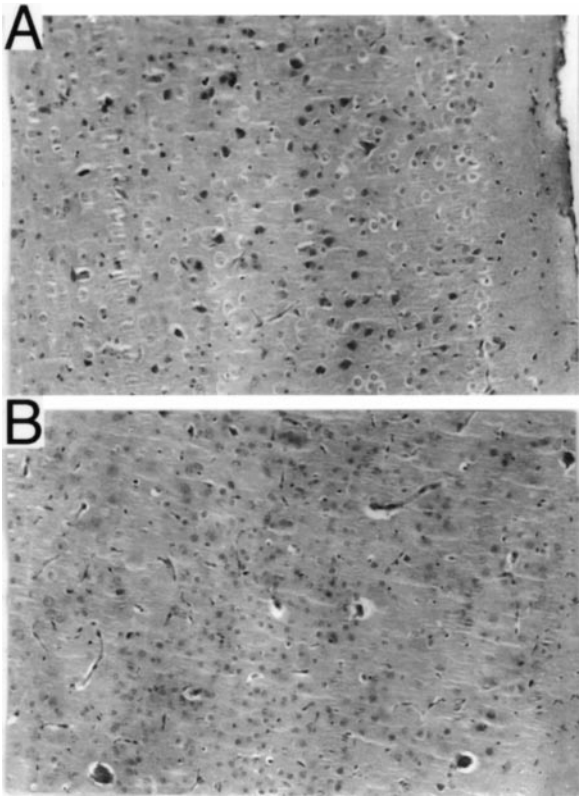

Fig. 2. Pathological section-staining (HE) of cerebral cortex (magnification: 100×). **A:** Post-ischemia. **B:** Non-ischemic control.

the 3,867 bp deletion accumulated as the mice aged.

PCR Analysis Detects the Same Deletion in mtDNA From Ischemic Mice

We next tested for the presence of the 3,867 bp deletion after ischemia. When the ischemic mice were compared to pseudo-operated controls, there was a dramatic increase in the presence of the 3,867 bp deletion in both young and old ischemic mice, as shown in Figure 3B and summarized in Table II. Twelve of 17 young ischemic mice and 11 of 11 old ischemic mice showed the 0.63 kb PCR product. This suggests that cerebral ischemia is capable of generating the same mitochondrial deletions found to be associated with aging.

Quantitation of Mutant mtDNA

There was some variation in the ratio of deleted to intact mtDNA molecules among the samples which showed the 0.63 kb fragment. Therefore, the relative amounts of mutant (deleted) and wild-type (intact) mtDNA in each sample were determined by densitometry. The data were normalized and the percentage of mutant mtDNA in total mtDNA was calculated for each group, as shown in the last column in Table II. The proportion of mutant mtDNA increased with age and with ischemia. Statistical analysis of the data showed that the differences between the groups were significant ($P < 0.05$).

We also pooled samples of mtDNA from all the individuals within each group, and then carried out the PCR amplification on each pool. The results are graphed in Figure 4. Thus, comparing all the mtDNA molecules, the percentage of mtDNA carrying the 3,867 bp deletion was increased in the old relative to the young groups, in the ischemic relative to the non-ischemic groups, and in the old ischemic relative to the young ischemic group.

To insure that the PCR products represented the correct regions of the mtDNA genome, they were digested with the restriction endonuclease Hinf I. This enzyme would be expected to cut the 0.47 kb and 0.63 kb pieces into two common fragments plus one or two unique fragments, respectively [Bibb et al., 1981]. The correct restriction patterns were obtained, as shown in Figure 5. A Southern blot was also carried out, using nuclear DNA, total DNA, or mtDNA as the target and the 0.47 kb or 0.63 kb PCR product as labeled probe. As shown in Figure 6, both probes hybridized to a single 16 kb band in the total or mtDNA lanes, but not in the nuclear DNA lanes. Together these control experiments confirmed the specificity and fidelity of the PCR products amplified from mtDNA.

TABLE III. Location of mtDNA Deletions and Associated PCR Products

Deletion[a]	Direct repeats	Deletion size (bp)	PCR product (bp)
a	8884–8896, 13120–13132	4,236	261
b	9089–9103, 12956–12970	3,867	630
c	9553–9566, 13279–13292	3,726	771

[a]a, b, and c refer to intervals shown in Figure 1. Direct repeats are numbered according to the mtDNA nucleotide sequence in Bibb et al., 1981.

