M.J. Bogusz (Ed.), *Forensic Science*
Handbook of Analytical Separations, Vol. 2

CHAPTER 20B

Mitochondrial DNA in forensic genetics

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20B.1 INTRODUCTION

Mammalian mitochondrial DNA (mtDNA) is a closed circular molecule that contains 16 569 base pairs (bp). The entire human mitochondrial DNA sequence has been determined [1], functions and gene products have been assigned to all mitochondrial genes including 13 protein-coding, 2 rRNA, and 22 tRNA genes. The two strands of the circular mtDNA have an asymmetric distribution of Gs and Cs generating heavy (H) and light (L) strands. Each strand is transcribed from one predominant promoter, PL and PH1, located in the control region (CR) which includes the displacement (D)-loop generated by the synthesis of a short piece of H-strand DNA named 7S DNA (Fig. 20B.1).

D-loop is approximately 1.2 kilobases (kb) in length. The region is highly polymorphic and contains two well-characterised hypervariable (HV) regions: HVI and HVII. Most mtDNA variation is concentrated in this HV region. mtDNA identification is usually based on these sequence differences in the HV region.

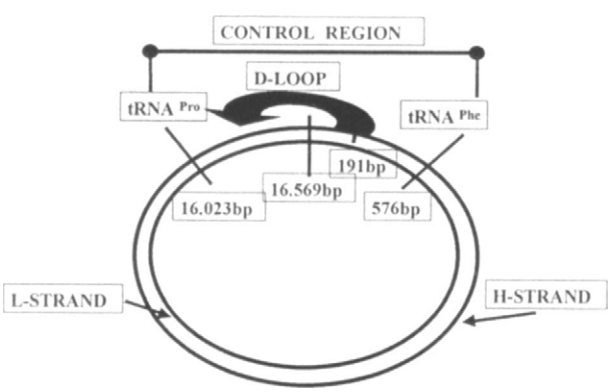

Fig. 20B.1. Scheme showing the situation of the control region in the mitochondrial DNA molecule.

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The mtDNA is maternally inherited [2]; because of this it does not go through recombination with each generation, representing only the maternal ancestry of an individual.

It has the following characteristics:

- Robustness: the mtDNA is more robust that nuclear DNA.
- Large amount of molecules per cell, 1000–10 000: there are a large number of copies compared to chromosomal DNA, providing results when genomic DNA does not.
- Rapid rate of evolution with a 5–10 times higher mutation rate than nuclear DNA [3].
- Maternal inheritance, not recombination, giving the possibility to study family members from the same maternal lineage.

The mtDNA has become very important in anthropological and evolutionary research as well as in forensic genetics.

20B.2 FORENSIC APPLICATIONS

In forensic genetics nuclear DNA is the first choice for routine casework. Nevertheless, sometimes, the nuclear DNA is degraded or cannot be recovered in sufficient amounts to be typed, and mtDNA analysis became the only method which can provide some information about casework. For this reason, mtDNA analysis is increasingly being used in forensic casework ([4,5], among many others).

Analysis of the mtDNA control region is an efficient method for the study and comparison of bones, old and degraded DNA and telogenic hairs [4–9].

In these cases, samples of mtDNA variation can be analysed using a variety of strategies. The combination of PCR amplification with direct DNA sequencing is usually the ultimate choice for identification and it has proved to be a reliable and reproducible method in forensic casework.

Analysis of mtDNA is a valid method to be applied in forensic genetics; however, its study presents some problems that we would like to show in this chapter. Problems such as mutation rate, heteroplasmy, homopolymeric tracts, statistical approach, etc. All of them are important for the reliability of the mtDNA results.

20B.3 MUTATION RATE

The evolution rate of mtDNA is 5–10 time higher that nuclear DNA. It may be due in part to the mitochondrial oxidative metabolic pathways, which provide most of the energy to the cell. The mtDNA is prone to oxidative damage mainly because: (a) the mitochondria consumes >90% of the oxygen that enters the cell, and free oxygen radicals may thus preferentially cause damage to mtDNA; (b) there is a lack of protective histones in the mitochondrial DNA molecule; and (c) mitochondria are less efficient than the nucleus in repairing DNA damage and replication errors [10].

