Int J Legal Med (1998) 111 : 292–298

ORIGINAL ARTICLE

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Mitochondrial DNA polymorphisms: a study of 50 French Caucasian individuals and application to forensic casework

Received: 10 March 1997 / Received in revised form: 4 February 1998

Abstract The analysis of mitochondrial DNA polymorphisms has proved to be efficient on highly degraded samples or samples having little or no genomic DNA such as hair shafts. In order to use this very sensitive method, the authors first established a database by analysing the mitochondrial DNA polymorphism in 50 French white Caucasian individuals, applied the analysis to different types of samples that can be found in forensic investigations and finally performed this method on two forensic cases involving the discovery of highly putrefied unidentified remains.

Key words Mitochondrial DNA · Polymorphism · Forensic · White Caucasian

Introduction

In order to improve human identification methods based on DNA analysis, many techniques have been developed. Minisatellite and microsatellite PCR amplifications allowed the analysis of small nuclear DNA samples but are inefficient for highly putrefied samples. On the contrary, the analysis of mitochondrial DNA polymorphism may be efficient on these kinds of highly putrefied samples. Mitochondrial DNA is a 16 kb molecule which has been fully sequenced (Anderson et al. 1981) and contains two very polymorphic segments (HVR1 and HVR2) located in the non-coding region (Greenberg et al. 1983; Vigilant et al. 1989). The mitochondrial DNA is strictly maternally inherited in humans (Giles et al. 1980) and is haploid, thus permitting the direct sequencing of the PCR products. A single cell contains many mitochondria (about 500) and each mitochondrion possesses many DNA molecules, so that 1000 to 10000 DNA molecules can be found in each human cell (Bogenhagen and Clayton 1974) in contrast to a single copy of nuclear DNA. All of these features of the mitochondrial DNA make it particularly interesting for human identification, especially when the remains are highly putrefied. The method described by Sullivan et al. (1991) consists of a two-step amplification, the amplification of a 1333 bp fragment from the D-loop region and the separate amplification of the two hypervariable regions HVR1 and HVR2, followed by the automatic sequencing of the PCR products. A sequence may be obtained even if the 1333 bp fragment cannot be detected on an agarose gel with less than 10 ng of DNA (Sullivan et al. 1991). This technique has already been applied successfully to numerous types of samples such as semen, hair shafts, blood, hair, skin, faeces, bone, buccal cells, teeth, bloodstains and semen stains on various substrates and hairs subjected to specific treatments (Sullivan et al. 1991, 1992; Hopgood et al. 1992; Ginther et al. 1992; Piercy et al. 1993; Holland et al. 1993; Wilson et al. 1995 a, b; Stoneking et al. 1995; Boles et al. 1995; Hopwood et al. 1996, Ivanov et al. 1996).

In this paper the mitochondrial DNA polymorphism in 50 French Caucasian individuals is presented as well as a comparison with other published population samples. Furthermore, the technique has been tested on different types of samples such as bloodstains, urine, hair roots and hair shafts, saliva on envelopes, buccal swabs, nails, bones and teeth and applied to two forensic cases.

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Materials and methods

Sample extraction

Blood samples were collected in EDTA from 50 unrelated individuals of white Caucasian French origin and kept frozen until extraction. The samples were washed twice with 1 ml of 1 × SSC by centrifugation for 1 min at 10000 rpm. After discarding the supernatant, 375 µl of 0.2 M sodium acetate, 10 µl of proteinase K (10 mg/ml, Boehringer Mannheim) and 12.5 µl of 20% SDS (Sigma) were added to the pellet for an overnight incubation at 56 °C. The DNA was then purified by addition of 120 µl phenol, precipitated by 2 volumes of cold absolute ethanol, washed with 1 ml of 70% ethanol and resuspended in 400 µl of 1 × TE buffer.

