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Report

Heteroplasmy of the Human mtDNA Control Region Remains Constant during Life

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In a longitudinal, retrospective study, we monitored the level of heteroplasmy at nucleotide position (nt) 309 and nt 16189 of the control region of human mtDNA. As a unique source of DNA, we analyzed multiple cervical-cell samples collected, during 1 or 2 decades, from four women with heteroplasmy at either nt 309 or nt 16189. According to accurate, quantitative analysis by solid-phase minisequencing, the level of heteroplasmy remained stable in the cervical-cell samples from all four women during the time studied. We also analyzed autopsy samples from several different tissues, all containing nt 309 in heteroplasmic form, of one of the women, who was deceased. On the basis of our results, heteroplasmy in the control region of mtDNA seems to be inherited and is not the result of somatic age-related accumulation.

Disease-causing mutations in mtDNA are typically heteroplasmic, with normal and mutant sequences coexisting at variable levels in the same individual (Wallace 1999). Heteroplasmy at neutral polymorphic nucleotide positions of human mtDNA has been observed only sporadically (Comas et al. 1995; Bendall et al. 1996; Ivanov et al. 1996; Jazin et al. 1996; Parsons et al. 1997). Recent studies have, however, suggested that heteroplasmy of neutral mtDNA sequence variants may be more common than has been postulated (Calloway et al. 2000; Howell and Smejkal 2000; Tully et al. 2000) and that the frequency of heteroplasmic polymorphisms increases with age, at least when studied either in cell cultures (Michikawa et al. 1999) or in population groups at different ages (Calloway et al. 2000). To address this question in vivo, we performed a longitudinal retrospective study in which we monitored the fluctuation of heteroplasmy levels of sequence variants in the human mtDNA control region in the same four individuals during 1 or 2 decades of life.

Cervical-cell samples that are collected every 3d year from Swedish women, to screen for cervical cancer, served as a unique source of DNA for the study. Initially, mtDNA in leukocytes from 36 women was screened for heteroplasmy by solid-phase minisequencing (Syvänen et al. 1993) at eight polymorphic nucleotide positions (nt) reported, in previous studies (Bendall and Sykes 1995; Parsons et al. 1997), to occur in heteroplasmic form. The eight analyzed polymorphic variants were G185A, A189G, del309C, T16089C, C16092T, T16093C, C16189T, and C16192T. PCR and minisequencing primer sequences and reaction conditions are available from the authors, on request. The minisequencing method allows quantitative determination of heteroplasmy of mtDNA, at a level of ⩾1%–2% (Suomalainen et al. 1993; Juvonen et al. 1994). In the present study, we were able to detect heteroplasmy, at a level of 0.5% T (see fig. 1). In the initial screen, heteroplasmic nucleotide variations were observed at 6 of the 288 sites analyzed. The C309T variant was observed in three individuals, at levels of 80%, 3%, and 50%. C16093T was seen in one individual (at a level of 28%) and C16189T in another individual (at a level of 98%). Interestingly, one individual was heteroplasmic at both nt 309 (at a level of 3%) and nt 16189 (at a level of 97%) (data not shown), indicating that one mitochondrial genome contains the 309C and 16189C variants and that the other mitochondrial genome contains the 309T and 16189T variants.

For three of the women with detected heteroplasmy

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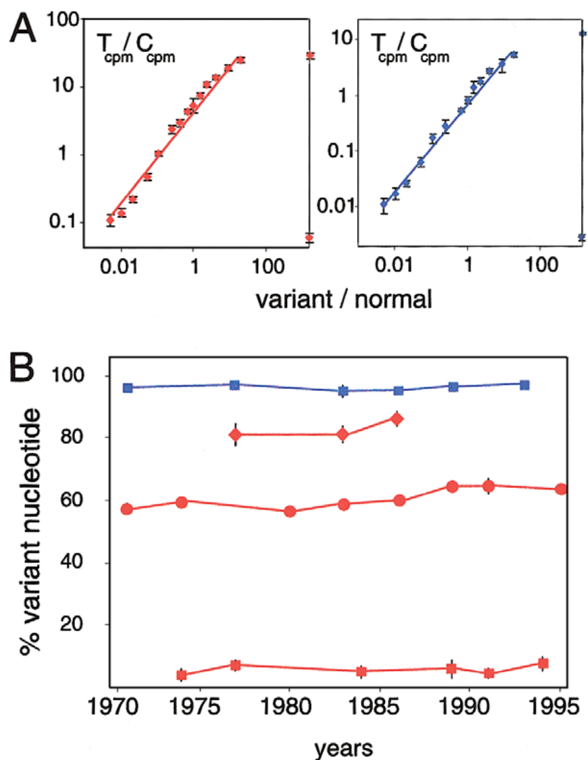

