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Telomere Length Varies By DNA Extraction Method: Implications for Epidemiologic Research–Letter

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In their recent article (1), Cunningham and colleagues 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 to 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 utilizes 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. TL and mtDNA copy number were measured in triplicate relative to nuclear DNA using quantitative PCR; assay methods have been described (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®, 5.1% for ReliaPrep™; mtDNA copy number: 3.8% for QIAamp®, 4.4% for ReliaPrep™).

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As shown in Table 1, we found that samples extracted using QIAamp® had significantly shorter leukocyte TL compared to 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 to ReliaPrep™ (medians of 212 and 184, respectively, P=0.005). The correlation between paired samples was moderately high for TL (spearman rho = 0.71), and weaker for mtDNA copy number (spearman rho = 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 of the samples in a given study should be extracted using the same method to ensure comparability between subjects in the measurements of these analytes.

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Table 1

Differences in leukocyte telomere length and mitochondrial DNA copy number by DNA extraction method in paired samples from the same subject

	Distributions of measurements									Spearman rho[2] (95% CI)
	N	Min	10th	25th	50th	75th	90th	Max	P-value[1]	
Telomere length										
QIAamp®	40	0.77	0.96	0.99	1.13	1.27	1.42	1.72	< 0.001	0.71 (0.51–0.84)
ReliaPrep™	40	1.08	1.22	1.34	1.48	1.65	1.84	2.15		
mtDNA copy number										
QIAamp®	48	82	149	179	212	265	341	372	0.005	0.46 (0.21–0.66)
ReliaPrep™	48	94	137	157	184	230	271	462		

Notes: CI, confidence interval; mtDNA, mitochondrial DNA

[1] Wilcoxon signed-rank test

[2] Spearman rank correlation coefficients evaluating agreement between measurements of the same analyte in paired samples of DNA extracted from the same source material using different methods