Table 1 Sequence of the primers used for mitochondrial DNA amplification of the HVR1 and HVR2 regions

Primer	Sequence from 5′ to 3′
L15926	TCA AAG CTT ACA CCA GTC TTG TCT TGT AAA CC
H00580	TTG AGG AGG TAA GCT ACA TA
L15997	CAC CAT TAG CAC CCA AAG CT
H16401	TGA TTT CAC GGA GGA TGG TG
L00029	GGT CTA TCA CCC TAT TAA CCA C
H00408	CTG TTA AAA GTG CAT ACC GCC A
M13(-21)L15997	TGT AAA ACG ACG ACG GCC AGT CAC CAT TAG CAC CCA AAG CT
M13(-21)H16401	TGT AAA ACG ACG ACG GCC AGT TGA TTT CAC GGA GGA TGG TG
M13(-21)L00029	TGT AAA ACG ACG ACG GCC AGT GGT CTA TCA CCC TAT TAA CCA C
M13(-21)H00408	TGT AAA ACG ACG ACG GCC AGT CTG TTA AAA GTG CAT ACC GCC A

Saliva on envelopes was transferred to swabs moistened with sterile water. DNA from bloodstains, buccal swabs and saliva from the envelopes was extracted after cutting the stain or the swab into small pieces (approximately 3 mm^2) in 0.05 M Tris pH8, with 150 µg/ml proteinase K and 1% SDS. After 2 h incubation at 37 °C, the liquid was recovered in a microtube, extracted twice with phenol/chloroform and once with chloroform/isoamylalcohol and concentrated on Centricon 30 (Amicon) (Hopkins et al. 1994).

DNA from hair roots was extracted by incubation in 200 µl of 20% chelex at 56 °C overnight. The mixture was boiled for 8 min at 100 °C, centrifuged for 3 min at 10 000 rpm and the supernatant was recovered in a clean microtube (Walsh et al. 1991).

Hair shafts (1 cm) were digested in 0.5 ml PBS 1×, 40 µl 0.5 M DTT and 15 µl proteinase K (10 mg/ml) for 6–8 h at 56 °C. Then 40 µl 0.5 M DTT and 15 µl proteinase K were added and incubated for 6–8 h at 56 °C. After centrifugation at 10 000 rpm, the supernatant was transferred to a clean microtube and the DNA was purified by extracting twice in phenol/chloroform and twice in n-butanol.

Fingernails were cut in 1–2 mm pieces, rinsed once with 1 N NaOH and twice with water. Then, 500 µl of buffer (10 mM Tris-HCl pH 8.3, 50 mM KCl, 0.45% NP-40, 0.45% Tween-20, 0.01% gelatin), 7.5 µl proteinase K (10 mg/ml) and 25 µl 10% SDS were added for a 16 h incubation at 37 °C. After one phenol/chloroform extraction, 4 µl RNaseA was added for another 30 min incubation at 37 °C. The DNA was then extracted once with phenol/chloroform and once with chloroform/isoamylalcohol (Kaneshige et al. 1992).

Urine samples (20 ml) were centrifuged for 10 min at 3000 g. After removing the supernatant, the cells were resuspended in 20 ml 1 × SSC for 20 min at room temperature, centrifuged for 10 min at 3000 g and digested in 1.5 ml 1 × PBS and 25 µl proteinase K (10 mg/ml). The DNA was purified by extracting twice in phenol/chloroform and once in n-butanol (Prinz et al. 1993).

Dental pulp was cut into small pieces and incubated in a solution containing 2 ml/g 1 × PBS, 60 µl/g proteinase K (10 mg/ml) overnight at 37 °C, extracted twice with phenol/chloroform and once with chloroform/isoamylalcohol.

Bones were first powdered in liquid nitrogen and incubated in a solution containing 2 ml/g 0.5 M EDTA pH8, 200 µl/g proteinase K (10 mg/ml) and 10 µl/g triton ×100 overnight at 37 °C. The DNA was purified by extracting twice in phenol, twice in phenol/chloroform and once in chloroform/isoamylalcohol, and concentrated on Centricon 30 (Amicon) (Gill et al. 1994).

All DNA samples, except those from whole blood, were precipitated with cold ethanol and recovered in 30 µl of sterile water, in order to concentrate them.

PCR amplification

The technique used was first described by Sullivan et al. (1991) and the sequences of all the primers involved are listed in Table 1. This two-stage technique represents a good strategy to obtain specific PCR products from intact DNA, as the specificity is increased by the use of internal primers during the second round of amplification.

