International Congress Series 1288 (2006) 843–845

www.ics-elsevier.com

Length heteroplasmy in the HVI control region

A. Sucena [a], T. Ribeiro [b], H. Geada [a,*]

[a] *Legal Medicine and Forensic Sciences, Faculty of Medicine, University of Lisbon, Lisbon, Portugal*
[b] *Forensic Genetics Laboratory, National Institute of Legal Medicine, Lisbon Delegation, Lisbon, Portugal*

Abstract. The variability in np16184–16193 HVI region presents two different types of polymorphism: sequence variability and length variability with length heteroplasmy. It is important to define properly the HVI homopolimeric regions for a correct classification of homopolimeric track defining the number of As and Cs and the various population types identified by adjacent bases to the homopolimeric track.

Keywords: Mitochondrial DNA; HVI region; Poli-C; Sequence variability; Length heteroplasmy

1. Introduction

MtDNA is generally accepted as a tool for forensic identity testing and evolutionary studies. It is used to analyse biological samples where the quality and/or quantity of nuclear DNA content is low. In order to properly interpret results, one needs to appreciate mtDNA features such as nomenclature, heteroplasmy, recombination and haploid maternal inheritance. Forensic community has adopted a common language to describe the variation observed in human populations naming mtDNA sequences by referring to a standard sequence—Cambridge Reference Sequence.

HVI mtDNA region have one citosine segment at np16184–16193, which in accordance with CRS possessed a timine at np16189. However, some mtDNA sequences have a transition T–C at np16189 or show some kind of sequence variability which is characterized by the number and position of Cs and Ts (CxTx) in that region [1,2].

The variability in np16184–16193 HVI region presents two different types of polymorphism: sequence variability considering distinct timine positions not interfering with results and length variability with length heteroplasmy with no timine position in this region giving rise to complex patterns in this area. So it is important to define properly the HVI homopolimeric regions [3] for a correct classification of homopolimeric track

* Corresponding author. Tel.: +351 21 8811800; fax: +351 21 8864493.
E-mail address: hgeada@dlinml.mj.pt (H. Geada).

0531-5131/
doi:10.1016/j.ics.2005.11.046

Fig. 1. Sequence variability at HVI np 16183–16193 region characterized by the number and position of timines T (CxTx) at this poli-C mtDNA region: A—C5TC3T; B—C5TC2TC; C—C2TC7.

defining the number of As and Cs and the various population types identified by adjacent bases to the homopolimeric track.

2. Materials and methods

A population study was performed in 98 maternally unrelated individuals from South Portuguese population. DNA was extracted from bloodstains with Chelex 100. HVI mtDNA region was amplified in a 9700 GeneAmp Thermal Cycler (Perkin Elmer) using HVI primers L15997(A1)/H16395(B1) [4]. PCR was carried out in a 25 ul reaction mixture consisting of 2.5 ul GeneAmp 10× Gold Buffer, 1.5 mM $MgCl_2$, 200 μM dNTPs, 100 μM each primer, 2 U of Taq Gold. The amplification was carried out at 95 °C for 11 min, followed by 36 cycles of 95 °C for 10 s, 60 °C for 30 s and 72 °C for 30 s and a final extension of 72 °C for 10 min. Prior to sequencing, PCR products were purified with Microcon YM100®.

Sequencing fragments were generated using Bigdye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems). Sequencing was carried out at 96 °C for 1 min followed by 25 cycles of 96 °C for 10 s, 50 °C for 5 s and 60 °C for 4 min. Removal of excess products was accomplished with Ethanol/EDTA as recommended by the Bigdye®

Table 1
Polymorphism at np16181–16193 with a T–C transition at np16189

CRS	181	182	183	184	185	186	187	188	189	190	191	192	193	Nomenclature	Sample number
	A	A	A	C	C	C	C	C	T	C	C	C	C		
Hc1I									C					A3C10	4
Hc2I									C				.1C	A3C11	5
Hc3I									C				.2C	A3C12	3
Hc4I									C				.3C	A3C13	1
Hc5I			C						C					A2C11	4
Hc6I			C						C				.1C	A2C12	6
Hc7I			C						C				.2C	A2C13	4

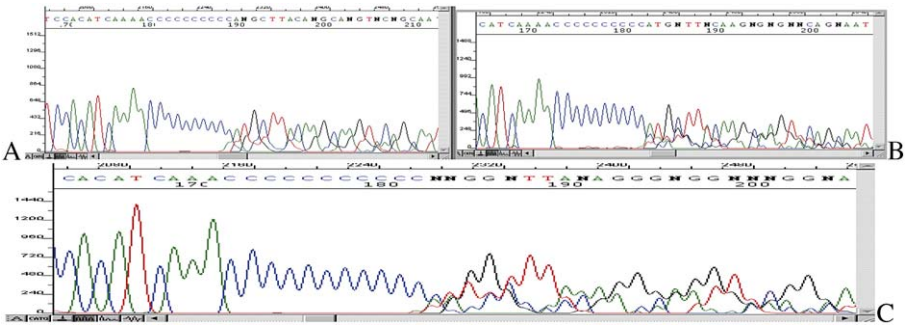

Fig. 2. Length heteroplasmy at HVI poli-C region characterized by the absence of timine T at np16183–16193 and the presence of several mitochondrial molecule populations (mp). A—A3C10 2mpC10C11; B—A3C10 3mp C10C11C12; C—A2C11 4mpC9C10C11C12.

Kit Manual. Fragments were analysed using an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems), with Filter Set E, POP4 Polymer and a 36 cm capillary array.

3. Results and discussion

Concerning HVI mtDNA region, the Cambridge Reference Sequence structure at np16183–16193 is AC5TC4 but some mtDNA samples studied show a T–C transition at np16189. Two possibilities can then be emphasized: sequence variability or length heteroplasmy.

3.1. Sequence variability

The presence of a timine at np16184–16193, different from np16189, in eight samples gives rise to five different CxTx structures in our study (Fig. 1): with a T–C transition at np16189–C2TC7 (2 samples), C3TC6 and with a timine at np16189–C4T2C4, C5TC2TC (2 samples) and C5TC3T (2 samples).

3.2. Length heteroplasmy

Due to T–C transition at np16189 and no presence of timine at np16184–16193, a homopolimeric 10–13 poli-C track may be obtained, rising problems concerning sequence reading and nomenclature as several population molecules can be detected (Table 1 and Fig. 2).

HVI length heteroplasmy at the homopolimeric poli-C track should be characterized by the number of Cs and adjacent polymorphic A sites well defined in the electropherogram. The number of molecule populations observed is designated through analysing adjacent bases—especially the first three bases adjacent to the homopolimeric C track.

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