In general the mechanism by which mtDNA mutation arises and becomes fixed in mammalian maternal lineages is not fully understood. These processes are relevant not only to the investigation of mtDNA diseases, but also to the analysis of human popu-

TABLE 20B.1

MUTATION RATES IN THE MITOCHONDRIAL DNA CONTROL REGION

	Number of families (lineages)	Mut. obs.	Generations (meiosis)	Mut./gen.[a]	Mut./site/gen.
Mutation rates (studies in families; empirical rates)					
Howell et al., 1996 [12]	4	1	81	1/81	1.08×10^{-5}
Soodyall et al., 1997 [13]	5	0	108	0/108	–
Bendall et al., 1996 [11]	[b]	7[c]	–	1/25	1.2×10^{-6} 2.7×10^{-5}
Parsons et al., 1997 [14]	134	10	327	1/33	2.7×10^{-5}
Jazin et al., 1996 [15]	33	0	288	0/238	–
Mutational rates (philogenetical studies)					
Ward et al., 1991 [16]	–	–	–	1/222	4.14×10^{-6}
Vigiland et al., 1991 [17]	–	–	–	1/638	1.44×10^{-6}
Stoneking et al., 1992 [18]	–	–	–	1/399	2.3×10^{-6}
Tamura and Nei, 1993 [19]	–	–	–	1/919	1.7×10^{-6}
Hasegawa et al., 1993 [20]	–	–	–	1/918	1.9×10^{-6}
Horai et al., 1995 [21]	–	–	–	1/429	2.16×10^{-6}

[a] Assuming a generational time of 20 years.
[b] 180 twins and 293 individuals unrelated.
[c] 7 mutations were heteroplasmic.

lations (where estimates of mtDNA sequence diversity are used to date demographic events) and for forensic genetics in identification cases [11].

There exists a large disparity of the mutation rate in mtDNA according to different authors. Some examples of the differences between phylogenetic and genealogical studies are shown in Table 20B.1.

What could account for the disparities between the observed substitution rates and those derived from phylogenetic analysis? Some theories have been proposed to explain these differences [14]:

(1) That generational studies observed substitutions predominantly at mutational 'hot spots', while phylogenetic estimates reflect rates averaged over all sites. Because this work involves a very restricted period of evolutionary time, it is reasonable to suspect that. Nevertheless, the authors think that hot spots alone do not explain the high observed mutation rate.

(2) It may be due to new substitutions, which while common between generations, are usually eliminated through random genetic drift before reaching an appreciable frequency in the population.

(3) An inherent mechanism makes some substitutions revert relatively rapidly to the more stable original state.

(4) It is also possible that some CR substitutions are deleterious and over time are selectively removed from the population. This would result in a low population frequency, despite a relatively high substitution rate.

(5) The mutation rate in mtDNA is not the same as the fixation rate of the mutation and this can also explain the difference between studies.

20B.4 HETEROPLASMY

Studies of human mitochondrial DNA transmission in families have revealed that multiple mtDNA genotypes (heteroplasmy) may be present in the same individual and even in the same sample from the same individual [11,22,14].

Most mammals are homoplasmic, suggesting that mitochondrial mutations arising in one molecule can rapidly spread to fixation within an individual [23]. A bottleneck hypothesis has been proposed to explain these observations, in which at some stage of oogenesis or embryogenesis a small number of mtDNA molecules determine the cytoplasmic genotype of the next generation. This would allow a mutation arising in a single molecule to become fixed in a lineage within a small number of generations [23,24].

Heteroplasmic point mutations have been thought to be very rare or absent in normal populations [25], and therefore most segregation studies in humans have shown heteroplasmy point mutations in human diseases [12,26,27]. Moreover, in the last few years, several authors have proved that heteroplasmic mutations in the control region are more widespread than has been reported before [11,14,21,28–30].

