International Congress Series 1239 (2003) 547–551

Variability of the mitochondrial loci nt00073 and nt16519 in populations of Germany, Syria, Cameroon, Japan, Vietnam and Peru—a study using the RFLP and Light Cycler™ technique

U. Szibor [a], R. Schneider-Stock [b], C. Augustin [c], M. Benecke [d], S. Elias [b], S. Harada [e], O. Landt [f], U. Lass [f], Pham Hung Van [g], K. Schmerbach [a], H. Wittig [a], R. Szibor [a,*]

[a] *Institute of Legal Medicine, Otto-von-Guericke-University, Leipziger Strasse 44, D-39120 Magdeburg, Germany*
[b] *Institute of Pathology, Otto-von-Guericke-University, Magdeburg, Germany*
[c] *Institute of Legal Medicine, University of Hamburg, Hamburg, Germany*
[d] *International Forensic Research and Consulting, Cologne, Germany*
[e] *Institute of Community Medicine, University of Tsukuba, Japan*
[f] *TIB MOLBIOL, Berlin, Germany*
[g] *Institute of Medicine, University of Hanoi, Hanoi, Viet Nam*

Abstract

We examined the mitochondrial (mt) loci nt00073 and nt16519 which show a considerable variability among European populations. These polymorphisms are easily detectable by means of PCR-based RFLP analysis. The study was also aimed at establishing the Light Cycler™ technique for application to forensic mt analysis. In analysing the loci nt00073 and nt16519, both methods provide very easy access and yield compatible results. We found that nt00073 is highly variable in German and Syrian populations and fairly homogeneous among Vietnamese, Japanese, Peruvians and Cameroonians. Locus nt16519 is highly variable in all populations investigated. However, in contrast to nt00073, its contribution to the mitochondrial potential for discriminating between various ethnic populations is rather negligible.

Keywords: mtDNA; SNP; RFLP; Light Cycler; Population data

* Corresponding author. Tel.: +49-391-6715812; fax: +49-391-6715810.
E-mail addresses: forensic@benecke.com (M. Benecke), reinhard.szibor@medizin.uni-magdeburg.de (R. Szibor).

0531-5131/03 © 2003 Elsevier Science B.V. All rights reserved.
PII: S0531-5131(02)00570-8

1. Introduction

The present study was carried out to evaluate markers which can be used in differentiating between ethnic populations. Such markers are needed in skeleton identification as well as in general forensic case work. The study was also aimed at establishing the Light Cycler™ technique for application to forensic mitochondrial (mt) analysis.

We examined the mt loci nt00073 and nt16519 which show a considerable variability in European populations. Both transitions create restriction sites. Hence, these polymorphisms are easily detectable by means of the PCR-based RFLP analysis Method I.

In addition, we used the innovative Light Cycler™ technique that is based on the principle of melting curve analysis [1] Method II.

2. Materials and methods

2.1. Method I

Template DNA was amplified by means of the primers L16517 (CGACATCTGGTTCCTACTTCAGG) and H 00097 (GGTGCTCCGGCTCCAGC) yielding PCR fragments of 189 bp in length. Aliquots were separately digested by *Hae*III and *Apa*L1, electrophoresed and silver stained. The transition at locus L16519 T→C creates an *Hae*III restriction site (GGCC). The transition at locus L00073 A→G creates an *Apa*L1 restriction site (GTGCTC). Hence, these enzymes detect the mutations by cleaving the 189-bp PCR product in 165+24 and 153+36 bp fragments, respectively.