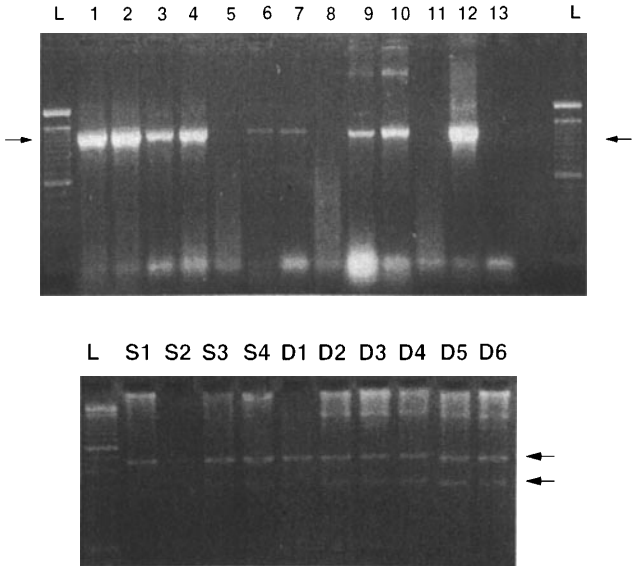

Fig. 1 Electrophoresis results of (top) amplification of the 1333 bp fragment; lanes 1 to 4: buccal swabs; lanes 6 and 7: fingernails; lanes 9 and 10: urine samples; lanes 5, 8 and 11: negative extraction controls for buccal swabs, fingernail and urine samples respectively; lane 12: amplification positive control; lane 13: amplification negative control; L: 100 bp ladder (Gibco BRL) and of (bottom) asymmetric amplification of the HVR1 400 bp region; S1: hair; S2–S4: bones; D1–D6: teeth; L: 100 pb ladder (Gibco BRL)

In the first round of amplification, the primers L15926 and H00580 give rise to a 1333 bp fragment containing both hypervariable regions HVR1 and HVR2. The reaction was performed with 5 µl of DNA in a 25 µl volume containing 1 × PCR buffer (Gibco BRL), 1.5 U of Taq polymerase (Gibco BRL), 400 ng/µl of BSA (Gibco BRL) and 1 µM of each primer (Genset, France). Thirty cycles of amplification (45 s at 94 °C, 1 min at 50 °C and 5 min 30 s at 72 °C) were performed on a GeneAmp PCR System 9600 (Perkin Elmer). A 10 µl aliquot was visualized under UV light after electrophoresis at 100 V for 2 h in 1 × TBE buffer on 1.5% agarose gels (Seakem) using ethidium bromide staining (Fig. 1, top).

In the second round of amplification, chimeric primers containing the sequence complementary to the universal -21M13 primer at their 3′ end, allowed PCR products to be obtained which can be sequenced directly using a commercially available universal -21M13 primer. The ratio between the normal primer and the chimeric primer (1 µM vs. 20 nM) generated single stranded DNA, thus avoiding double strand sequencing or the need to purify single stranded DNA on Dynabeads (Dynal Oslo, Norway). An aliquot of 0.5 µl from the first amplification was used directly without purifi-

cation (Hopgood et al. 1992) in a 50 µl volume containing 1 × PCR buffer (Gibco BRL), 2.5 U of Taq polymerase (Gibco BRL), 100 ng/µl of BSA (Gibco BRL), 1 µM of normal primer and 20 nM of chimeric primer. The primer pairs used were L15997/M13 (-21)H16401 and M13(-21)L15997/H16401 for HVR1 and L00029/ M13(-21)H00408 and M13(-21)L00029/ H00408 for HVR2. The amplification (45 s at 94 °C, 1 min at 60 °C for HVR1 and 53 °C for HVR2, and 3 min at 72 °C) was performed for 32 cycles in the 9600 system (Perkin Elmer). The PCR product was run on 3% Nusieve agarose gels after 45 min of migration at 100 V in 1 × TBE and visualized by ethidium bromide staining (Fig. 1, bottom).

DNA sequencing

The single stranded DNA products obtained through the asymmetric PCR were sequenced using the DNA Sequencing Kit Dye Primer Cycle Sequencing Ready Reaction -21M13 (Applied Biosystems) and analysed on a 6% polyacrylamide denaturing gel on an automated DNA sequencer model 373A (Applied Biosystems) using the 373A software. The sequences were then compared to the reference sequence (Anderson et al. 1981) using the Sequence Navigator software (Applied Biosystems).

Results

All of the 50 French Caucasian individuals studied showed a sequence different from the reference sequence (Anderson et al. 1981) when both hypervariable regions HVR1 and HVR2 were studied together (Fig. 2). Among the differences observed, some were systematically found in all individuals (e.g. an A to G transition at position 263 and a C insertion between positions 310 and 314) and others were very frequent (e. g. an A to G transition at position 73 and the insertion of 1, 2 or 3 C between positions 302 and 308).

