RFLP IN CONJUNCTION WITH ANATOMICAL TRAITS IN INDIVIDUALISATION OF BONE

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ABSTRACT

RFLP of D4S139 and D14S1, loci is used to individualize a tibia bone seized in a homicide case. Bone was sexed with a Y specific probe. Anatomical traits were eleborately studied to conclude identification.

INTRODUCTION

RFLP analysis is an unequivocal approach for individualisation of biological materials. The conclusiveness of evidence depends upon recovery of DNA from sample, number and nature of loci analysed and DNA prints used for comparison. DNA profiles of body fluids their stains and soft tissue are frequently prepared using multilocus or a battery of highly polymorphic locus specific probes and compared with suspects or of his/her parents to fix the identity of sample (Gill P. 1985). Without adequate amount of high mol. wt. DNA and proper controls the DNA profiling is insignificant. The identity of deceased from a bone in similar situation is established with only pAW101 and pH 30 two loci specific probes and using 102(d)2 (Singh L et al 1980) a Y specific probe. Anatomical traits, i.e. age, height and nature of callus present on an old healed fracture of tibia were examined for conclusive identification.

MATERIALS & METHODS

Examination of Bone

A left tibia and a small piece of maxilla containing 5 teeth (3 PM + 2M) seized in a homicide case were X-rayed and examined to ascertain height and age. A callus present on lower extremity of tibia was X-rayed and examined to know nature and time of fracture (Krogman & Iscan 1986).

DNA Extraction and Profiling

The bone marrow (BM) of tibia was scrapped out mechanically into TES buffer. The extracted marrow alongwith blood samples

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of mother (M), brother (B), sister (S) was subjected for DNA analysis by standard method (Gautreau et al 1983). 1.0ug DNA from each sample was digested with EcoRI and Hind III separately. DNA samples (BM, M, BM+M, S, B) alongwith human control of known allele composition, lambda size marker were electrophoresed in lane 1 to 7 respectively. After transfer and baking on nylon membrane blots of EcoRI and Hind III digested DNA were hybridized with pAW101 (Nakamura et al 1988) and pH30 (Milner et al 1989) probes respectively. The hybridized blots were washed stringently with 0.1 x SSC, 0.1% SOS and exposed for autoradiography.

Sexing

100ng of bone DNA was dotted on nylon membrane alongwith DNA of M, B & S and known control of both sexes and hybridized with P32 labelled 102(d) 2 Y-specific probe to ascertain sex of source.

Band Matching and Statistical Interpretation

The size of bands in DNA print of bone M, B and S were computed with relative mobilities of DNA size marker. The bands of bone were compared with bands in profiles of M, S and B. The probability of relationship was calculated using (Lynch 1988), a database of allele frequency for South Indian population prepared for both the probes (data unpublished). The probability value of involvement of two persons of a particular age, sex and height and with similar abnormality - like limping as reported in this case of suspected deceased in a homicide case was also calculated. The final conclusion of the identity of source of bone was drawn by considering (i) Probability of relatednes between bone and M, S & B DNA Profiles; and (ii) probability of any other individual of same anatomical traits involved in homicide.

RESULT AND DISCUSSION

On visual comparison of DNA profiles developed by pAW101 with EcoRI restricted DNA and pH30 with Hind III digested DNA, it is clear that one band (12kb) for pAW101 is comparable with a band of mother's profile, other band of bone matches with the non-maternal (paternal) band in one of the two siblings. Similarly, one band (8kb) of bone is comperable with one band of mother's profile, other band matching with the siblings band. Bone marrow DNA did not hybridize with 102(d) 2 probe indicating that bone belongs to a human female. On examination of tibia maxilla and tooth sections and their radiographs, it was found that tibia and maxilla may belong to a human female of 25±5 years age group and 5'.1"±1" height may be having a healed fracture on lower extremity of tibia which may cause limping as in the case of suspected deceased. Since the mean probability of presence of any allele of D4S139 in two unrelated individuals is 3.6×10^{-2} and allele of D14S1 is 2.7×10^{-3}, this suggests that tibia belongs female child of M and true sibling of B&S (probability 1.23×10^{-8}).

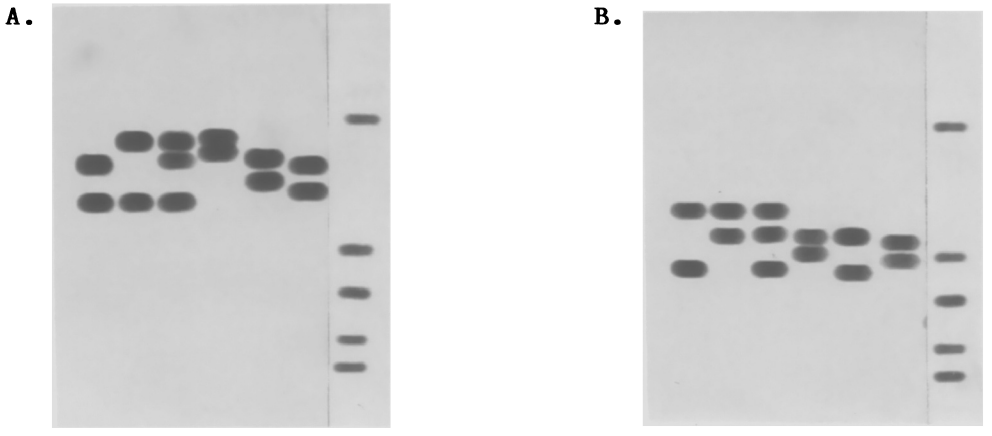

Figure 1(A)&(B): DNA Profile of a bone individualization case. DNA profile of bone marrow : Lane 1; Mother, Sister and Brother : Lane 2,4,5; Bone marrow + Mother : Lane 3; Known control : Lane 6; Size marker : Lane 7; (A) DNA profile with pAW101, (B) DNA profile with pH30

The chance of any other lady of 25±5 age group and 5'.1"±1" of height in India having a 6-8 year old healed fracture in lower end of tibia leading in limping worked out to be 1.8×10^{-7}. From DNA profiling and anatomical traits study, tibia is found to belong to suspected deceased (probability 1.7×10^{15}). It is evident from this study that a bone can be successfully individualized, with RFLP analysis at two loci, provided it bears specific characteristics which are discernable on examination.

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