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Report

Point Mutations of the mtDNA Control Region in Normal and Neurodegenerative Human Brains

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Recent observations in cultured human fibroblasts suggest that the accumulation of point mutations in the noncoding control region of mtDNA may be important in human aging. We studied the mtDNA control region in brain tissue from 31 normal elderly individuals, from 35 individuals who had Alzheimer disease, and from 47 individuals who had dementia with Lewy bodies. We found no evidence that these somatic mtDNA point mutations accumulate either in the brains of normal elderly individuals or in the brains of individuals with neurodegenerative disease.

It has been suggested that the accumulation of somatic mtDNA mutations may contribute to the aging process. Various mtDNA deletions accumulate in postmitotic tissues, including skeletal muscle (Cortopassi and Arnheim 1990) and brain (Corral-Debrinski et al. 1992). Although these deletions are present at low levels (<2%) in whole tissue, mutations may clonally expand, reaching high levels within individual cells (Brierley et al. 1998). When the percentage of these mutations exceeds a critical threshold within a single cell, this may cause a defect of mitochondrial oxidative metabolism and may ultimately lead to cell death.

Point mutations in the noncoding mtDNA control region have also been detected in human fibroblasts from normal, elderly individuals (Michikawa et al. 1999). These mutations were not detected in fibroblasts from young individuals, and longitudinal studies showed that these mutations appeared with advancing age. One particular mutation (T414G) reached high levels (as high as 50%) and was detected in fibroblasts from more than half the older subjects. Although the mutations in that study were not associated with any obvious phenotypic effects, they occurred in the region that is crucial for mtDNA replication and transcription, thereby raising the possibility that they may have a detrimental effect on mitochondrial function within single cells.

The experiments of Michikawa et al. (1999) were performed on cultured fibroblasts from skin, a tissue in which cell proliferation continues throughout life. We have a long-standing interest in the role of mtDNA mutations in aging, particularly in postmitotic cells. Furthermore, like others (e.g., Pennisi 1999), we were concerned about the significance of these findings for aging in the human brain and for neurodegenerative disease. We therefore studied the mtDNA control region in DNA extracted from frozen brain tissue of both normal elderly individuals and individuals with neurodegenerative disease.

Postmortem control brain tissue was from individuals (n = 31; mean age [SD] 71.7 [10.21] years) who showed no clinical evidence of dementia before death and who had only age-associated pathological abnormalities without Lewy bodies. Of the control brain tissue samples, 20 were from individuals who died at ⩾70 years of age. Individuals with neurodegenerative disease were classified, on the basis of clinical examination before death, as having either Alzheimer disease (AD) (n = 35; mean age [SD] 77.9 [9.55] years) or dementia with Lewy bodies (DLB) (n = 47; mean age [SD] 79.1 [5.51] years), and the diagnosis was confirmed at autopsy, with the use of established neuropathological criteria (Perry et al. 1996). Individuals who had extensive