For the HVR1 region, 44 different haplotypes were observed in 50 individuals of which 41 were unique, 2 were found in 2 individuals (individuals 26 and 35, and individuals 46 and 49) and 1 was found in 4 individuals (6, 24, 38 and 43). For the HVR2 region, 32 different haplotypes were distinguished among the 50 individuals of which 23 were unique, 5 were found in 2 individuals (3 and 20, 10 and 14, 28 and 40, 29 and 45, 35 and 50), 1 was found in 3 individuals (9, 15 and 23), 2 in 4 individuals (individuals 1, 11, 33 and 43, and individuals 5, 7, 21 and 42) and 1 in 6 individuals (6, 19, 26, 30, 36 and 41). The mean pairwise difference for the French Caucasian population sample studied here was 8.38 (Fig. 3).

Application to different kinds of samples

This technique has been successfully performed on different kinds of samples such as bloodstains, urine, hair roots and hair shafts, saliva on envelopes, buccal swabs, nails (which were collected from laboratory staff), bones (from a corpse at the Institute of Legal Medicine of Strasbourg) and wisdom teeth (kindly provided by the Faculty of Ondotology of Strasbourg) (Table 2). Sequences obtained for different types of samples from the same individual (blood and urine from individual A; blood and hair roots from individual B; blood, hair roots, hair shafts and saliva from individual C; blood, buccal swab, hair roots, saliva and fingernails from individual D; blood, buccal swab, urine, hair roots, hair shafts, saliva and fingernails from individual E) were the same, thus showing the somatic stability. A sequence was obtained even though no DNA was detected on yield gels (data not shown) for hair shafts, nails or saliva on envelopes.

All of the 15 individuals studied, except 2, showed sequences different from each other and different from the reference sequence (Anderson et al. 1981). Two individuals (K and O) showed the same sequence, which was different from the reference sequence and, after investigating the origin of these two samples (which were wisdom teeth), we found that these two individuals were brothers thus explaining the identical mitochondrial sequences.

All the variations from the reference sequence (an A to G transition in position 263 and an additional C between positions 310 and 314), observed in the 50 French Caucasian individuals were also found in all the individuals tested here.

Application to forensic casework

The method has already been applied to two forensic cases. The first case involved highly putrefied remains of a human female head, legs and the lower part of a trunk, which were found in different places. In order to establish if the head and legs belonged to the same victim, we analysed the HVR1 region only on the DNA extracted from 0.5 or 1 g of bone powder and from leg muscle. The comparison of the three samples sequences with the reference sequence was only possible over 178 bp and showed a C to T transition at position 16218 from the skull, a G to C transversion at position 16129 and a deletion between positions 16180 and 16183 from the leg muscle (Table 3, top). The two bone samples showed the same sequence which was different from the one obtained from the leg muscle, thus proving that the legs and the head belong to two different victims.

The second case concerned an unidentified victim discovered in France in September 1995. The corpse was highly putrefied, the only samples available for identification were hair, putrefied muscle attached to the femur and thigh bone. The mitochondrial DNA typing of the HVR1 region only was performed on these samples. Sequences were obtained from the putrefied muscle and from the bone (0.65 and 1.35 g) but not from the hair. The sequences, which were aligned over 250 bp, were all identical and showed four mutations in comparison to the reference sequence: a G to C transversion at position 16129, an A to C transversion at position 16183, a C to T transition at positions 16188 and 16250, and an A to G transition at position 16275 (Table 3, bottom). The victim has not yet been identified, but the 250 bp sequence on the HVR1 region should allow the comparison with blood DNA sequence obtained from any possible maternal relative.