A well known case is that of the analysis of human bones from the family of Tsar Nicholas II [28] where a single heteroplasmic point mutation at position 19 169 (T/C) was found. Comas et al. [29] found two heteroplasmic point mutations in the CR in an individual. Sullivan et al. [30] studied twelve hairs and saliva from a single individual. They found that saliva and seven hairs had a C in the position 16 093, three hairs had a C/T in the same position and two hairs showed a T. Bendall et al. [11] studied 180 pairs of twins and 293 unrelated individuals and found three pairs of twins with heteroplasmy mutations (position: 16 192 (C/T), 16 222 (C/T) and 16 293 (C/T) and one pair of twins with two heteroplasmic point mutations (16 262 (C/T), 16 293 (A/G). In the unrelated individuals they found three individual with a heteroplasmy point mutation (position: 16 192 (C/T), 16 256 (C/T), 16 311 (T/C). Parsons et al. [14] studied 327 generational events and they found a family with two heteroplasmic point mutations in positions 16 092 (C/T) and 16 256 (T/C). Gocke et al. [22] studied 96 mother/children pairs and they found three heteroplasmy pairs, two of them were a length polymorphism and one was a heteroplasmy C/T in the 15 945 position.

Heteroplasmy can be present in different proportions in different tissues from the same individual. In this way, Bendall et al. [31] described one individual with variable levels of a heteroplasmic point mutation in different hair roots. At this moment, it seems that, over all, in hairs the heteroplasmy is not rare even between different pieces of a single hair [32].

The importance of this fact in genetic forensics is evident above all when we are comparing samples from an individual in relation with a criminalistic case or a body identification through maternal lineage. We need to always be aware of this mtDNA behaviour.

As we can see, heteroplasmic point mutation in the control region of mtDNA is not rare and its observation depends on the quality of the sequence method that we are using in the laboratory.

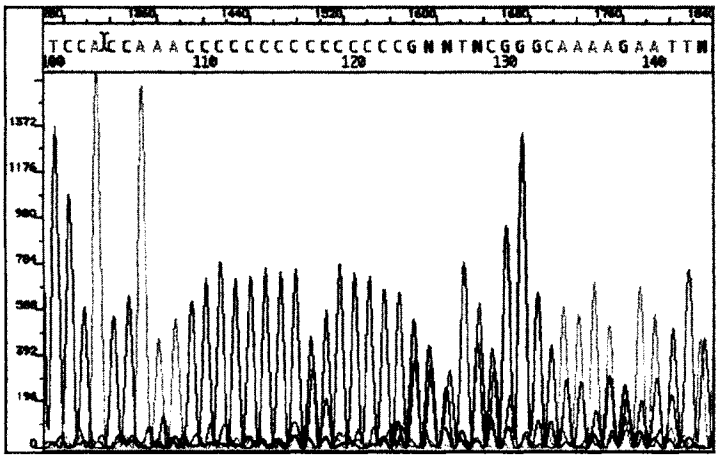

Fig. 20B.2. Length heteroplasmy in the homopolymeric tract at the HVII region (positions: 303–315).

20B.5 HOMOPOLYMERIC TRACTS

The two hypervariable segments of the control region contain some homopolymeric tract. In the HVI region there is a cytosine stretch from base position 16 184 to 16 193 interrupted at position 16 189 by a thymine, and in HVII another from base position 303 to 315 interrupted at position 309 by a thymine. In both homopolymeric tracts a variant commonly found in population screening is a T-to-C transition. These tracts are often heteroplasmic, having populations of mtDNA molecules differing in number of cytosines within the cytosine stretch [23,33].

Bendall et al. [23] studied individuals with a T-to-C transition at nt 16 189 relative to the Cambridge reference sequence [1]. All samples with this transition showed length polymorphism in the homopolymeric tract and are thus heteroplasmic. Tracts are predominantly 10, 11 or 12 nt long.