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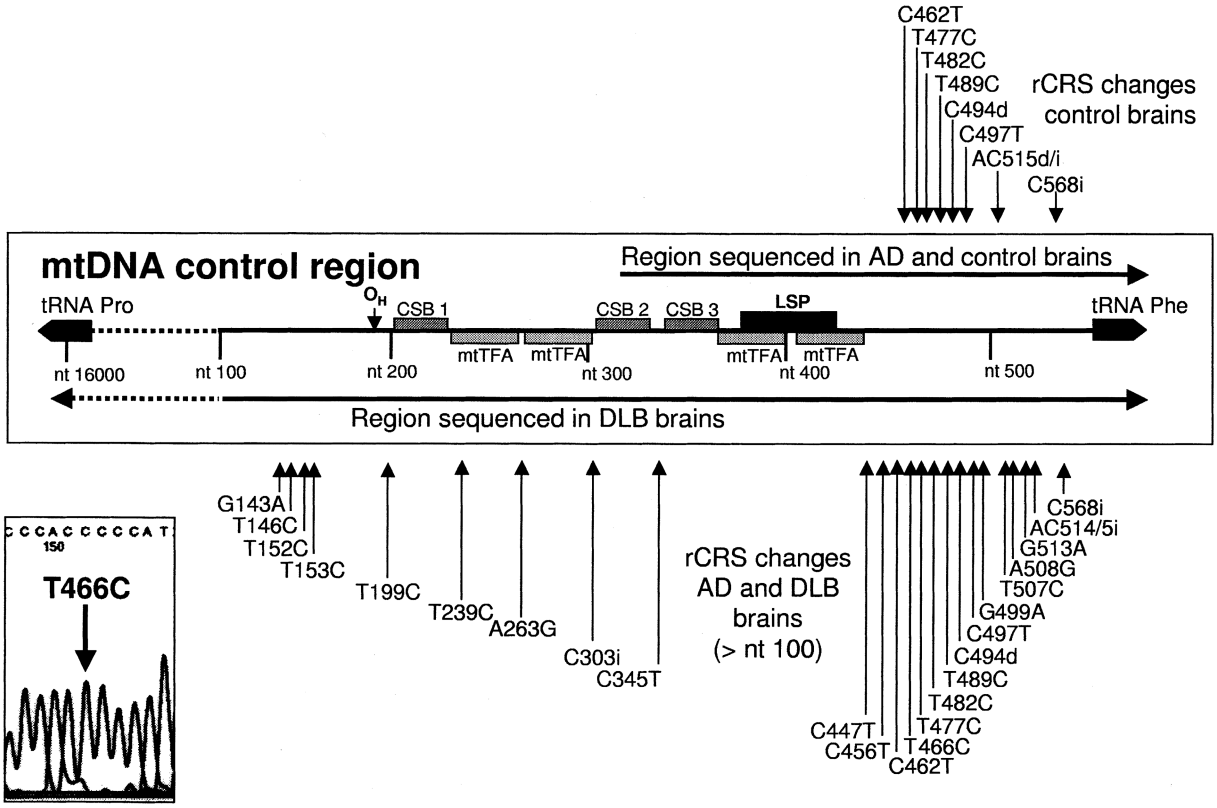

Figure 1 The mtDNA control region, showing the position of the sequence changes seen in brain tissue isolated from elderly normal individuals (n = 31) and from individuals with either AD (n = 35) or DLB (n = 47), at nt 100–576 (numbered according to the revised Cambridge Reference Sequence [Anderson et al. 1981; Andrews et al. 1999]). CSB = conserved sequence blocks; d = deletion; i = insertion; LSP = light-strand promoter; mtTFA = binding site for mitochondrial transcription factor A; O_H = origin of heavy-strand replication; tRNA Phe = tRNA phenylalanine gene; tRNA Pro = tRNA proline gene.

mixed pathology, including those with multiple cerebral infarcts, were excluded.

DNA was extracted from 300 mg of frozen brain tissue. The most prominent age-associated control-region mutations reported by Michikawa et al. (1999) mapped to the region between nucleotides (nt) 333 and 785 of the control region (A386G, 383i, and T414G). We therefore sequenced nt 333–785 of the control region in the brain DNA samples, with the use of M13-tagged primers, Big-Dye terminator cycle sequencing, and an ABI 377 automated DNA sequencer (PE Biosystems), as described elsewhere (Andrews et al. 1999). Serial-dilution experiments indicate that low levels (≤20%) of mtDNA heteroplasmy cannot be reliably detected by our automated sequencing protocol. This limitation raised the possibility that we did not detect mutations that reached high percentage levels within a small number of neurons. To determine whether low-frequency mutations were undetected in our nucleotide-sequencing approach, we analyzed the T414G transversion in the brain tissues of 37 normal elderly persons and the brain tissues of 35 individuals who had AD, by use of fluorescent primer-extension reaction ($_f$PEA), according to a modification of the protocol of Fahy et al. (1997), with the use of a FAM-labeled primer ttg ggt ggt gac tgt taa aag tgc (details available on request from P.F.C.). This technique allows the detection of low levels (<1%) of mtDNA heteroplasmy when the reaction products are electrophoresed through a 12% denaturing polyacrylamide gel on an ABI 373 genetic analyzer (PE Biosystems).