Two Other Deletions are Detected in Old Ischemic Mice

We next asked whether the other deletions predicted in Table III were present in any of the mice. By changing the PCR conditions, we were

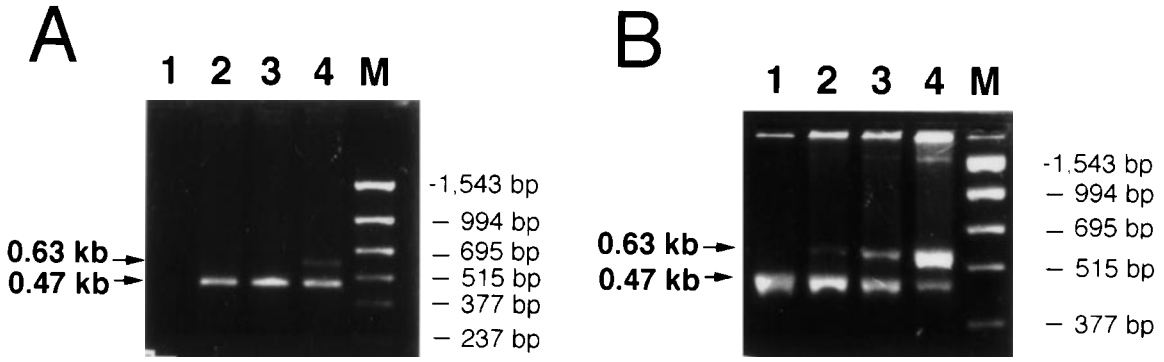

Fig. 3. PCR products indicate the presence of 3,867 bp mtDNA deletions. Amplified PCR products were separated on a 1.5% agarose gel and stained with ethidium bromide. 0.63 kb fragment indicates the presence of the 3,867 bp deletion. 0.47 kb fragment indicates wild-type sequence with no deletion. **A:** Different age groups. **Lane 1:** Blank (no template); **lane 2:** weaning; **lane 3:** young; **lane 4:** old; **lane M:** PCR markers. **B:** Ischemic vs. non-ischemic. **Lane 1:** young non-ischemic; **lane 2:** old non-ischemic; **lane 3:** young ischemic; **lane 4:** old ischemic; **lane M:** PCR markers.

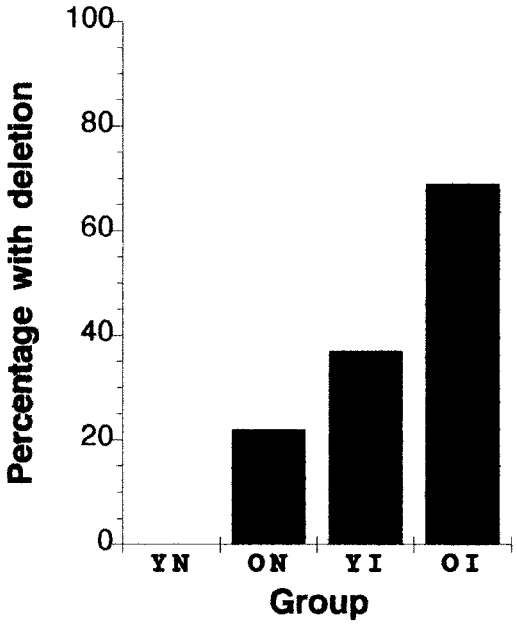

Fig. 4. The percentage of mutant mtDNA in total mtDNA within each group increases with age and ischemia. mtDNA from all the individuals in a group was pooled and subjected to PCR analysis. Densitometric quantitation was performed on the 0.63 kb and 0.47 kb products, the data were normalized and the percentage of the 0.63 kb product (indicating deletion) was calculated for each group. YN, Young, non-ischemic; ON, old, non-ischemic; YI, young, ischemic; OI, old, ischemic.

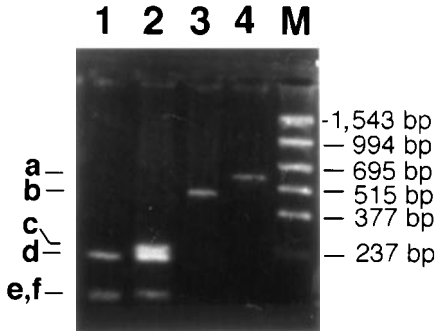

Fig. 5. PCR products digested with Hinf I. The letters a-f denote the migration of DNA fragments of the following molecular masses, in bp: (**a**) 630; (**b**) 472; (**c**) 248; (**d**) 217; (**e**) 129; (**f**) 127. **Lane 1:** Digest of 0.47 kb PCR product (the predicted fragments were d–f); **lane 2:** Digest of 0.63 kb product (the predicted fragments were c–e and a 36 bp piece which was too small to be visualized); **lane 3:** 0.47 kb product, undigested; **lane 4:** 0.63 kb product, undigested; **lane M:** PCR markers.

able to amplify products of 0.77 kb and 0.26 kb, indicating the presence of the 3,726 bp and the 4,236 bp deletion, respectively. As shown in Figure 7, these deletions were both found in four out of 11 old ischemic mice. They were not present in the young or old non-ischemic controls, nor in the young ischemic mice. This suggested that these deletions result with less frequency from ischemia and not at all from aging.