Figure 1 *A,* Minisequencing standard curves for determination of the heteroplasmy levels of the C309T (*red*) and C16189T (*blue*) variants of human mtDNA. The standard curves were prepared by mixing plasmids containing sequence-verified cloned variant and normal mtDNA fragments in known proportions, followed by PCR with a biotinylated primer and minisequencing primer extension in streptavidin-coated microtiter-plate wells with [^{3}H]dNTPs as label (Syvänen et al. 1993). The ratio between the amount of [^{3}H]dTTP and [^{3}H]dCTP incorporated into the minisequencing reaction is plotted as a function of the ratio between the two sequence variants. Mean values, with SDs (coefficient of variance <10%), are plotted. The signal ratios from control samples containing only one of the sequence variants are given on the vertical axes on the right. *B,* Fluctuation of heteroplasmy levels of the C309T (*red*) and C16189T (*blue*) variants of mtDNA in cervical-cell samples from four individuals during 9–25 years. Each data point represents the mean values from three separate assays, with the interassay range of variation indicated by the vertical line. Quadruplicate samples of each PCR product were analyzed in each assay.

in their leukocyte samples, we were able to retrieve several cervical-smear samples collected during 20–25 years. From a fourth woman, who was deceased and had been affected by spinocerebellar ataxia type 7, we obtained autopsy samples from 14 different tissues, in addition to three cervical-cell samples taken during 9 years. DNA was extracted from the papanicolaou-stained cell smears (Josefsson et al. 1999) from the four women and from ∼100 mg of the tissue samples of the deceased woman. mtDNA fragments spanning nt 309 and nt 16189 were amplified by PCR, from 200 ng of DNA. C309T is a deletion of a C residue in a C homopolymer stretch (Bendall and Sykes 1995), and the first nucleotide (a T residue) following the homopolymer stretch is detected in the minisequencing reaction. The relative amounts of the T and C sequence variants at nt 309 and nt 16189 were determined accurately by minisequencing-quantification standard curves, analyzed in parallel (fig. 1*a*). The quantitative analysis demonstrated that the level of heteroplasmy of the C309T and C16189T variants remained unaltered in cervical cells in all four individuals during the time studied (fig. 1*b*). The autopsy tissue samples contained the C309T variant in heteroplasmic form, at levels of 84%–91% (fig. 2).

Our longitudinal analysis, during 1 or 2 decades, of four individuals with different levels of heteroplasmy in the control region of mtDNA, combined with the data from autopsy samples from several tissues of one of the individuals, suggests that heteroplasmy in the mtDNA control region is inherited and remains stable throughout life, without selection for either of the variants. On the basis of our study, heteroplasmy in the mitochondrial control region is rare and does not seem to be the result of somatic accumulation of sequence variation, as has been proposed elsewhere (Michikawa et al. 1999; Calloway et al. 2000). Our findings are important and reassuring for forensic identity testing and human evolutionary studies, which, so far, have been extensively based on the analysis of polymorphic sequence variation in the control region of mtDNA.

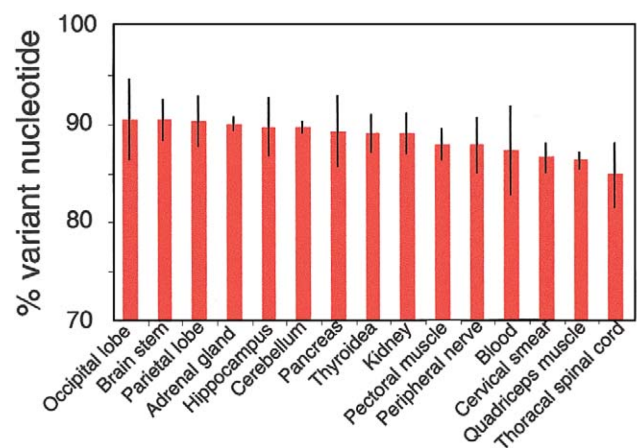

Figure 2 Heteroplasmy levels of the C309T variant of human mtDNA in autopsy tissue samples from one individual. Mean values of three separate assays are shown, and quadruplicate samples of each PCR product were analyzed in each assay.

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