APPLICATION OF DNA POLYMORPHISMS TO THE DETERMINATION OF PATERNITY

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Abstract

The Mendelian inheritance of polymorphic DNA sequences makes them useful for paternity determinations. We examined the DNA polymorphisms associated with the genetic loci D14S1 and HRAS1 in more than 100 paternity cases. The objective of these tests was to first determine the phenotypes (ie. the size of polymorphic fragments) of the mother, child and alleged father and second to use this information in assignment of paternity. In addition, the HLA haplotype of these individuals was established and used to determine paternity. A comparison of these two methods for the analysis of paternity cases indicated: 1) the two DNA polymorphisms used in this study excluded the same number of alleged fathers from paternity cases as HLA, 2) both methods resulted in similar paternity indices.

Introduction

Biological testing to help determine paternity includes ABO, HLA, and protein polymorphism analysis. In combination, these tests can exclude or include an alleged father with a high degree of certainty (1). Current technology allows the direct examination of DNA from specific heritable changes through the use of restriction endonucleases and specific probes (2). This report examines the application of DNA restriction fragment length polymorphism analysis to the assignment of paternity.

Materials and Methods

Sample Origin

Blood samples were collected at the New York Blood Center in New York City as either non-related volunteer blood donors or as paternity trios. Information concerning race was provided. All samples were collected using potassium EDTA as anticoagulant.

DNA Purification

DNA was isolated from the white cells. First the red cells were lysed by the addition of 4 volumes of 4°C blood lysis buffer (0.32 M sucrose; 10 mM Tris-HCl, pH

Advances in Forensic Haemogenetics 1
Edited by B. Brinkmann and K. Henningsen

7.6; 5 mM MgCl; 1.0% Triton X-100) followed by centrifugation at 2,000 x g for 10 minutes at 4°C. The nuclei were resuspended in 1 - 2 cc of DNA lysis buffer (10 mM Tris-HCl, pH 7.4; 10 mM NaCl; 10 mM MgCl) containing 100 ug/ml Proteinase K and 1.0% SDS. After 4 to 16 hours incubation at 37°C with gentle rocking, sodium perchlorate was added to 1.0 M. Samples could be stored at 4° C for months or processed into DNA by extracting twice with phenol-chloroform (1:1) followed by two extractions with chloroform, then dialysis against 3 changes of a 1000 fold excess of TE (10 mM Tris-HCl, pH 7.4; 1 mM EDTA). DNA concentrations were determined by absorbence at 260 nm.

Restriction Endonuclease Digestion, Electrophoresis, and Hybridization

Five micrograms of each DNA was digested to completion with a five fold excess of Eco R1 or Taq 1 (Bethesda Research Laboratories) restriction endonuclease using conditions recommended by the manufacturer. Samples were routinely concentrated by the addition of ammonium acetate to 0.3M and 2.5 volumes of cold 95% ethanol, stored at -20°C overnight, and the DNA pelletted by a 10 minute centrifugation in a microcentrifuge (10,000 x g). After briefly drying the pellet, the DNA was redigested with a five fold enzyme excess in a final volume of 20 microliters.

Digested DNAs were size fractionated at room temperature by agarose gel electrophoresis in recirculated TAN buffer (10 mM Tris-HCl, pH 7.9; 5mM sodium acetate; 1 mM EDTA). Eco R1 digested DNA was electrophoresed in 0.4% agarose at 0.6 volts/cm for 65 hours while Taq 1 digested DNA was electrophoresed in 1.2% agarose at 1.8 volts/cm for 20 hours. The length of the agarose gels varied from 15 to 22 cm. Appropriate DNA size markers were included and DNA was visualized by ethidium bromide staining with ultraviolet illumination.

After soaking the gel in denaturation solution (0.3 M NaOH, 0.5 M NaCl) followed by neutralization solution (0.5 M Tris-HCl, pH 7.0; 0.4 M NaCl), the DNA was transferred to a nylon membrane (Zetabind, AMF Cuno). Specific cloned DNA sequences were labelled with ^{32}P by nick translation and hybridized to the the nylon filters using standard procedures (3). After washing away unhybridized probe, the filter was exposed to x-ray film (Kodak, X-omat) at -70° C in a cassette with Lightning Plus screens (DuPont) for 20 to 72 hours. Fragment sizes were determined by relative mobility to standards using a digitizing pad interfaced with a DEC PDP-11 computer.

