POSSIBILITY OF PATERNITY TESTING USING RFLP ANALYSIS ON A VERY SMALL AMOUNT OF MATERIAL

Y. Yamada,[1] M. Kobayashi,[2] T. Takatori,[3] M. Nagao,[4] H. Ohira,[1] I. Yamamoto,[1] S. Ohtani[1]

1. Department of Forensic Dental Medicine, Kanagawa Dental College, Yokosuka-City, 238-8580, Japan
2. Department of Forensic Medicine, Graduate School of Medicine, University of Tokyo, Bunkyou-Ku, 113-0033, Japan
3. National Research Institute of Police Science, Kashiwa-City, 277-0882, Japan
4. Department of Legal Medicine, Nagoya City University Medical School, Mizuho-Ku, 467-8601, Japan

ABSTRACT

Paternity testing by DNA analysis was carried out using dental pulpal and chorionic villous tissue from two children respectively, and fresh blood samples obtained from the alleged parents. DNA was extracted spectroscopically from the pulp of an upper wisdom tooth (16μg) and the chorionic villi (53μg). The RFLP method was used for DNA analysis of the parent-child relationships because both of the DNAs extracted had a high molecular weight. Distinct bands were detected with ^{32}P-labelled multi-locus (Myo) and single locus (pYNH24) DNA probes. In the case of the dental specimen all of the bands of the child's DNA were found to be derived from either of the alleged parents, demonstrating a consistent parent-child relationship (the probability of established paternity was 99.86%) whilst in the case of the villous specimen the father-child relationship was denied. This procedure can provide much information using very little material for analysis but where the samples are in a good condition. **(J Forensic Odontostomatol 2001;19:1-4)**

INTRODUCTION

The forensic application of DNA analysis has increased over the past few years[1] and there have been great advances in the identification of individuals using PCR (polymerase chain reaction) analysis which is the most widely used method of DNA analysis in forensic medicine. Identification of DNA polymorphism has also become a popular test in forensic science because of its increased sensitivity. Conversely, DNA fingerprinting[2-6] based on the restriction fragment length polymorphisms (RFLP) analysis is a minor forensic procedure but it may provide more information than PCR.

Conventional paternity testing is based on the detection of as many blood type genetic markers as possible in order to increase the accuracy of the result. If fresh blood samples are not available from the alleged father, mother or child, or if tissues other than blood are the only materials available only limited blood types are detectable and it is difficult to ensure the accuracy of the test result.

We were asked to give an expert opinion of paternity testing by DNA analysis using pulp tissue from a wisdom tooth (case 1), chorionic villous tissue (case2) and fresh blood samples obtained from each of the alleged four parents. The wisdom tooth was from a 22 year-old male extracted as a result of pericoronitis and stored for a period of 2 years. DNA fingerprinting was used because the quantities of both the pulpal and villous tissues were extremely small, and because it may provide more information than PCR. Many laboratories, when presented with these small samples would initially use the PCR procedure.

The main purpose of this study was to confirm the possibility of using the RFLP method with a combination of multi-locus (DNA fingerprinting) and single locus DNA probes for analysis of these extremely small samples.

MATERIALS AND METHODS

Paternity testing by DNA analysis was carried out using three types of tissue sample: pulpal tissue (dry weight 2.4 mg) isolated from the pulp cavity of an undecayed upper wisdom tooth kept at room temperature in a dry environment for 2 years following extraction[7] (case1), chorionic villous tissue (fresh

weight 500 mg) obtained by sampling under ultrasonography at 14 weeks of gestation (case2) and fresh blood samples obtained from each of the four alleged parents.