Replication slippage is thought to create length polymorphisms in these homopolymeric tracts.

The mixture of different length variants is often difficult to interpret creating problems in the sequence analysis, as stops and ambiguities in the sequence. For this reason, the sequence cannot be properly analysed. The problem can be solved most of the time by sequence analysis in the opposite direction [33] (Fig. 20B.2).

20B.6 TECHNICAL APPROACH

The common method to study mtDNA is through polymerase chain reaction (PCR). Using this technique several protocols can be used: asymmetric PCR, overlapping fragment PCR, seminested PCR, nested PCR, etc.

A variety of methods have been proposed for detecting variation in mtDNA HV regions from the amplified product. In the forensic genetics laboratories, screening methods of

point mutation polymorphisms are very important; however, sequence analysis is the chosen method when we need to obtain all information about a specific sample.

20B.6.1 Point mutation polymorphism: screening methods

As we said before, a variety of methods have been proposed for detecting variation in mtDNA HV regions without sequencing, such as restriction enzyme analysis [34–36], hybridization with allele-specific probes [37], oligonucleotide ligation assay and oligonucleotide ligation solid-phase minisequencing [30].

Electrophoretic methods for studying conformational DNA changes [38,39] can analyse a high percentage of the variation in a cheap and simple way. Among all the methods for screening mutations, single strand conformation polymorphism (SSCP) analysis [40] is the most widely used for clinical or forensic applications.

As everybody knows, polymorphisms in mtDNA are mainly sequence variants and all methods that can detect point mutations can be used as a screening procedure to analyse mtDNA.

Screening methods are important in forensic genetics laboratories because it is not uncommon to receive in a single case 50 or more hairs to be analysed by mtDNA. It is obvious that control region sequencing of mtDNA is the more informative method but it would be impossible to do this analysis in each sample because the technique is expensive and time consuming.

Some alternative strategies to DNA sequencing have been developed. Among these, SSCP is the most used method. It is based on the different conformations, which single stranded DNA adopts when the samples are previously denaturalized and run in native gels. Using this method, a large number of samples can be analysed in an easy and quick way. However, the advantages of this method are counterbalanced by the fact that standardization is difficult when SSCP analysis is performed. In addition SSCP fails to recognize variation unless a variety of electrophoretic methods are tried out.

Three varieties of SSCP have been described for analysing mtDNA variation: basic SSCP in polyacrylamide gels, SSCP analysis of superposed restriction enzyme fragments (RE-SSCP) and fluorescent SSCP of overlapping fragments (FSSCP-OF). The basis of each method as well as their advantages and disadvantages are described in this chapter.

20B.6.1.1 Single strand conformation polymorphism (SSCP) analysis

PCR-SSCP is one the simplest methods for mutation detection and therefore for screening of mtDNA variation. In this method, the target sequence of interest is amplified by PCR and separated as single stranded molecules by electrophoresis in non-denaturing polyacrylamide gels. Sequence variants usually show differences in mobility, and the presence of mutation is revealed as the appearance of new bands in silver-stained polyacrylamide gels. This mobility shift is believed to be caused by mutation-induced changes of tertiary structure of the single stranded DNA.

Unlike heteroduplex analysis [38,41] or other methods based on the conformation of DNA molecules, SSCP patterns are quite reproducible if the same electrophoretic

conditions (buffer and temperature) are used. Therefore, the use of semiautomatic systems with precast gels (i.e. PhastSystem, APB; Hoeffer) is recommended [39].

SSCP analysis was proposed by different groups [39,42,43] as an alternative strategy for studying mtDNA variation. In general, SSCP is considered as a very practical method for screening the mtDNA variation in casework.

20B.6.1.2 RE-SSCP (restriction enzyme–single strand conformation polymorphism)

This represents a different strategy for reducing the drawbacks just mentioned and for analysing mtDNA variation in the control region [44].

