Identification of Fire Victims by Using DNA Amplification (PCR)

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INTRODUCTION

The most widely used approach for individualization at the DNA level is analysis of variable number of tandem repeats (VNTR) loci by restriction fragment length polymorphisms (RFLP) via southern blotting (Jeffreys 1985). Although, at present, this analysis has a major impact on identity testing, it has certain limitations. These are: (i) sufficient amount of relatively high-molecular weight (hmw) DNA is required; (ii) the RFLP-technique is comparatively laborious and time consuming; and (iii) the resolution capacity of agarose gel electrophoresis is limited.

The polymerase chain reaction (PCR) technique (Mullis 1987) offers a potential alternative to overcome the limitations of the RFLP analysis. The use of PCR can provide greater sensitivity and specificity for genotyping/phenotyping techniques. PCR obviates the need for radioisotopic detection, and it also reduces the time and laboratory work required for DNA analyses. Moreover, the PCR enables the analysis of extensively degraded samples (Bugawan 1988).

In this study the use of the PCR for genetic characterization of extensively burned fire victims was evaluated. Two different loci were analyzed using the PCR. First, the analysis of amplified fragment length polymorphisms (Amp-FLPs) at the D1S80 (pMCT118) locus (Kasai 1990). Second, a sequence polymorphism at the HLA-DQα locus (Bugawan 1988).

MATERIALS AND METHODS

Autopsy cases

The autopsy series comprised ten extensively charred fire victims. Identification by external macroscopic examination was impossible in all cases and identity could only be determined indirectly by personal effects, eyewittness statements or by forensic odontological means.

DNA extraction

Tissue specimens from the ten extensively burned fire victims were collected during autopsy and maintained at -20°C until subjected to DNA extraction. DNA extraction from each crude tissue specimen (158-1055 mg) was performed by using phenol-chloroform extraction method. DNA quality and quantity were estimated on agarose test-gels containing ethidium bromide (1 mg/ml) by direct comparison with molecular weight markers (EcoRI-HindIII digested lambda, Promega) and a dilution series of lambda DNA (Promega).

Amp-FLP analysis of the D1S80 locus

Amplification of D1S80 locus was performed using the primers described by Kasai et al (1990). The 28-mer primer was 5'-GAAACTGGCCTCCAAACACTGCCCGCCG-3'

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and the 29-mer primer was 5'-GTCTTGTTGGAGATGCACGTGCCCCTTGC-3'. Each reaction was composed of 100 ng of DNA in 10mM Tris-Cl, pH 8.3, 50mMKCl, 1.5mM MgC12, 0,01 % gelation, 1.0 μM of each primer, 200 μM of each dNTP and 2.5 U of Taq I polymerase (AmpliTaqTM DNA polymerase, Perkin-Elmer Cetus). The total volume of each reaction was 50 μl. The PCR cycles consisted of denaturation at 95°C for one minute, primer annealing at 65°C for one minute and primer extension at 70°C for eight minutes. A total of 25 cycles was carried out in a Perkin-Elmer Thermocycler.

The analysis of amplified D1S80 alleles was carried out on rehydratable polyacrylamide gels (4 % T, 3 % C, 400 u thick). The gels were rehydrated in 33mM tris-sulfate, pH 9.0 and 7 % glycerol for at least one hour. The trailing ion (0.14M tris-borate, pH 9.0) and bromophenol blue were contained in 2 % (w/v) agarose plugs placed on the cathodal and anodal edges of the gel. Distance between anodal and cathodal agarose plugs was 10 cm. A small volume (2.5 to 5.0 μl) of the amplification product was absorbed into 2.5 x 5.0 mm fiberglass applicator tabs (Pharmacia-LKB) and applied onto the gel surface one cm from the cathodal agarose plug. In each gel a ladder-set of known alleles was run parallel to the amplified DNA samples in every fifth lane. The electrophoretic running conditions were as previously described (Budowle 1991). The separated fragments were detected using a simple silver staining procedure also described elsewhere (Allen 1989; Budowle 1991).

HLA-DQα genotyping

HLA-DQα genotyping was performed with AmpliType™ HLA-DQα Forensic DNA Amplification and Typing Kit (Cetus Corporation, CA, USA) according to manufactures recommendations as well as the procedure published elsewhere (Bugawan 1988).

RESULTS

The qualitative evaluation of the extracted DNA from soft tissues indicated that all samples had significant degradation. The specimens from femoral muscle, psoas muscle and bone marrow yielded DNA from 500 ng/100 mg of crude tissue to greater than 6000 ng/100 mg of crude tissue. The yield of DNA extracted from post mortem blood ranged DNA from 250 ng/100 μl to 1000 ng/100 μl of blood.

All samples produced interpretable D1S80 and HLA-DQα profiles and the results were consistent in various tissues from each individual. The detected genotypes and their combined frequency in the Finnish population are shown in Table 1.

In cases no 1 and 2 a sister and a brother were burned to death in a fire at their home. Peripheral blood samples were obtained from the parents. Mendelian inheritance was demonstrated for alleles at both loci. A family perigree with genotypes is shown in Figure 1.

