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Mitochondrial DNA analysis of formalin-fixed paraffin-embedded tissue samples: Effect of formalin on DNA stability and its implications in genetic studies

S. Cardoso [a], Z.S. Quintero-Niño [b], X. Elcoroaristizabal [c], I. Guerra-Merino [b], M.M. de Pancorbo [c,*]

[a] Servicio General de Investigación Genómica, Banco de ADN. Centro de Investigación y Estudios Avanzados CIEA "Lucio Lascaray", UPV/EHU, Vitoria-Gasteiz, Spain
[b] Servicio de Anatomía Patológica, Hospital Txagorritxu, Vitoria-Gasteiz, Spain
[c] BIOMICs Research Group, Centro de Investigación y Estudios Avanzados CIEA "Lucio Lascaray", UPV/EHU, Vitoria-Gasteiz, Spain

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ABSTRACT

Formalin-fixed paraffin-embedded tissue (FFPET) samples are widely employed in Molecular Epidemiology and Forensic Genetics. However, the effects of formaldehyde on mitochondrial DNA (mtDNA) still remain unexplored. Our aim was to determine the presence of alterations in mtDNA caused by the process of fixation with formalin. FFPET, blood samples and fresh tissue samples were collected from autopsies. Segment HVSIa within the displacement loop (Dloop) and a segment of the coding region of the mtDNA were amplified and sequenced. Changes were not observed in the coding region. However, analysis of HVSIa revealed the existence of numerous differences between FFPET samples and their corresponding reference sequences from blood and/or fresh tissue. These results point to readdress the use of FFPET samples in studies of the Dloop of the mtDNA and urge to act with caution in the resolution of practical cases in Forensic Genetics.

1. Introduction

Formalin-fixed paraffin-embedded tissue samples (FFPET) represent one of the most important sources of biological material in Molecular Epidemiology and Forensic Genetics [1,2], especially for post-mortem identification. Several studies have demonstrated that formaldehyde (H_2CO), the principal component of formalin, causes alterations in nuclear DNA [3–5]. However, its effects over mitochondrial DNA (mtDNA) still remain unexplored. The structure of mitochondrial genome might cause a greater susceptibility to the effect of fixation in formalin, especially within the control region (Dloop) that remains uncoiled and accessible longer than coding region. Considering the previous reports regarding alterations in nuclear DNA caused by formalin-fixation and paraffin-embedding, it is important to ascertain its effects on mtDNA sequence alteration. This study aimed to determine the presence of alterations in the Dloop and coding region of mtDNA on FFPET samples.

2. Materials and methods

Samples were collected from 10 autopsies for a total of 85: 50 FFPET samples, 10 blood samples from heart cavity and 25 from fresh tissue. For autopsies AT01 to AT05 blood samples and FFPET samples were available, whereas for autopsies AT06 to AT10 we also had at disposal the corresponding fresh tissue samples. In all the cases five tissues were considered for analysis: heart (A), lung (F), colon (R), brain (Y) and muscle (AA). Fixation time of FFPET samples varied from one week to eight weeks. After DNA extraction, segment HVSIa within the displacement loop (Dloop) and a segment of the coding region (nps 12,362–12,602) of the mtDNA were amplified and sequenced in an ABI 3130 Genetic Analyzer (Applied Biosystems).

3. Results

Sequence alterations were not detected within the coding region. However, analysis of HVSIa from np 16,019 to np 16,236 revealed the existence of numerous differences between FFPET samples and their corresponding reference sequences from blood and/or fresh tissue (Table 1). The majority of these alterations were point heteroplasmies by transition (Fig. 1). Point heteroplasmies by transversion and base transitions were also observed. Most of the alterations occur in well known hotspots. Thus, nucleotide positions 16,111, 16,126, 16,129, 16,183, 16,187, 16,189, 16,192 and 16,223, among others, have been described as hotspots in several studies [6]. These eight nucleotide positions account for 75% of the alterations observed and would have occurred as a direct result of the process of fixation in formalin.

* Corresponding author. Tel.: +34 945014528; fax: +34 945014458.
E-mail address: marian.mdepancorbo@ehu.es (M.M. de Pancorbo).