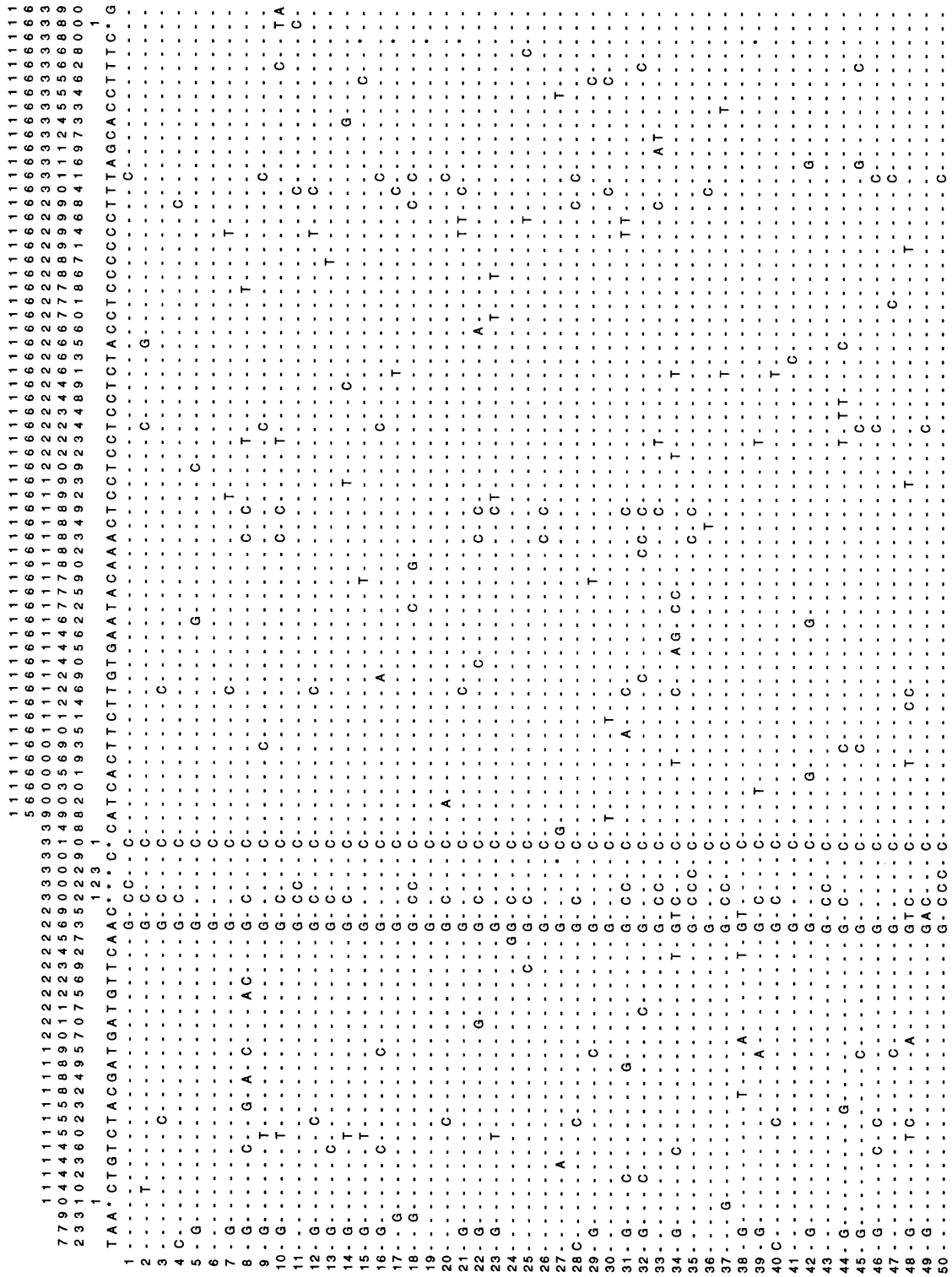

Fig. 2 Mitochondrial DNA polymorphism in 50 French Caucasian individuals. The polymorphisms have been numbered according to the reference sequence (at the top) (Anderson et al. 1981). Insertions are noted by stars and dashes indicate positions identical to the reference sequence

Only two of the seven different polymorphisms found in these two cases have already been observed in the French Caucasian population sample (16129 once and 16183 six times) and the study of the HVR1 region only was sufficient to discriminate the different individuals.