DISCUSSION

The identification of remains of fire victims is generally attempted by recognizing personal effects, individualizing marks (e.g. scars, tottoos, signs of known disease) and/or dental records. However, due to effects of heat and severe lacerations of the body, the identification by usual forensic means is not always possible.

The obvious limitation of RFLP analysis of DNA from post-mortem soft tissues is that the DNA can degrade rapidly. Thus, no result or minimal data may be obtained. The use of PCR based techniques may be a viable alternative for post-mortem DNA analysis in forensic cases. Virtually any defined short DNA sequence potentially can be analyzed easily and rapidly using PCR. Moreover, with PCR the amount of DNA is not as limiting a factor as it is with the RFLP methodology. Therefore, the effect of

DNA degradation will not be as pronounced with PCR compared with RFLP typing.

In the present study we have shown that PCR based analysis of D1S80 and HLA-DQα loci can be performed succesfully on DNA extracted from different soft tissue specimen from cadavers burned beyond recognition. Sufficient quantity of DNA was extracted from all tissues for PCR analysis (from 500 ng/100 mg crude tissue to grater than 6000 ng/100 mg crude tissue and 250–1000 ng/100 μl blood). On crude tissue specimen (femoral of psoas muscle and bone marrow) we found no remarkable difference between the samples and DNA yield, but post-mortem blood yielded less DNA than crude tissue in each case. DNA from all tissues was typeable and the results were consistent from tissue to tissue in each body studied. A parentage test was performed on the DNA from two victims where identity was confirmed by traditional means. The DNA data also suggests that the victims were the children of the parents. The consistency of D1S80 and HLA-DQα types in the different tissues and the Mendelian inheritance of alleles in the family study provide support that results from PCR based typing methods are reliable.

In addition to the ability to type DNA from soft tissues of charred human remains, Amp-FLP and HLA-DQα dot blot technologies enable resolution of discrete alleles. Analytical systems that provide correct genotyping and minimal measurement error permit evaluation of the goodness of fit of genotype distributions of the particular locus. The D1S80 and HLA-DQα loci satisfy the assumption of Hardy-Weinberg equilibrium (Sajantila et al., submitted; Sajantilaet al., in press). Thus, the actual allelic frequencies can be used to predict genotype frequencies.

In conclusion, PCR based techniques can provide a means for typing DNA derived from soft tissues of fire victims. The technology is simple and can provide data in an expeditious manner. When relatives are available, this approach may be useful for potential identification of human remains. We currently are performing additional validitation studies involving PCR typing methodologies and investigating additional genetic markers that may prove useful for individualization.

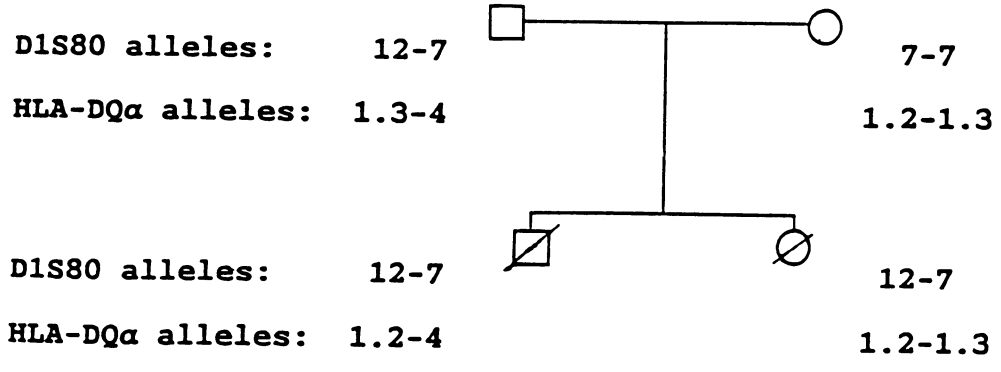

Figure 1. A pedigree of the paternity test performed in cases 1 and 2. The D1S80 and HLA-DQα alleles and the Mendelian inheritance are shown

Table 1.

Summary of the genotypes detected with Amp-FLP of D1S80 locus and HLA-DQα locus. Combined genotype frequencies are calculated from the genotype frequencies obtained in a Finnish population sample

case no.	genotype D1S80	HLA-DQα	combined genotype frequency (D1S80 and HLA-DQα)
1	12-7	1.2,1.3	5.8×10^{-4}
2	12-7	1.2,4	2.4×10^{-3}
3	19-1	1.1,2	3.0×10^{-4}
4	7-7	1.1,2	3.3×10^{-3}
5	11-7	1.1,4	1.6×10^{-3}
6	11-4	1.1,4	5.4×10^{-4}
7	7-1	1.2,3	1.4×10^{-2}
8	19-1	3,4	1.3 x 10-3
9	11-1	1.1,2	1.7×10^{-3}
10	7-7	1.1,1.2	7.7×10^{-3}

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