mtDNA sequencing identified numerous base substitutions within the control region, with reference to the revised Cambridge mtDNA sequence (Anderson et al. 1981; Andrews et al. 1999) (fig. 1). All the sequence changes appeared to be homoplasmic on the sequencing chromatogram. With the exception of one change (T466C), all these changes have been detected before in blood or bone samples from healthy young individuals (N.H. and T.J.P., unpublished observations; HVR-Base of the Max Planck Institute for Evolutionary Genetics; MITOMAP: a Human Mitochondrial Genome Database; also see Mitosearch mtDNA Analysis Software, version 2.0 [available from the Federal Bureau of Investigation]). The T466C polymorphism is 3′ to the light-

strand promoter region and its associated mitochondrial transcription factor A binding site (fig. 1). It was seen in the brain tissue of one patient with AD and also appeared to be homoplasmic on the sequencing chromatogram. Private mtDNA polymorphisms are not uncommon (e.g., see Anderson et al. 1981; Andrews et al. 1999), and it is likely that all the sequence changes that we detected in the mtDNA control region of the brain DNA are maternally inherited mtDNA polymorphisms. Sequencing did not detect the A386G, 383i, or T414G polymorphisms in any of the 113 samples of brain DNA. $_f$PEA did not detect the T414G mutation in the brain tissue of either control subjects or individuals with AD.

We did not detect any of the mtDNA control region mutations reported by Michikawa et al. (1999) either in brain tissue of normal, elderly individuals or in brain tissue of individuals with neurodegenerative disease. How can we reconcile this discrepancy? Although it is possible that we did not detect infrequent mutations by the use of automated mtDNA sequencing, the $_f$PEA assay effectively excludes the presence of significant levels of the T414G mutation in the brain tissue samples. This was the most frequent control-region mutation reported by Michikawa et al. (1999); it was detected in fibroblast cell lines from 8 of 14 individuals >65 years of age, and it reached levels as high as 50%.

It is possible that we did not detect some mtDNA mutations in brain tissue because they were so detrimental as to cause neuronal cell death. We think that this explanation is unlikely, because, even at high percentage levels, the control-region mutations in the fibroblast cell lines had no discernible phenotypic effect (Michikawa et al. 1999). Also, if the mutations (such as the T414G transversion) were causing neuronal loss, one would expect to detect the mutation in a whole-brain homogenate, because some brain cells would contain intermediate or low levels of mutant mtDNA.

Another possibility is that there may be phenotypically significant mtDNA mutations in the brain but that these occur in segments of the control region that are outside the span of our sequencing primers. This possibility also seems unlikely, because we have sequenced the entire control region (nt 16074–16569 and 1–576) in brain DNA from the 47 individuals with DLB (Chinnery et al. 2000). In a search for any of the eight polymorphisms that Michikawa et al. (1999) observed in fibroblasts, we found one patient with DLB who was homoplasmic for the T146C mutation and four individuals with DLB who were homoplasmic for the T152C mutation. We have seen both these base changes in blood samples from young control subjects (T146C in 23% of 4,360 sequences from an ethnically diverse population and in 10% of 1,961 sequences from a white population; T152C in 28% of 4,360 sequences from an ethnically diverse population and in 19% of 1,961 sequences from a white population), and, in the present study, it is most likely that they are inherited polymorphisms in the individuals who had DLB.

Finally, it is possible that the frequent occurrence of high percentages of mutant mtDNA that were seen by Michikawa et al. (1999) arose because those authors studied fibroblast cell lines in culture. Rapid changes in the percentages of both pathogenic and neutral heteroplasmic mtDNA mutations have been seen in cultured fibroblast cell lines, despite the fact that the level of heteroplasmy may remain relatively constant in vivo (e.g., see Bidooki et al. 1997). However, random drift can explain the high frequency of the T414G mutation seen by Michikawa et al. (1999) only if the mutations were present, at moderately high levels, in most of the fibroblasts in vivo. It therefore seems most likely that there is some form of selection bias—either in quiescent fibroblasts in vivo or in the fibroblast cultures—that favors the mtDNA molecules containing the novel control region–sequence variants. The latter scenario is appealing because of the nonphysiological conditions that accompany fibroblast cell culture. However, on the basis of the extensive analyses reported here, we conclude that such selection does not act in brain neurons. In conclusion, our findings in human brain tissue do not support the hypothesis that somatic mtDNA control region mutations contribute to the aging process in vivo.

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Electronic-Database Information

URLs for data in this article are as follows:

HVR-Base of the Max Planck Institute for Evolutionary Genetics, http://www.eva.mpg.de/hvrbase/

MITOMAP: a Human Mitochondrial Genome Database, http://www.gen.emory.edu/cgi-bin/MITOMAP

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