International Congress Series 1261 (2004) 413–415

www.ics-elsevier.com

Sequence Polymorphisms of Mitochondrial Control Region DNA in Argentine population

S.E. Filippini, A. Castro, M.G. Fraga, F. Gagliardi, C. Echenique, A.M. Di Lonardo*

Banco Nacional de Datos Genéticos, Hospital Dr. C.G. Durand, J.B. Ambrosetti 743 Planta Baja, 1405 Buenos Aires, Argentina

Abstract. As mitochondrial analysis offers great potentials for individualization in forensics, it is sometimes the more convenient and more successful method to be considered, in particular, with low DNA samples. That is the reason why today, many efforts are made to build up mitochondrial databases for forensic purposes. In our study, we analyzed the Sequence Polymorphisms of Mitochondrial DNA Control Regions, HV1 and HV2, in Argentine individuals, most of them from the city of Buenos Aires, with the aim of creating our own database.

Keywords: Mitochondrial DNA; Forensics; Population; Polymorphisms

1. Introduction

Sequencing of Mitochondrial DNA D-Loop Region [1,2] has been incorporated since 1993 in our laboratory to study maternal lineage. Since then, it has become a tool of choice for forensic casework. Automated DNA Sequencing of PCR products have made mitochondrial DNA analysis easier and faster. The aim of this study is to analyze Sequence Polymorphisms of Mitochondrial Control Region DNA [3] in our population, and for that purpose, we studied the D-Loop Hypervariable Regions 1 and 2 (HV1 and HV2). Statistical interpretation of the results let us create a database with sequences of our own population.

2. Materials and methods

We studied 565 unrelated Argentine individuals, most from the city of Buenos Aires. DNA was extracted by Miller's method (salting out) and by chloroform/phenol/isoamyl alcohol extraction method.

Mitochondrial DNA D-Loop Region of approximately 1.300 bp was amplified from blood samples using the following primers: L-15926 and H-00580. For forensic samples, we amplified three fragments of HV1 Segment (253, 156 and 192 bp) and three fragments of HV2 Segment (233, 227 and 172 bp) using the following primers: L-15997/H-16255; L-

* Corresponding author. Tel.: +5411-4-982-1716; fax: +5411-4-982-0625.
E-mail address: bndg@infovia.com.ar (A.M. Di Lonardo).

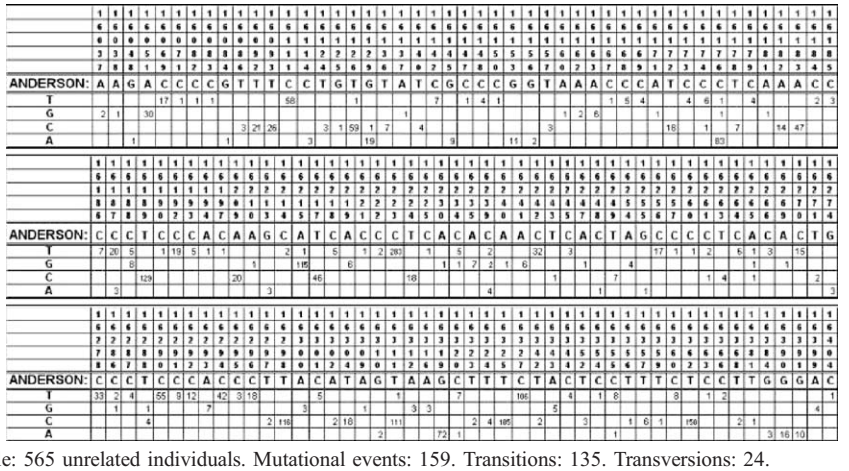
0531-5131/
doi:10.1016/S0531-5131(03)01646-7

16099/H-16255; L-16209/H-16401; L-00030/H-00262; L-00140/H-00366; and L-00241/H-00412, respectively. PCR was performed in a GeneAmp PCR System 9700® (Perkin Elmer). PCR product was purified using Microspin S-300 HR Columns (Amersham/Pharmacia). Sequencing reaction was performed with "Big Dye Terminator Cycle Sequencing Kit" (PE BioSystems). Templates were purified using Centrisep-Spin Columns and analyzed in an ABI Prism 310 Genetic Analyzer (PE Applied BioSystems).

3. Results and discussion

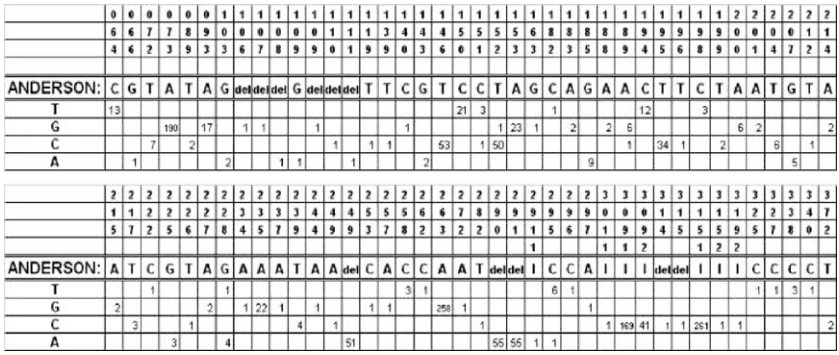
We analyzed the Sequence Polymorphisms of Mitochondrial Control Region DNA in 565 unrelated individuals. We studied HV1 Region in all the samples and HV2 Region in 261 of them. For HV1 Region, we analyzed 390 bp (16023–16412), and for HV2 Region, 342 bp (060–401). In HV1 Region, we observed 325 different haplotypes (57.5%) and 262 individuals showed a unique haplotype (46.4%).The Cambridge Reference Sequence (CRS) was the most frequent haplotype observed, present in 35 unrelated individuals (11%). We calculated Genetic Identity ($P = 0.013$) and Genetic Diversity ($h = 0.9887$). We also found 143 polymorphic sites with 159 mutational events, being 135 of them transitions (84.9%) and 24 transversions (15.1%), and, in 13 polymorphic sites, we could observe both types of mutational events (8.17%). In our population, HV1 Region did not present either insertions or deletions. Transition C → T at position 16223 was the most frequent substitution, found in 283 individuals (50%).This mutational event is present in Amerindian, Asian and African haplotypes [4]. The haplotype 16223-T, 16298-C, 16325-C, 16327-T was frequent in our population (8.9%), as well as that described in Amerindians [5]. In 129 individuals, we found a transition C → T at position 16189 showing the polycitosine tract (from 16184 position to 16193 position). We found heteroplasmies in five samples at positions 16187C/T, 16210 A/G, 16261 C/T, 16265 C/T and 16179 T/C. All the mutational events found in HV1 Region are described in Table 1. The analysis of HV2 Region in 261 unrelated individuals displayed 146 different haplotypes, a value of $P = 0.017$ (Genetic

