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Polymorphisms analysis of mitochondrial DNA in coding area

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Abstract. *Objective:* To analyze mitochondrial DNA (mtDNA) polymorphisms in coding area and provide a theoretical basis to apply in Forensic Science. *Methods:* The primers of 8162F/8483R and 13070F/13299R were designed according to the Anderson's sequence. Using PCR-sequencing method to detect polymorphisms of mtDNA nt8162–8483 and nt13070–13299. *Results:* The lengths of the amplicons were 322 bp and 230 bp, respectively. There were 24 mitochondrial haplotypes defined by 21 variable positions in both regions. The gene diversity was estimated at 75.11%, and the probability of two randomly selected individuals having identical mtDNA types was 25.64%. *Conclusions:* The polymorphic sites within mtDNA coding area can be useful in combination with mtDNA control region in order to increase the discriminational power. © 2005 Published by Elsevier B.V.

Keywords: Mitochondrial DNA; Coding area; Single nucleotide polymorphisms; Discriminational power

1. Introduction

Mitochondrial DNA (mtDNA) sequencing has allowed investigators to derive genetic information from forensic samples where nuclear-based analyses have failed; for example, degraded samples, old bone fragments or hair shafts without roots. Currently, mtDNA for forensic testing consists primarily of portions of the control region, most often targeting the hypervariable regions one and two (HV1/HV2), but poor discrimination power remains a problem. The only solution would appear to be to find more polymorphic sites within mtDNA. The suggestion has been made that besides the mtDNA control region, the polymorphisms within mtDNA coding area should be used for forensic biologists in order to increase the discrimination power of mtDNA. In this study, we have sequenced the

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Table 1
The primer sequence

Primer	Primer sequence	Primer	Primer sequence
8162F	5′TACGGTCAATGCTCTGAAATC 3′	13070F	5′CCCTACTCCACTCAAGCACT 3′
8483R	5′TTGGTGAGGGAGGTAGGTG 3′	13299R	5′TTGGTTGATGCCGATTGT 3′

mtDNA coding area nt8162–8483 and nt13070–13299 of 100 unrelated healthy Han Chinese individuals. We will present the single nucleotide polymorphisms (SNP) sites and 9-bp length-polymorphism of the mtDNA intergenic COII/tRNA^{Lys} region, which may be of crucial importance to forensic testing.

2. Materials and methods [1]

Total genomic DNA was extracted from blood sample with a 5% final concentration of Chelex 100 beads following published protocols [2]. Primer pairs 8162F/8483R and 13070F/13299R, which were designed according to the Anderson's sequence [3] (Table 1), were used to amplify 322 bp and 230 bp fragments of the mtDNA coding area, respectively (marked mtco I and mtco II). After purification and sequencing reaction, samples were analyzed on ABI Prism 377 sequencer.

3. Results and discussion

In mtco I, 12 sites were variable and in mtco II, 9 sites were variable. A total of 14 and 12 different haplotypes were observed in mtco I and mtco II, respectively, in which 5

Table 2
Polymorphic sites of mtco I and mtco II

Position	Variant	Frequency (%)
8271	9-bp deletion	15
8271	9-bp insertion	1
8273	C/T	1
8277	T/C	1
8291	A/G	1
8343	A/G	1
8404	T/C	2
8410	C/T	2
8414	C/T	1
8440	A/G	1
8459	A/G	1
13135	G/A	2
13145	G/A	2
13152	A/G	3
13182	T/C	1
13215	T/C	1
13227	C/T	1
13260	T/C	1
13263	A/G	7
13269	A/G	2

haplotypes of mtco I and 4 haplotypes of mtco II were shared by more than 1 individual. The most common haplotypes of mtco I and mtco II were found in 60 and 82 individuals respectively. Among the combination of mtco I and mtco II, 24 haplotypes were identified: 17 types were unique, the most common type was observed in 47 individuals. Gene diversity calculated from our data using the equation $h=(1-\sum x_i^2)n/(n-1)$ (where n is sample size and x_i is the frequency of i-th mtDNA type) [4], was 60.79% for mtco I, 32.57% for mtco II and 75.11% for both regions combined. The probability of two randomly selected individuals from a population having identical mtDNA types ($P=\sum x_i^2$) was 39.82% for mtco I, 67.76% for mtco II and 25.64% for both regions combined. The location, variants and frequency of the mutations are listed in Table 2. Previous researches [1,4] show that the polymorphism of mtDNA coding area is less than that of mtDNA control region. Therefore, more efficient polymorphic sites should be used to provide an improved discrimination power for forensic mtDNA testing.

As forensic markers, they should be phenotypic neutral to avoid landing investigators into serious situations of medical genetic privacy and ethnics, especially for mtDNA coding area whose mutation often correlated with an increased risk of some disease. The sites listed in Table 2 were all searched by using the web Mitmap (http://www.mitomap.org/cgi-bin/mitomap/tb18gen.pl), stating that these polymorphic variants were found to be "silent" and had nothing to do with disease.

In mtco I segment, the intergenetic COII/tRNA^{Lys} region contains two tandemly repeated copies of 9-bp sequence, which may produce multiple deletions and insertions. In our study, 15 individuals with 9-bp deletions, 1 individual with 9-bp insertion were detected.

With the whole mtGenome sequences being researched, we are optimistic that the polymorphism sites within mtDNA coding area will be useful in combination with control region SNPs so as to increase the discrimination power of mtDNA.

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