2.2. Method II

For each SNP, primers and two probes (sensor and anchor) were designed (Table 1). The 3′ end of one probe was labelled with a donor fluor, whereas the 5′ end of an adjacent

Table 1
Parameters of primers and probes

		Position	T_m (°C)
nt16519			
mt F	CgACATCTggTTCCTACTTCAgg	15909–15931	58.3
mt A	ggTgCTCCggCTCCAgC	16096–16080	62.2
Sensor [C]	CTTCAgCg**C**CATAAAgCCTAAAT X	15925–15947	59.8
L16519 Anc	LC Red640-CCCACACgTTCCCCTTAAATAAgACA p	15950–15975	63.1
nt00073			
mt FM	CgACATCTggTTCCTACTTCAgC	15909–15931	58.4
mt Bmt	TCCAgCggCCCgCAAT	16085–16070	63.2
Sensor [G]	gTgCA**C**ACCCCCC X	16061–16049	50.4
L00073 Anc	LC Red640-CgAAAATACCAAATgCATggAgAgCTCCC p	16045–16017	68.8

probe was labelled with an acceptor fluor. Fluorescence resonance energy transfer occurs only when both probes hybridize to the amplicon. Therefore, SNP alleles are detectable by measuring the relevant melting curves.

Light Cycler conditions: Ingredients were used with the following final concentrations: 16.6 mM $(NH_2)_4SO_4$, (GC [Genecraft]) 3.125 mM $MgCl_2$ (GC), 0.5 mM dNTP (GC), 0.5 μM sense primer (TIB [TIB MOLBIOL])), 0.5 μM antisense primer (TIB), 0.15 μM 3′FL probe (TIB) 0.15 μM 5′LC probe (TIB), 12 ng BSA (Sigma), 2.5 U *Taq* Polymerase (GC).

Experimental protocol: Initial denaturation (regarding nt16519 and nt00073 analyses): 95 °C/3 min; cycle amplification (regarding nt16519 analysis): 40×95 °C/8s, 60 °C/8s (single), 72 °C/15s; cycle amplification (regarding nt00073 analysis): 40×95 °C/5s, 60 °C/8s (single), 72 °C/15s; melting analysis (regarding nt16519 and nt00073 analysis): 95 C/20s, 38 °C/20s. 85 °C/0s ramping: 0.2 °C/s (continuous); fluorescence settings: F1=1; F2=15; F3=35.

Allele detection: Temperature curves enable detection of the nt16519 alleles T and C at 45.3 and 49.5 °C, respectively.

2.3. Samples

DNA extracted from blood spots of 150 Germans (Ger), 100 Syrians (Syr), 100 Japanese (Jap), 100 Vietnamese (Viet), 100 Peruvians (Per), and 60 Bantu-speaking Cameroonians (Cam) were examined to establish the frequency of the transitions A→G L00073 and T→C at L16519.

3. Results and discussion

Studies of the relevant literature revealed that the nt00073 locus is a highly polymorphic site in Caucasian populations [2] and lowly variable in non-Caucasian populations, such as Japanese and Korean populations [3,4]. Due to its localisation within HVII, this polymorphism is well investigated. The nt16519 locus is situated outside the HVI and HVII regions, i.e. outside the most commonly sequenced regions. Hence, population data for this polymorphic site are rather rare.

This study also compared the suitability of PCR-based RLFP technique and Light Cycler™ technique for mtSNPs investigations. In analysing the loci nt00073 and nt16519 both methods provide very easy access and yield compatible results. But since the Light Cycler™ procedure involves less work and a lower risk of carry-over contamination, it must be considered superior.

We found that nt00073 is highly variable in German and Syrian populations and fairly homogeneous among Vietnamese, Japanese, Peruvians and Cameroonians. The χ^2 test reveals highly significant differences between Caucasian and non-Caucasian populations.

Locus nt16519 is highly variable in all populations investigated (Table 2). However, in contrast to nt00073, its contribution to the mitochondrial potential for discriminating between various ethnic populations is rather negligible. Nonetheless, the nt16519 is one

Table 2
Population study results for mtDNA loci nt00073 and nt16519 (for abbreviations and symbols, refer to the Table 3)