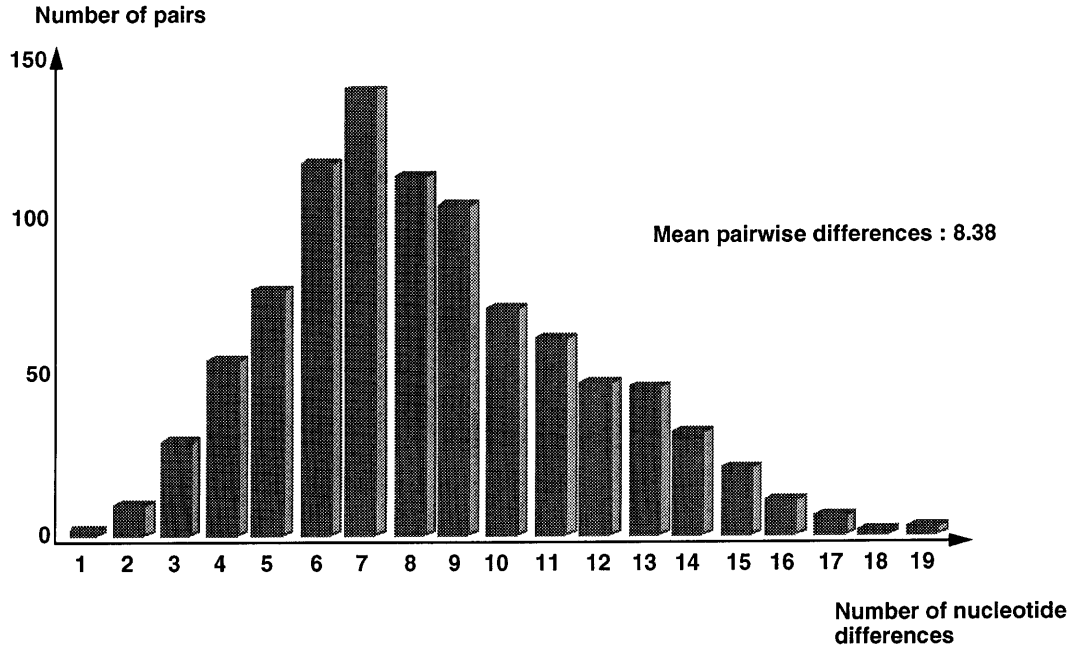

Fig. 3 Number of pairwise differences between the 50 French Caucasian individuals

Table 2 Kinds of samples analysed for each individual in the application

Sample Individual	Blood	Buccal swab	Urine	Hair roots	Hair shafts	Saliva	Finger-nails	Bone	Dental pulp
A	×		×						
B	×			×					
C	×			×	×	×			
D	×	×		×		×	×		
E	×	×	×	×	×	×	×		
F	×								
G	×								
H	×								
I								×	
J									×
K									×
L									×
M									×
N									×
O									×

Table 3 Polymorphic sites observed in the two forensic cases

Case 1					
Position	16129	16180	16218		
Reference sequence	G	A	C		
Bone (0.5 g)	G	A	T		
Bone (1 g)	G	A	T		
Leg muscle	C	–	C		
Case 2					
Position	16129	16183	16188	16250	16275
Reference sequence	G	A	C	C	A
Muscle	C	C	T	T	G
Bone (0.65 g)	C	C	T	T	G
Bone (1.35 g)	C	C	T	T	G

Discussion

According to the restriction sites published by Wallace et al. (1991), only 21 of the 88 polymorphisms (18 for HVR1 and 3 for HVR2) can be detected by RFLP analysis (Table 4) thus showing that mitochondrial DNA sequencing may detect about four times more polymorphisms than RFLP analysis, as already published by Horai and Hayasaka (1990) for Japanese individuals. The HVR1 region is the most variable with 59 polymorphic sites versus only 31 for the HVR2 region (66% for HVR1 and 34% for HVR2), as already observed in India (60% for HVR1 and 40% for HVR2; Bamshad et al. 1996) and in Argentina (56% for HVR1 and 44 % for HVR2; Ginther et al. 1993). Out of the 88 variable sites, only 5 (195, 295, 16129, 16265 and 16380.1) showed many possible substi-

Table 4 Polymorphic sites that can be detected by RFLP polymorphism: few polymorphisms present in the French Caucasian individuals described can be detected by the gain or loss of a particuliar enzyme restriction site