The idea behind this method is to choose fragments of adequate length when performing SSCP analysis, selecting a set of restriction enzymes (RE) which yield fragments of prefixed lengths after digestion of mtDNA by the appropriate enzyme or set of enzymes. The conformational changes due to single mutations are therefore detected without changing the electrophoretic protocol, but changing the relative position of the mutations within the fragment (Fig. 20B.3).

The fragments obtained must be of an appropriate size for SSCP analysis and at the same time of different sizes in order to avoid overlapping of SSCP patterns. The combination of several restriction enzymes is important, since single mutations can be solved by exchanging its relative position in the fragment using the same electrophoretic system.

This has significant time and cost advantages compared with the multiple electrophoretic conditions necessary when searching for unknown genetic variation in a large number of individuals.

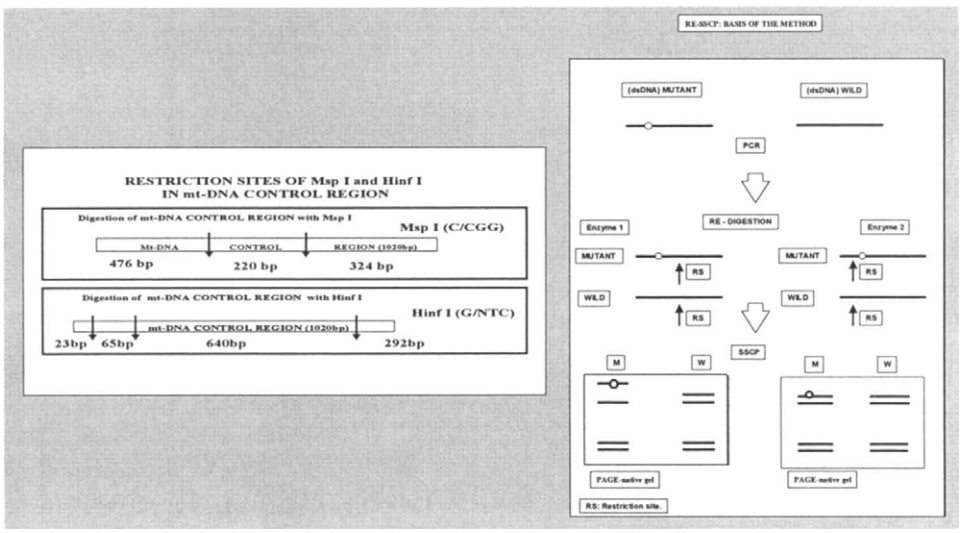

Fig. 20B.3. Basis of the RE-SSCP method. Two different enzymes are used to distinguish a single mutation in two individuals. While the mutation can not be detected with enzyme 2 after SSCP analysis, it is clearly distinguished from enzyme 1.

20B.6.1.3 Minisequencing

This technique is based on a multiplex fluorescent minisequencing, which detects both sequence and length polymorphism. The concept of fluorescent minisequencing is to characterise a particular polymorphic site within the PCR product by annealing to it a primer whose 3'end is one base upstream from the site in question. A single base extension reaction is then performed utilizing dideoxynucletides labelled with four distinguishable dyes [30,45]. This method detects ten substitution polymorphisms and two length polymorphisms in the control region: an oligo-G region at position H00309 and a dinucleotide repeat at position L00524

20B.6.1.4 Fluorescent SSCP of overlapping fragments (FSSCP-OF)

Another novel SSCP method of screening has been denominated fluorescent SSCP of overlapping fragments (FSSCP-OF) [46]. FSSCP-OF combines two complementary strategies:

(a) One is related with the PCR amplification of overlapping fragments. It is well known that the relative position of a specific mutation in a fragment is highly decisive for the conformation of the single strand during the run. If a specific mutation is situated in the overlap region, this polymorphism will be present in two relatively different positions of two different fragments. It increases enormously the probability of detecting this polymorphism. The overlapping fragments strategy is one of the most commonly used in forensic routine and it has quickly gained success because of its simplicity and facility to analyse highly degraded samples. It implies that the same strategy can be used for screening and for ulterior sequence.