Table 1
Mutational events in HV1 Region



Sample: 565 unrelated individuals. Mutational events: 159. Transitions: 135. Transversions: 24.

Table 2
Mutational events in HV2 Region



	0	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	2	2	2	2
	6	6	7	7	8	9	0	0	0	0	0	0	1	1	1	3	4	4	4	5	5	5	5	6	8	8	8	8	8	9	9	9	9	9	0	0	0	0	1	1
	4	6	2	3	9	3	3	6	7	8	9	9	0	1	9	9	0	3	6	0	1	2	3	3	2	3	5	8	9	4	5	6	8	9	0	1	4	7	2	4
ANDERSON:	C	G	T	A	T	A	G	del	del	del	G	del	del	del	T	T	C	G	T	C	C	T	A	G	C	A	G	A	A	C	T	T	C	T	A	A	T	G	T	A
T	13																			21	3				1					12			3								
G				190		17		1	1			1						1					1	23	1		2		2	6						6	2				2
C			7		2								1		1	1			53		1	50							1		34	1		2			6		1	
A		1					2			1	1			1				2									9											5			

	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	3	3	3	3	3	3	3	3	3	3	3
	1	1	2	2	2	2	2	3	3	3	3	4	4	4	5	5	5	6	6	7	8	9	9	9	9	9	0	0	0	1	1	1	1	1	2	2	3	4	7
	5	7	2	5	6	7	8	4	5	7	9	4	9	9	3	7	8	2	3	2	2	0	1	1	5	6	7	1	9	9	4	5	5	5	9	5	7	8	0	2
																								1				1	1	2			1	2	2					
ANDERSON:	A	T	C	G	T	A	G	A	A	A	A	T	A	A	del	C	A	C	C	C	A	A	T	del	del	I	C	C	A	I	I	I	del	del	I	I	I	C	C	C	C	T
T			1				1											3	1								6	1										1	1	3	1	
G	2					2		1	22	1		1				1	1			258	1								1													
C		3			1						4		1									1								1	169	41	1	1	261	1	1					2
A				3			4							51										55	55	1	1														

Sample: 261 unrelated individuals. Mutational events: 85. Transitions: 58. Transversions: 8. Insertions: 7. Deletions: 11.

Identity) and a value of $h = 0.9866$ (Genetic Diversity). We observed 69 polymorphic sites and 85 mutational events corresponding to 58 transitions (68.2%) and 9 transversions (10.6%). In contrast to HV1 Region, HV2 Region presented insertions and deletions.

The insertion of an additional cytosine at the cytosine tract 311–315 was found in all individuals, while 64.75% presented the insertion of an additional cytosine at the 303–309 cytosine tract. We observed 51 individuals sharing three deletion positions (249, 290 and 291), which are described in Mapuches [6]. This HV2 haplotype is associated with the following HV1 haplotype: 16223-T, 16298-C, 16325-C, 16327-T. In one case, we observed a 6-bp deletion (from nucleotide 106 to nucleotide 111), also found in Chibcha population (http.www.mitomap.org). All the mutational events found in HV2 Region are described in Table 2.

Considering the complete haplotype (HV1 and HV2) from 261 unrelated individuals, 224 different haplotypes were distinguished with $P = 0.0058$ (Genetic Identity) and $h = 0.9979$ (Genetic Diversity). A number of 107 polymorphic sites were detected in HV1 Region, and 69 in HV2 Region. We also analyzed the mutational events present in HV2 Region in association with the most frequent HV1 haplotypes in our population. For example, our most frequent HV1 haplotype, CRS (common in Europeans) [7], was associated with HV2 haplotypes which did not present the point mutation 073-G. Complete sequence analysis (HV1–HV2) showed a high level of diversity (99.8%), which let us conclude that sequencing of Mitochondrial DNA D-Loop Region is an essential marker to be considered in human identification.

References

[1] S. Anderson, et al., Nature 290 (1981) 457–465.
[2] R.L. Cann, et al., Nature (London) 325 (1987) 31–36.
[3] R. Hopgood, et al., BioTechniques 13 (1992) 82–92.
[4] E. Watson, et al., American Journal Genetics 61 (1997) 691–704.
[5] A. Torroni, et al., Human Genetics 53 (1993) 563–590.
[6] C. Ginther, et al., DNA fingerprinting, in: S.D.J. (Ed.), State of the Science, 1993.
[7] A. Salas, et al., European Journal of Human Genetics 6 (1998) 365–375.