Localisation	Nucleotide	Mutation	Original sequence	Mutated sequence	Enzyme	Consequence
146	T	C	CCTC	CCCC	MnII	loss
257	A	G	GCAC	GCGC	HhaI	gain
263	A	G	GCCAC	GCCGC	Fnu4HI	loss
16051	A	G	GGTACC	GGTGCC	KpnI	loss
16051	A	G	GTAC	GTGC	RsaI	loss
16069	C	T	GACTC	GACTT	HinfI	loss
16093	T	C	GTAT	GTAC	RsaI	gain
16124	T	C	AATATT	AATATC	SSpI	loss
16126	T	C	GTAC	GCAC	RsaI	loss
16129	G	A	GGTACC	AGTACC	KpnI	loss
16129	G	C	GGTACC	CGTACC	KpnI	loss
16180	A	G	TCAA	TCGA	TaqI	gain
16189	T	C	CCTC	CCCC	MnII	loss
16209	T	C	GTAC	GCAC	RsaI	loss
16222	C	T	CCTC	TCTC	MnII	loss
16223	C	T	CCTC	CTTC	MnII	loss
16224	T	C	CCTC	CCCC	MnII	loss
16278	C	T	GATACC	GATATC	EcoRV	gain
16304	T	C	GTAC	GCAC	RsaI	loss
16311	T	C	GTAC	GCAC	RsaI	loss
16356	T	C	CTTC	CCTC	MnII	gain
16368	T	C	CCATGG	CCACGG	NcoI	loss
16390	G	A	GGTCC	AGTCC	AvaII	loss

tutions and a 20:1 transition/transversion ratio was detected in the 50 French Caucasian individuals. The ratio of transversion (5%) is a little higher in this sample than the samples from Japan (Horai and Hayasaka 1990), India (Bamshad et al. 1996), Italy (Francalacci et al. 1996) and England (Piercy et al. 1993) (3%, 3%, 2% and 2% respectively). The mean pairwise difference (8.38) for the French Caucasian population sample studied here is similar to that observed for the British sample (8.48; Piercy et al. 1993) but much higher than those observed for other Caucasian populations i.e. 3.15 for Basques (Bertranpetit et al. 1995), 4.22 for Sardinians (Di Rienzo and Wilson 1991), 3.77 for north Italians (Barbujani et al. 1996) and 5.03 for Tuscans (Francalacci et al. 1996).

In conclusion, the analysis of the mitochondrial DNA polymorphism in 50 French white Caucasian individuals showed variations from the reference sequence published by Anderson et al. (1981). In particular, an A to G transition in position 263 and a C insertion between positions 310 and 314 were systematically found in all individuals, as already observed by Piercy et al. (1993) for a British white Caucasian population sample. The HVR1 region is much more discriminating than the HVR2 region with 41 unique haplotypes and 59 polymorphic sites for HVR1 versus 23 unique haplotypes and 31 polymorphic sites for HVR2.

Acknowledgements This work was supported by a grant from the ANRT (Association Nationale de la Recherche Technique). I wish to thank all the laboratory members who kindly provided samples and also Professor Kaess who kindly provided wisdom teeth samples.

References

Anderson S, Bankier A T, Barrell B G, De Bruijn M H L, Coulson A R, Drouin J, Eperon I C, Nierlich D P, Roe B A, Sanger F, Schreier P H, Smith A J H, Staden R, Young I G (1981) Sequence organization of the human mitochondrial genome. Nature 290:457–465

Bamshad M, Fraley A E, Crawford M H, Cann R L, Busi B R, Naidu J M, Jorde L B (1996) MtDNA variation in caste populations of Andhra Pradesh India. Hum Biol 68:1–28

Barbujani G, Stenico M, Excoffier L, Nigro L (1996) Mitochondrial DNA sequence variation across linguistic and geographic boundaries in Italy. Hum Biol 68:201–215

Bertranpetit J, Sala J, Calafell F, Underhill P A, Moral P, Comas D (1995) Human mitochondrial DNA variation and the origin of Basques. Ann Hum Genet 59:63–81

Bogenhagen D, Clayton D A (1974) The number of mitochondrial deoxyribonucleic acid genomes in mouse L and human HeLa cells. J Biol Chem 249:7991–7995

Boles T C, Snow C C, Stover E (1995) Forensic DNA testing on skeletal remains from mass graves: a pilot project in Guatemala. J Forensic Sci 40:349–355

Di Rienzo A, Wilson A C (1991) Branching pattern in the evolutionary tree for human mitochondrial DNA. Proc Natl Acad Sci USA 88:1597–1601

Francalacci P, Bertranpetit J, Calafell F, Underhill P A (1996) Sequence diversity of the control region of mitochondrial DNA in Tuscany and its implications for the peopling of Europe. Am J Phys Anthropol 100:443–460

Giles R E, Blanc H, Cann H M, Wallace D C (1980) Maternal inheritance of human mitochondrial DNA. Proc Natl Acad Sci USA 77:6715–6719