(b) Fluorescent labelled DNA fragments can be easily obtained by using labelled primers in PCR. The use of fluorocromes implies a high sensibility when these fragments are loaded in an automatic sequencer, with the additional advantage of sequencing technology. Moreover, if the fluorocromes for the dye-labelled primers are combined in the correct way and the fragments are coamplified in the same tube-reaction, the multiplexing products can be loaded in the same well.

Multicolour fluorescent technology for SSCP has other advantages over single-colour fluorescent labelling and radioactive methods. First, it can use the internal lane standards to align data from lane to lane and eliminate variability. This point is very important since SSCP bands can be read with high precision. The second advantage is that the two strands of DNA in the PCR amplicon can be labelled with different dyes making the reading of the chromatogram easier. Finally, the software to analyse multicolour technology permits better treatment of the results and the storing of data.

20B.6.2 Mitochondrial DNA sequencing protocols

The screening methods are very useful, but in order to have complete information about the mtDNA the best method is always the sequence analysis.

Various approaches for the direct sequencing of PCR products have been described that are based on the Sanger dideoxy chain termination method.

The dideoxy sequencing method [47] has been universally employed for sequencing. In recent years numerous improvements have been made to obtain good results in the sequence. Some rapid, robust and sensitive methods have been developed for the amplification and sequencing of mitochondrial DNA as cycle sequence, solid-phase sequence or developing automatic sequencers.

(1) *Cycle sequence.* At the moment, it is the most used method in forensic genetics laboratories. The method is a simple and powerful tool for sequencing double strand DNA. In this method the DNA is denatured, a primer is annealed, and then a complementary oligonucleotide is synthesized by a DNA polymerase until extension is terminated by incorporation of a dideoxynucleotide. In cycle sequencing this series of events occurs not once, but 20–30 times in succession. The result is a clearer and stronger sequence from DNA for less effort [48]. Some of the advantages of cycle sequencing are: (a) the signal increase caused by the inherent linear amplification; (b) sequencing at high temperature reduces false stops from template secondary structure; and (c) less non-specific background. The procedure improves the reliability and efficiency of sequencing DNA, and eliminates the problem of preparing single strand DNA. Even knowing that sequencing data quality is dependent on the integrity and purity of the template DNA, the properties of cycle sequencing allow the use of fewer amounts and less pure DNA as template, a very important characteristic when we are working with criminalistic casework.

(2) *Solid-phase method.* Normally PCR produces double strands; nevertheless sequencing reactions are best performed using a given single strand of DNA. This method permits to obtain high-quality templates. Strongly binding a single strand from a PCR reaction to a solid phase allows the remainder of the reaction components to be removed by washing. Normally streptavidin-coated magnetic beads have been used as the solid phase. Streptavidin has an extremely high affinity for biotin [49]. The strand to be immobilized on streptavidin-coated magnetic beads must therefore contain biotin, which is achieved by biotinylating its primer. Each strand of a PCR can be prepared separately for sequencing. The solid-phase method produces sequencing templates from PCR products with a very high quality.

(3) *Automatic sequence.* The efficiency of the sequence protocols has been greatly facilitated by the development of fluorescence-based dideoxy-nucleotide sequencing chemistries and instruments for real-time detection of fluorescence-labelled DNA fragments during gel electrophoresis [50]. Some advantages of this technology are that it eliminates the use of radioactivity, provides computer readable data in real time that can be analysed into a sequence assembly engine, is less time consuming and allows the possibility to reanalyse the data. On the basis of the number of fluorescent dyes used, the commercially available automated sequencers can be divided into two types: the method that uses single-label, four-lanes separation and the second method that employs four-label, single-lane separation.