DISCUSSION

Our study demonstrates a link between aging/ischemia and the presence of mtDNA deletions in mice, which may be relevant to humans. Recently, certain specific high-frequency mtDNA deletions, primarily in postmitotic tissues, have been shown to be associated with a number of human mitochondrial disorders characteristic of patients with rare, sporadically occurring degenerative diseases [Brown and Wallace, 1994; Kiechle et al., 1996]. The most common large-scale deletion in humans occurs between nucleotides 8482 and 13460, resulting in the loss of 4,977 bp [Arnheim and Cortopassi,

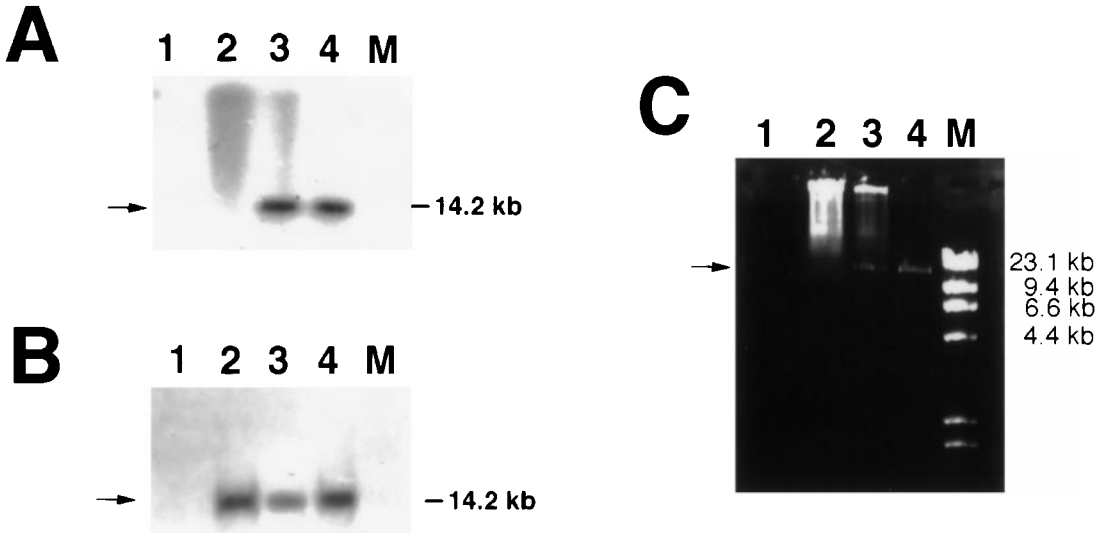

Fig. 6. Southern blot analysis of PCR products. Target DNA was digested with EcoR I, which cuts mtDNA at one site to produce a linear molecule. Each lane contained 10 ug of total DNA, 10 ug of nuclear DNA or 1 ug of mtDNA, as appropriate. Probes were radiolabeled with alpha-^{32}P-dATP. Markers were a Hind III digest of lambda DNA. The arrow shows the position of mtDNA after digestion. **A:** Autoradiograph of the blot. Probe was the 0.47 kb PCR product from wild-type mtDNA. **Lane 1:** No DNA; **lane 2:** nuclear DNA; **lane 3:** total DNA; **lane 4:** mtDNA; **lane M:** markers. **B:** Autoradiograph. Probe was the 0.63 kb PCR product from mtDNA carrying the 3,867 bp deletion. **Lane 1:** Nuclear DNA; **lane 2:** mtDNA, after DNase I digestion to remove nuclear DNA; **lane 3:** mtDNA, purified by electrophoresis; **lane 4:** total DNA; **lane M:** markers. **C:** Photograph of the ethidium bromide-stained DNA templates after digestion and electrophoresis on a 0.7% agarose gel. **Lane 1:** No DNA; **lane 2:** nuclear DNA; **lane 3:** total DNA; **lane 4:**, mtDNA; **lane M:** markers.

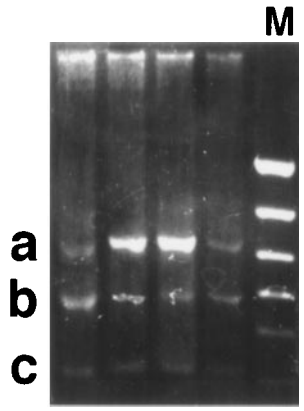

Fig. 7. PCR products indicate the presence of 3,726 bp and 4,236 bp mtDNA deletions in four of 11 old ischemic mice. PCR conditions were changed as described in Materials and Methods. **a**: 0.77 kb product indicating the 3,726 bp deletion; **b**: 0.47 kb internal control product; **c**: 0.26 kb product indicating the 4,236 bp deletion; M, PCR markers.