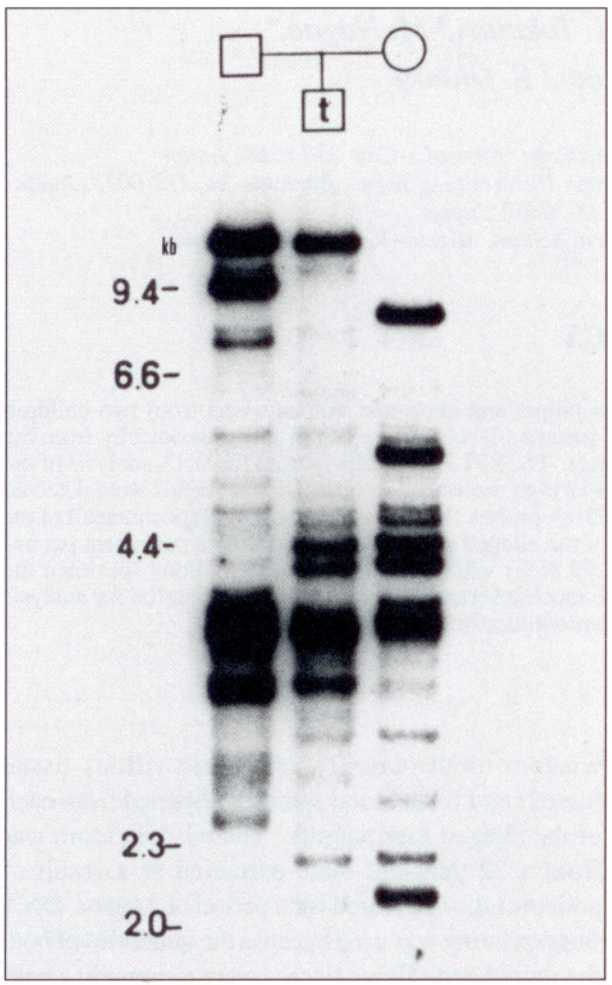

Fig.1: *DNA fingerprints used in paternity testing in an inclusion case (case1). DNA fingerprinting of blood DNA from the father (left) and the mother, and of DNA extracted from the son's tooth (t) is shown. Eliminating maternal bands allowed identification of paternal DNA fragments in the son's DNA. Molecular weight markers are given on the left in kilobases.*

The dental pulp, chorionic villous tissue and leukocytes that were isolated from the blood samples were incubated overnight in 1.5 ml microcentrifuge tubes at 50°C in lysis buffer containing proteinase K (0.1 mg/ml) and 2% SDS. The DNA was then extracted by phenol/chloroform and precipitated in 2 volumes of absolute ethanol. DNA from the alleged parents was extracted from lymphocytes in the fresh blood samples by the usual method. The DNA was pelleted by centrifugation at 3000 rpm for 20 min, air-dried then dissolved in TE (10 mM Tris, 0.1 mM EDTA) to form a DNA solution. To prevent the loss of possibility, DNA yield was determined by comparison to known amounts of intact human DNA after electrophoresis (1.0% agarose, 100V, 30 min.) and ethidium bromide staining. High-molecular-weight DNA extracted from each sample was digested with *Hae* III, electrophoresed on an agarose gel and hybridized with a ^{32}P-labelled multi-locus DNA probe Myo[8] and a single locus (or locus specific) DNA probe pYNH 24 in RFLP analysis, as described previously.[6]

RESULTS AND DISCUSSION

DNA was extracted spectroscopically from the upper wisdom tooth (16µg) and the chorionic villi (53µg). The RFLP method was used for DNA analysis of the parent-child relationships because both of the DNAs extracted had a high molecular weight (data not shown). Distinct bands were detected with ^{32}P-labelled multi-locus and single locus probes and detection of bands with one type of probe was followed by alkaline treatment to remove the probe and secondary detection with the other type of probe on the same filter.[6]

In the case of paternity testing with the dental specimen (case1), all bands obtained from DNA of the