As an example of the first method, Pharmacia ALF Sequencer supports one dye requiring four lanes to sequence one sample. Fluorescent labelling of molecules for DNA sequencing is achieved by the extension of fluorescently labelled primers or the incorporation of fluorescently labelled deoxynucleotides. During electrophoresis, the fluorescently labelled fragments in each lane migrate downwards through the gel. The

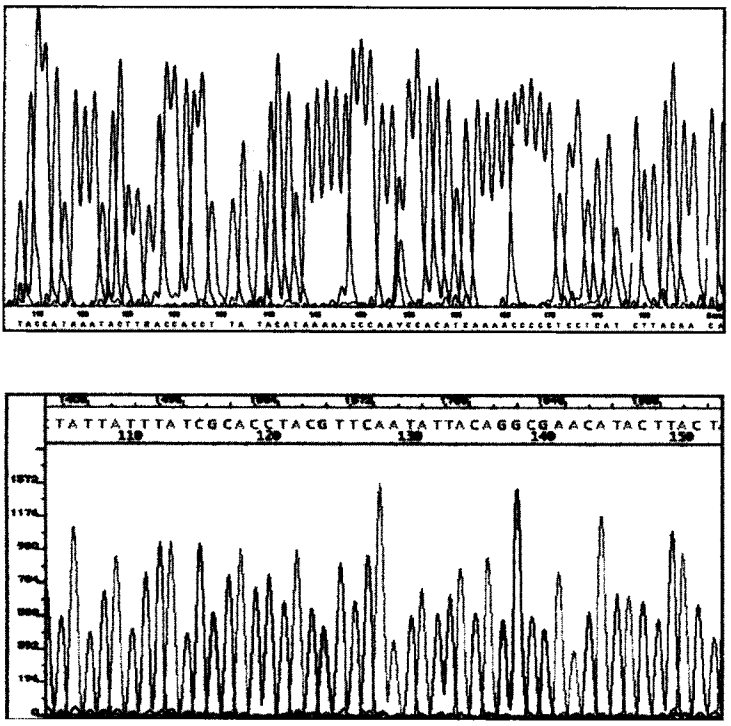

Fig. 20B.4. Sequence of the first hypervariable region (HVI) performed in an A.B.I. (Perkin-Elmer, Applied Biosystems) and in an A.L.F sequencer (APB, Uppsala, Sweden).

laser beam excites the fluorescently labelled DNA bands and the light emitted is detected by photodetectors located behind the gel. With four lanes being used for each clone, ten clones can be run simultaneously. The signals are collected and sent to the computer for storing and processing. Different colours are used to display each of the four bases, one peak representing each nucleotide in the sequence (Fig. 20B.4).

The second method is used by the Applied Biosystems automated DNA sequencer and utilises a multi-spectral approach in which four distinct fluorescent tags are detected in a single lane on the sequence gel [51,52]. The four tags are incorporated during the DNA sequencing reactions and may be present on either the 5'end of the sequencing primer ('dye-primer') or on the dideoxynucleotide triphosphate ('dye-terminators'). The ABI sequencer only requires one line per sample (Fig. 20B.4).

20B.6.3 High-density DNA array: chips for mtDNA

In 1996, Chee et al [53] described the analysis of the entire human mitochondrial genome using DNA arrays containing up to 135 000 probes complementary to the 16.6 kb of the human mitochondrial genome. These arrays, generated by light-directed chemical synthesis, have the resolution of a single base and the results are obtained in a few minutes.

Nowadays, it is relatively easy to prepare arrays of thousands of probes complementary to the entire mtDNA genome on a microchip for parallel processing through hybridization. Nevertheless, this technology needs to be optimized in order to be used in forensic routine. Without doubt, the future of mtDNA analysis is the development of new technologies, such as biochips, which permit the analysis of most of the variable positions along the entire mtDNA molecule, especially the control region.

20B.7 VALIDATION OF MITOCHONDRIAL DNA IN FORENSIC GENETICS AND THE STATISTICAL APPROACH

In the last few years, many efforts have been made to validate mtDNA in forensic genetics laboratories to solve routine casework. Thanks to these efforts, many laboratories are using mtDNA as a good method to solve important casework, always knowing its problems and using this analysis in those cases where nuclear DNA, for different reasons, can not be used.