Gill P, Ivanov P L, Kimpton C, Piercy R, Benson N, Tully G, Evett I, Hagelberg E, Sullivan K (1994) Identification of the remains of the Romanov family by DNA analysis. Nat Genet 6:130–135

Ginther C, Issel-Tarver L, King M-C (1992) Identifying individuals by sequencing mitochondrial DNA from teeth. Nat Genet 2:135–138

Ginther C, Corach D, Penacino G A, Rey J A, Carnese F R, Hutz M H, Anderson A, Just J, Salzano F M, King M-C (1993) Genetic variation among the Mapuche Indians from the Patagonian region of Argentina: mitochondrial DNA sequence variation and allele frequencies of several nuclear genes DNA. In: Pena S D J, Chakraborty R, Epplen J T, Jeffreys A J (eds) DNA fingerprinting: state of the science. Birkhauser, Basel Boston Berlin, pp211–219

Greenberg B D, Newbold J E, Sugino A (1983) Intraspecific nucleotide sequence variability surrounding the origin of replication in human mitochondrial. Gene 21:33–49

Holland M M, Fisher D L, Mitchell L G, Rodriquez W C, Canik J J, Merril C R, Weedn V W (1993) Mitochondrial DNA sequence analysis of human skeletal remains: identification of remains from the Vietnam war. J Forensic Sci 38:542–553

Hopgood R, Sullivan K M, Gill P (1992) Strategies for automated sequencing of human mitochondrial DNA directly from PCR products. Biotechniques 13:82–92

Hopkins B, Williams N J, Webb M B T, Debenham P G, Jeffreys A J (1994) The use of minisatellite variant repeat-polymerase chain reaction (MVR-PCR) to determine the source of saliva on a used postage stamp. J Forensic Sci 39:526–531

Hopwood AJ, Mannucci A, Sullivan KM (1996) DNA typing from human faeces. Int J Legal Med 108:237–243

Horai S, Hayasaka K (1990) Intraspecific nucleotide sequence differences in the major noncoding region of human mitochondrial DNA. Am J Hum Genet 46:828–842

Ivanov P L, Wadhams M J, Roby R K, Holland M M, Weedn V W, Parsons T J (1996) Mitochondrial DNA sequence heteroplasmy in the Grand Duke of Russia Georgij Romanov establishes the authenticity of the remains of Tsar Nicholas II. Nat Genet 12: 417–420

Kaneshige T, Takagi K, Nakamura S, Hirasawa T, Sada M, Uchida K (1992) Genetic analysis using fingernail DNA. Nucleic Acids Res 20:5489–5490

Piercy R, Sullivan K M, Benson N, Gill P (1993) The application of mitochondrial DNA typing to the study of white Caucasian genetic identification. Int J Legal Med 106:85–90

Prinz M, Grellner W, Schmitt C (1993) DNA typing of urine samples following several years of storage. Int J Legal Med 106: 75–79

Stoneking M, Melton T, Nott J, Barritt S, Roby R, Holland M, Weedn V, Gill P, Kimpton C, Aliston-Greiner R, Sullivan K (1995) Establishing the identity of Anna Anderson Manahan. Nat Genet 9:9–10

Sullivan K M, Hopgood R, Lang B, Gill P (1991) Automated amplification and sequencing of human mitochondrial DNA. Electrophoresis 12:17–21

Sullivan K M, Hopgood R, Gill P (1992) Identification of human remains by amplification and automated sequencing of mitochondrial DNA. Int J Legal Med 105:83–86

Vigilant L, Pennington R, Harpending H, Kocher T D (1989) Mitochondrial DNA sequences in single hairs from a southern African population. Proc Natl Acad Sci USA 86:9350–9354

Wallace D C, Lott M T, Torroni A, Shoffner J M (1991) Report of the committee on human mitochondrial DNA. Cytogenet Cell Genet 58:1103–1123

Walsh P S, Metzger D A, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513

Wilson M R, DiZinno J A, Polanskey D, Replogle J, Budowle B (1995a) Validation of mitochondrial DNA sequencing for forensic casework analysis. Int J Legal Med 108:68–74

Wilson M R, Polanskey D, Butler J, DiZinno J A, Replogle J, Budowle B (1995b) Extraction, PCR amplification and sequencing of mitochondrial DNA from human hair shafts. Biotechniques 18:662–669