Recombinant DNA Probes

The two DNA probes used in this study were pAW101 and

pLM0.8. The probe pAW101 (kindly supplied by R. White) consists of a 6.5 kilobase (kb) Eco R1 insert in the plasmid pBR322, derived from the D14S1 locus on chromosome 14, and is polymorphic with Eco R1 (4). The probe pLM0.8 is a 879 base pair Cla 1-Sph 1 insert in pBR322 derived from the 3' flanking region of the Harvey Ras oncogene. This locus is localized in chromosome 11 (5) and is polymorphic with Taq 1. Each probe was used as an insert, purified from the pBR322 vector.

Results and Discussion

The two probes used in this study detect RFLPs corresponding to unique sequences in the human genome that are inherited in a Mendelian fashion (4,6). With each probe, one (homozygote) or two (heterozygote) bands are seen in each individual.

Allele frequency data was collected for Caucasians, Hispanics, and Blacks from random, non-related blood donors from the New York City area. The number of alleles observed for the pLM0.8 probe hybridized with Taq 1 digested DNA was 18 with sizes ranging from 1.8 to 4.5 kb. The size of the fragments were measured to the nearest 10 base pairs. Under the standard conditions used to fractionate the DNA, the resolution of two adjacent alleles was approximately 40 base pairs in the 2.60 kb size region and 60 base pairs in the 4.0 kb region. However, an increased separation of the DNA fragments, by the use of longer gels, failed to reveal alleles varying in size by 20 or 30 base pairs. In all three racial groups, the 2.59 Kb allele predominated. Although most of the alleles were observed among the three races, the allele frequencies were different among the three groups. For the pAW101 probe hybridized with Eco R1 digested DNA we could resolve bands varying in size by 200-300 base pairs. Therefore, the D14S1 alleles could be grouped in over 30 size groups, ranging from 14.3 to 32.5 kb. In this size range the fragments were measured to the nearest 100 base pairs. For all three racial groups, the majority of fragments were between 14.3 and 17 kb, but again the frequency of the various DNA fragments varied among the groups.

The allele frequencies obtained for these two polymorphisms were used to calculate the likelihood that the allele present in both the child and the alleged father was the same.

The results of 102 paternity trios analysed for DNA RFLPs were compared with the HLA and protein determinations. DNA determinations were done in duplicate and the samples were displayed on gels with the child between the mother and alleged father flanked by size standards. With this type of layout it was easy to visualize which of the alleles of the child had been inherited from the mother and which one might correspond to the alleged father's alleles. If the child's paternal

allele alligned with one of the alleged father's alleles, he was included as a possible biological father. If on the other hand the child's paternal allele and the alleged father's alleles did not comigrate, he was excluded as an alleged biological father.

The following results were obtained. There were 29/102 (28.4%) alleged fathers excluded by DNA and also 29/102 excluded by HLA 25 of which were excluded by both HLA and DNA. The average paternity index of included alleged fathers based on the racial frequency of the observed alleles was 91.6% for DNA. This compares well with HLA A and B which has an average paternity index of about 90%. Although the estimation of paternity is subject to interpretation by the courts, a number of 90.0% is often accepted as evidence along with other proof that an alleged father is the true biological father.

There were 8 cases where there was disagreement between the DNA and HLA results. In 4 cases, the alleged father was excluded by DNA but included by HLA. In 4 cases, an alleged father was excluded by HLA but not DNA. These discrepancies point out the difficulty in relying on only a few genetic systems to establish biological paternity. In combination DNA and HLA would have excluded 33/102 (32.4%).

Of the 29 alleged fathers excluded by DNA RFLP analysis, 26/29 (89.7%) were excluded by Eco R1 and 11/29 (37.9%) were excluded by Taq 1 digestions. Thus as might be expected by the larger number of alleles, Eco R1 digested DNA hybridized with the pAW101 probe was much more informative in assignment of biological paternity. As a mater of fact, in only 2/29 (6.9%) cases were alleged fathers excluded by Taq 1 and not Eco R1 digestions. Finally, in 9/29 (31.0%) cases were alleged fathers excluded by both Eco R1 and Taq 1 digestions.

Conclusion

The assignment of paternity in legal situations is aided by biological testing of alleged fathers. The use of DNA restriction fragment length polymorphisms can help in these determinations. A comparison of the results obtained by HLA and DNA RFLP analysis indicated that DNA can help in the determination of biological paternity. Among 102 paternity trios examined by the two methods, similar numbers of alleged fathers were excluded by both methods and the paternity index calculated for both methods was comparable. These results indicate that DNA restriction length polymorphisms can add greatly to the assignment of biological paternity.

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