An experimental validation of the use of mitochondrial DNA analysis has been done by Wilson et al. [4]. The authors have studied chemical contaminant effects on DNA from blood and semen and the effect of typing DNA extracted from body fluid samples deposited on various substrates. They have also evaluated the mtDNA analysis performed on human hair shafts, including hair exposed to chemical treatment, contaminated with several body fluids, etc. The results obtained confirm that mtDNA typing using PCR and direct automated sequencing is a valid and reliable means of forensic identification.

In Europe, the European DNA Profiling Group (EDNAP) has also done much in order to validate mtDNA analysis. In an initial work [5] twelve European laboratories have participated. All of them have reported sequence results for the HVI region analysed in three bloodstains. Although each lab has used different technologies and strategies, all of them have reported the same results.

mtDNA analysis is a valid method for forensic genetics and it has been included in proficiency testing programs of the GEP-ISFG (Spanish and Portuguese Group of the International Society for Forensic Genetics).

A statistical approach to the analysis of mtDNA sequences in cases of coincidence of genetic profiles is a difficult task and it needs a great effort of co-operation between labs. To solve this problem one of the priorities is to perform population studies for the compilation (as large as possible) of population databases.

At this moment, in the last report of the National Research Council (NRC, [54]) the use of conservative frequencies for observed and unobserved haplotypes is recommended; moreover, nowadays it is an important subject for debate in the forensic community. In cases of maternity testing or identification through the analysis of family members related matrilinearly, the interpretation of differences in only one point mutation and the calculation of the exact probability, depend on the correct estimate of the mutation rate at the control region. Currently, several studies by the EDNAP group and other labs are being performed in order to know the somatic mutation rate.

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20B.8 ANTHROPOLOGICAL APPLICATIONS

Sequences from the mtDNA control region are highly variable within human populations and have been a primary source of information regarding the genetic structure, age and origin of modern *Homo sapiens*. MtDNA was the first DNA polymorphism studied in humans for evolutionary purposes. Due to the maternal inheritance and absence of recombination, the only source of new variation is mutation. In this way, the number of mutations, which separate two mtDNA sequences, reflects how related they are. This is the base of the phylogenetic reconstruction. The phylogenetic relationship of sequences can therefore be readily constructed by a variety of methods [55].

Initially, mtDNA was analysed by means of fragment length profiles produced by digestion with individual restriction enzymes of the entire mtDNA molecule. The first studies of human populations analysing RFLPs by Southern blots [56] showed differences between two African groups and Caucasian populations, Chinese and Amerind.

One of the most popular studies based on human mtDNA variation was the one which led us to know the 'African Eve' hypothesis, initially formulated by Cann et al. [57] and posteriorly supported and extended by other authors [58,59]. There are three main aspects derived from this hypothesis: (a) all mtDNA types in contemporary populations trace back to a single ancestor; (b) this ancestor probably lived in Africa; and (c) this ancestor probably lived about 200 000 years ago.

In the last few years, RFLP analysis has been substituted by sequence studies of the control region of the mtDNA. This method was first employed by Vigilant et al. [60] to study different populations from Africa, Europe, Asia and Australia. The results obtained were in concordance with those previously obtained from restriction analysis.

mtDNA studies have demonstrated a great utility for evolutionary studies. However, mtDNA is only informative with respect to the evolutionary history of the maternal lineages. These studies should be completed by means of the analysis of other nuclear and Y chromosome (paternally inherited) polymorphisms which can lead us to resolve many questions about human origins and the complicated pattern of human migrations during history.

20B.9 ACKNOWLEDGEMENTS

This work was supported in part by the grants from the Xunta de Galicia (XUGA 20816B96 and XUGA 20806B97). A.S. was the recipient of a grant from the Consellería de Educación y Ordenación Universitaria (Dirección Xeral de Universidades e Investigación of the Xunta de Galicia). The help of Meli Rodriguez is also acknowledged with appreciation.

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