1992; Wei, 1992]. This deletion was found, for example, in nearly 50% of ocular myopathy cases [Brown and Wallace, 1994] and in more than 50% of patients with alcoholic microvesicular steatosis [Fromenty et al., 1995]. Family studies of diabetes mellitus have shown deletions and other mtDNA mutations to be present at varying levels in various tissues of maternal relatives of patients [Ballinger et al., 1994]. Disease symptoms are usually associated with deletions in a large percentage of the mtDNA. For example, in an 11-year-old boy with Kearns-Sayre Syndrome who displayed growth delay, general muscle weakness, and ataxia, 75% of the mtDNA molecules in the peripheral blood had a large deletion [Fischel-Ghodsian et al., 1992]. Furthermore, it has been reported that Kearns-Sayre Syndrome patients had at least 20–80% deleted mtDNA molecules before symptoms of myopathy were seen [Holt et al., 1989] and deleted mtDNA ranged from 6% to 67% in different tissues of various family members of the patients [Ponzetto et al., 1990]. In short, the severity of symptoms seems to depend in part on a high ratio of deleted to normal mtDNA molecules.

Although there has been some evidence in the literature that damage to mtDNA is also an important factor in aging, there has not yet been any proof of a direct relationship. One reason is the difficulty in estimating the amount of total mtDNA damage in various tissues of human subjects. Consequently, mouse models have been developed for the study of this question [Tanhauser and Laipis, 1995]. In the mouse, the 3,867 bp deletion which we have studied in this paper is similar to the prevalent 4,977 bp human deletion described above.

By PCR analysis, we were able to detect the 3,867 bp deletion in Balb/c mouse cerebral mtDNA. Our results indicate that the deletion was present in old mice but not in young or weaning mice. Those results are in agreement with the work in humans and with a study on other strains of mice [Tanhauser and Laipis, 1995]. We were further able to show in a reconstructed mouse model of cerebral ischemia that ischemia strikingly increased the incidence of mtDNA deletions. Moreover, aging increased the susceptibility of the mice to deletions caused by ischemia. That was true for the 3,867 bp deletion and for the two minor deletions. To our knowledge, this is the first report documenting a link between cerebral ischemia and mtDNA damage. Finally, there may be a common mechanism linking the cellular damage associated with ischemia and with aging, since we observed the 3,867 bp deletion under either condition. Conversely, the 4,236 bp deletion may require both conditions simultaneously, since it was not observed by us or by others [Tanhauser and Laipis, 1995] in old mice, but only by us in the mice which were both old and ischemic.

Although we were unable to measure absolute mtDNA concentrations, the use of the wild-type internal control allowed a comparison of the relative incidence of deletions in the various groups. In our experiments, the percentage of mtDNA molecules with deletions was relatively high in the group of old mice, in agreement with two recent studies [Muscari et al., 1996; Wang et al., 1997] and in conflict with other previous work [Tanhauser and Laipis, 1995]. The reason for the range of values seen by various investigators is as yet unclear. It may be due to differences in the strain of mice which we used and to the conditions under which the animals were raised. Our results with old mice would also be in agreement with the theory that one mtDNA mutation stimulates further mtDNA mutations through the increased production of free radicals as a result of inhibited electron transport [Wallace et al., 1995].

Our results with the ischemic mice suggest a higher rate of mtDNA deletion associated with cerebral ischemia, in agreement with the higher rates of deletions associated with some human diseases, as described above. At present we do not know why deletions accumulate so rapidly as a result of ischemia. Deletions at direct repeats are believed to occur as a result of slipped replication [Schon et al., 1989; Wallace, 1992] or recombination [Shoffner et al., 1989], while oxygen-free radical damage has been proposed as a general cause for mtDNA deletions [Arnheim and Cortopassi, 1992]. It may be that cerebral cells respond to ischemia with a burst of mitochondria replication in an attempt to increase energy production, thereby dramatically increasing the rate of mtDNA replication. Possibly in conjunction with the already damaged tissue and excess free radicals, this leads to a rapid accumulation of the mtDNA deletions which we observed. Older mice may be more susceptible to these effects because of general deterioration in their capacity for DNA repair.

In any case, our work provides an estimate of the changes in mtDNA which may occur with aging and ischemia. These observations can form the basis of future research into the aging process and the mechanisms of disease.

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