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Table 1
Variations observed in FFPET samples with respect to their reference samples (blood and/or fresh tissue). Only those samples with altered mtDNA sequence within each autopsy are shown. rCRS, revised Cambridge reference sequence.

Autopsy	Sample	rCRS												
		16061C	16111C	16117C	16126C	16129G	16183A	16187C	16189T	16192C	16193C	16213G	16223C	16224T
AT01	Blood	.	.	.	.	.	.	Y	.	.	.	.	.	.
	A	.	.	.	.	.	.	Y	Y	.	.	.	.	.
	F	Y	.	.	Y	.	M	.	Y	.	.	.	.	.
	R	Y	.	.	Y	.	.	.	Y	.	Y	.	.	Y
	Y	.	.	.	.	.	.	Y	.	.	.	.	.	.
	AA	.	.	.	.	.	.	Y	.	Y	.	.	.	.
AT02	Blood	.	.	.	.	.	.	.	.	T	.	.	.	.
	Y	.	.	.	.	.	.	.	.	.	.	.	.	.
	AA	.	.	.	.	.	.	.	.	Y	.	.	.	.
AT06	Blood	.	.	.	.	.	.	.	.	.	.	.	.	.
	AAtissue	.	.	Y	.		.	.	.	.	.	.	.	.
	AA	.	.	Y	.	.	.	.	.	.	.	.	.	.
AT07	Blood	.	.	.	.	.	.	.	.	.	.	.	.	.
	R	.	.	.	.	.	.	.	.	.	.	.	Y	.
AT08	Blood	.	.	.	.	.	.	.	.	.	.	.	.	.
	F	.	.	.	.	R	.	.	.	.	.	.	Y	.
	R	.	Y	.	.	R	.	.	.	.	.	R	Y	.
	AA	.	.	.	.	.	.	.	.	.	.	.	Y	.

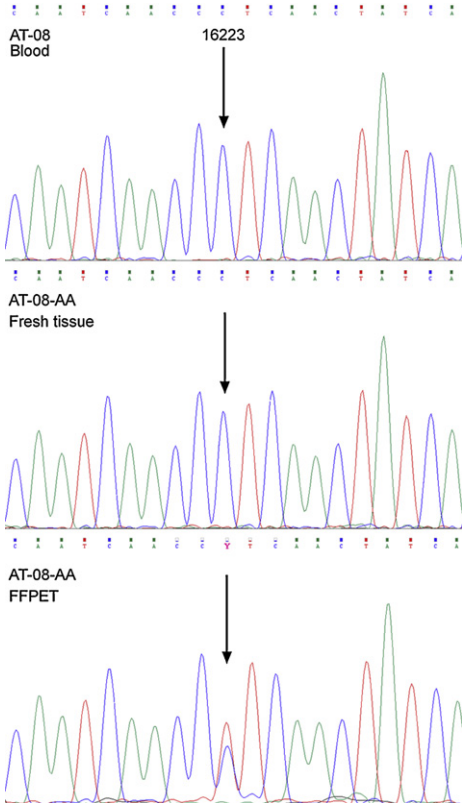

Fig. 1. Point heteroplasmy by transition was the most common variation observed in the FFPET samples with respect to their reference samples. The figure shows an example from autopsy AT08.

4. Discussion and conclusion

Mitochondrial DNA sequenced from FFPET samples compared to their reference samples of blood and/or fresh tissue shows non-constitutive genetic alterations. These modifications might be directly related to the process of tissue fixation with formalin. Alterations in the Dloop region are mainly associated to nucleotide positions described as hotspots [6]. This fact suggested that the open structure of the Dloop is more susceptible to changes caused by external agents, such as formalin used for the fixation of tissues prior to their embedding in paraffin. These results point to readdress the utility of FFPET samples used for Dloop analysis of the mtDNA and urge to act with caution in the resolution of practical cases in Forensic Genetics. In consequence, it would be advisable to use mtDNA coding region instead of the Dloop in forensic casework involving FFPET samples.

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Conflict of interest

None.

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