Telomere Length Varies by DNA Extraction Method: Implications for Epidemiologic Research—Letter

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In their recent article, Cunningham and colleagues (1) reported differences in leukocyte telomere length (TL) related to the method of DNA extraction, with shorter TL measurements among samples extracted using QIAamp (Qiagen) compared with those extracted using PureGene or phenol/chloroform methods. It is unclear whether such within-subject differences are also observed with other commonly used methods of DNA extraction, such as the Promega ReliaPrep Kit, or for other suspected DNA-based biomarkers of cancer risk, such as mitochondrial DNA (mtDNA) copy number.

To address these questions, we conducted a similar methodologic evaluation involving paired samples of genomic DNA freshly extracted from the same buffy coat source specimens using two different methods: the QIAamp DNA Blood Midi Kit from Qiagen and the ReliaPrep Large Volume HT gDNA Isolation Kit from Promega. The QIAamp Kit uses a standard column matrix for DNA capture and elution, while the ReliaPrep chemistry is based on magnetic bead capture of nucleic acid. We measured leukocyte TL in paired samples from 40 subjects and mtDNA copy number in paired samples from 48 subjects in the Research Donor Program at the Frederick National Laboratory for Cancer Research (Frederick, MD). TL and mtDNA copy number were measured in triplicate relative to nuclear DNA using quantitative PCR (qPCR); assay methods have been described previously (2, 3). Masked replicate QC samples (N = 8) from a single subject were interspersed to assess assay reproducibility; coefficients of variation were very low and did not differ by extraction method (TL: 5.4% for QIAamp and 5.1% for ReliaPrep; mtDNA copy number: 3.8% for QIAamp and 4.4% for ReliaPrep).

As shown in Table 1, we found that samples extracted using QIAamp had significantly shorter leukocyte TL compared with those extracted using ReliaPrep (medians of 1.13 and 1.48, respectively; P < 0.001). Conversely, for mtDNA copy number, levels were significantly higher in samples extracted using QIAamp compared with ReliaPrep (medians of 212 and 184, respectively; P = 0.005). The correlation between paired samples was moderately high for TL (Spearman r = 0.71) and weaker for mtDNA copy number (Spearman r = 0.46).

Our data corroborate the findings of Cunningham and colleagues and underscore the importance of taking...
DNA extraction method into consideration in epidemiologic studies investigating TL or mtDNA copy number in relation to cancer and other chronic diseases. Whenever possible, all the samples in a given study should be extracted using the same method to ensure comparability between subjects in the measurements of these analytes.

References


Disclosure of Potential Conflicts of Interest

R. Cawthon has ownership interest (including patents) in a patent on the qPCR method of measuring average TL and is a consultant/advisory board member of Telomere Diagnostics, Inc. No potential conflicts of interest were disclosed by the other authors.

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