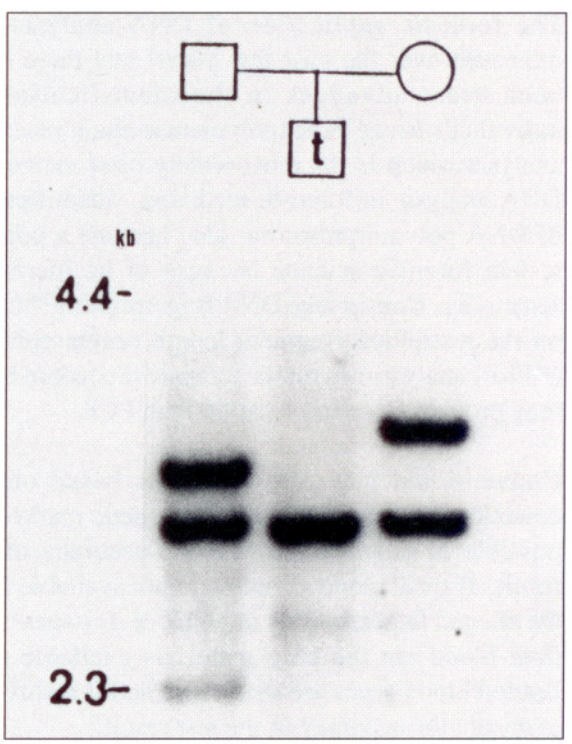

Fig.2: *Paternity testing as shown in Fig. 1. The nylon filter used in Fig. 1 was treated with NaOH, and the Myo probe was removed. The filter was hybridized again with the ^{32}P-labelled single locus DNA probe pYNH24.*

tooth were derived from both of the alleged parents, demonstrating a consistent parent-child relationship. The probability of established paternity was 99.86% using a gene frequency of multiple loci of 0.27[8], and 97.75% using a gene frequency of a single locus of 0.023[9] (Figs.1 and 2). In the case of the villous specimen (case2) the bands were identified with the multi-locus DNA probe in the child's DNA. The father-child relationship of this pair was denied (Figs.3 and 4).

Currently, DNA analysis is used as an auxiliary to blood type analysis in paternity testing and its use by RFLP cannot be accepted as a reliable, stand-alone procedure because of the problems with DNA probes[3] and the lack of sufficient basic data. Although multi-locus DNA probes can detect many bands and provide a large amount of information, the number of loci and alleles is not known, leading to inconclusive results. Conversely, single locus DNA probes can be used to calculate allele frequencies but can detect only 2 bands at most and therefore provide limited information.

Accurate blood type analysis for paternity testing was not feasible in these cases because unusual specimens were used. On the other hand almost all current DNA testing uses PCR[10] because sufficient high-molecular-weight DNA is usually available to allow for any type of DNA analysis. We therefore used the most fundamental technique of DNA analysis, RFLP (in a supporting capacity for PCR analysis), with a combination of multi-locus and single locus DNA probes for analysis of each sample. In this way the drawbacks of each type of probe (ambiguous loci and limited information) were compensated for by the other's benefits, and useful information was obtained. Although we used a radioisotope in terms of DNA marker detection,