Population	n	L 00073	Differences between populations χ^2					L16519	Differences between populations χ^2				
		G [n (f)]	Syr	Jap	Viet	Peru	Cam	C [n (f)]	Syr	Jap	Viet	Peru	Cam
Germ	150	76 (0.51)	0.2^{ns}	>70*	>66***	>51***	>23***	96 (0.64)	2.0^{ns}	5.5^{*}	0.1^{ns}	0.1^{ns}	0.9^{ns}
Syr	100	59 (0.59)	–	>51*	>48***	>34***	>13***	55 (0.55)	–	0.7^{ns}	2.5^{ns}	2.5^{ns}	0.04^{ns}
Jap	100	100 (1.00)	–	–	1.0^{ns}	6.2*	>14***	49 (0.49)	–	–	5.9*	5.9*	0.9^{ns}
Viet	100	99 (0.99)	–	–	–	3.7^{ns}	>10**	66 (0.66)	–	–	–	0^{ns}	1.4^{ns}
Peru	100	94 (0.94)	–	–	–	–	2.5^{ns}	66 (0.66)	–	–	–	–	1.4^{ns}
Cam	60	52 (0.87)	–	–	–	–	–	34 (0.57)	–	–	–	–	–

Significance level: ns—not significant.
* Significance level: $p<0.05$.
** Significance level: $p<0.01$.
*** Significance level: $p<0.001$.

of the most polymorphic mitochondrial sites. Haplotyping of nt00073 and nt16519 provides a high power of discrimination (PD), in particular in the Caucasian populations (Table 3).

Table 3
Population study results for mtDNA haplotypes regarding loci nt00073 and nt16519

Population	n	Haplotype L00073 – L16519				Differences between populations χ^2					PD
		A–T	A–C	G–T	G–C	Syr	Jap	Viet	Peru	Cam	
		n (f)	n (f)	n (f)	n (f)						
Germ	150	30 (0.20)	44 (0.29)	24 (0.16)	52 (0.35)	0.09^{ns}	>23***	>29***	>23***	9.6**	0.73
Syr	100	25 (0.25)	16 (0.16)	20 (0.20)	39 (0.39)	–	>24***	>31***	>24***	>10**	0.72
Jap	100	0	0	51 (0.51)	49 (0.49)	–	–	∅	>24***	3.4^{ns}	0.50
Viet	100	0	1 (0.01)	34 (0.34)	65 (0.65)	–	–	–	1.1^{ns}	4.4*	0.46
Peru	100	2 (0.02)	4 (0.04)	32 (0.32)	62 (0.62)	–	–	–	–	0.6^{ns}	0.51
Cam	60	2 (0.03)	6 (0.10)	24 (0.40)	28 (0.47)	–	–	–	–	–	0.61

Abbreviations and symbols: Germ (Germans); Viet (Vietnamese), Jap (Japanese); Peru (Peruvians); Cam (Cameroonians).
A (A at locus L00073 A); G (G at locus L00073 [transition]); T (T at locus nt16519); C (C at locus nt16519 [transition]); PD (power of discrimination).
Significance level: ns—not significant.
* Significance level: $p<0.05$.
** Significance level: $p<0.01$.
*** Significance level: $p<0.001$.

References

[1] C.T. Wittwer, K.M. Ririe, R.V. Andrew, D.A. David, R.A. Gundry, U.J. Balis, The LightCycler: a microvolume multisample fluorimeter with rapid temperature control, Biotechniques 22 (1997) 176–181.
[2] S. Lutz, H.J. Weisser, J. Heizmann, S. Pollak, Location and frequency of polymorphic positions in the mtDNA control region of individuals from Germany, Int. J. Legal Med. 111 (1998) 67–77.
[3] S. Horai, K. Murayama, K. Hayasaka, S. Matsubayashi, Y. Hattori, G. Fucharoen, S. Harihara, K.S. Park, K. Omoto, I.H. Pan, MtDNA polymorphism in east Asian populations, with special reference to the peopling of Japan, Am. J. Hum. Genet. 59 (1996) 579–590.
[4] H. Pfeiffer, R. Steighner, R. Fisher, H. Mornstad, C.L. Yoon, M.M. Holland, Mitochondrial DNA extraction and typing from isolated dentin—experimental evaluation in a Korean population, Int. J. Legal Med. 111 (1998) 309–313.