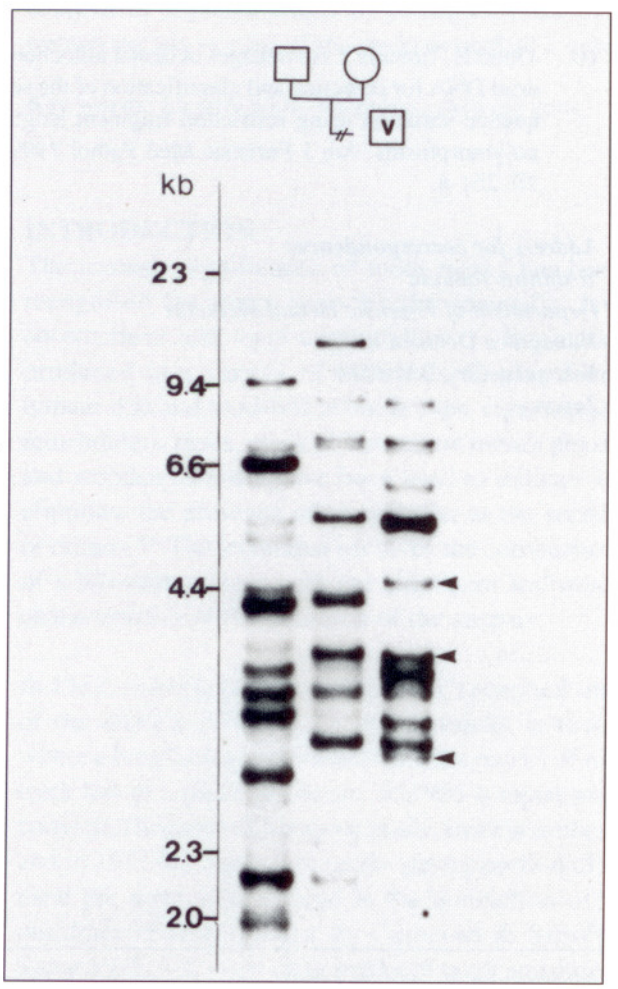

Fig.3: *Paternity testing in prenatal diagnoses with the ^{32}P-labelled multi-locus DNA probe Myo using Hae III in an exclusion case (case2). DNA fingerprints of blood DNA from the father (left) and the mother, and of DNA extracted from the chorionic villi (v) are shown. The paternal fragments are arrowed.*

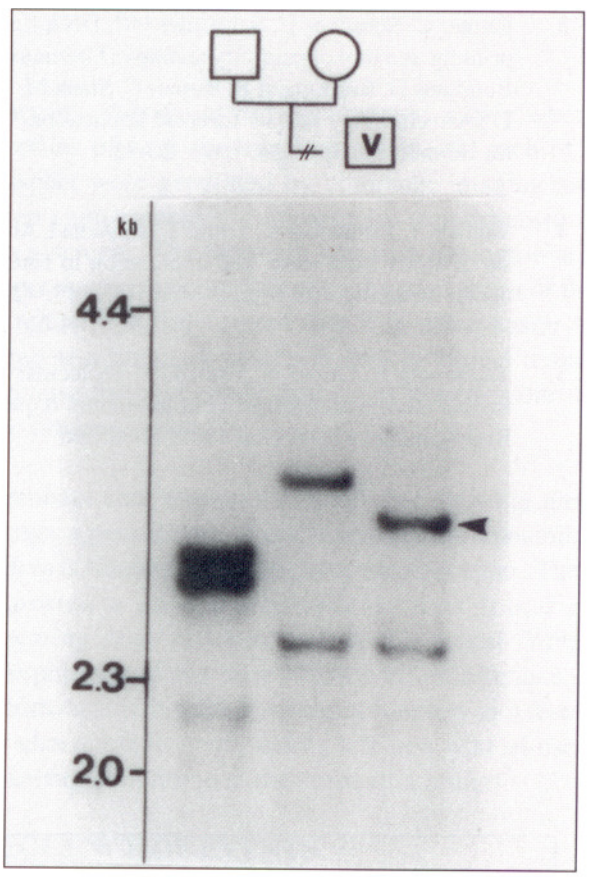

Fig.4: *Paternity testing in prenatal diagnoses as shown in Fig.3. The filter was rehybridized with the ^{32}P-labelled single locus DNA probe pYNH24. The paternal fragments are arrowed*

highly sensitive non-isotopic probes detected by chemiluminescence is a conceivable procedure for mainstream use, but the DNA analysis using a radioisotope is rarely carried out today, and would be in the future.

DNA analysis in paternity testing based on RFLP is restricted by the condition of the sample but if analysis is possible RFLP will provide much more information than DNA analysis based on PCR and provide improved clarification of a parental relationship.

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Address for correspondence:
Yoshihiro Yamada
Department of Forensic Dental Medicine
Kanagawa Dental College
Yokosuka-City, 238-8580
Japan

ERRATUM: An error occurred in the last edition of this journal Volume 18, No.2, December 2000. On Page 46 in the article entitled "Wounding dynamics in distorted bitemarks: two case reports" the authors should read: "S. Sakoda, M.Q. Fujita, B-L. Zhu, S. Oritani, K. Ishida, M. Taniguchi and H. Maeda" - all from Japan.