THE APPLICATION OF DNA-PRINT FOR THE ESTIMATION OF PATERNITY.

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Abstract

We have applied the method of DNA polymorphism analysis to resolve paternity cases. DNA samples were isolated from the blood of mother, child, and alleged father, digested with the restriction endonuclease Pst 1, size separated by agarose gel electrophoresis, and hybridized with four recombinant DNA probes which recognize hypervariable regions in the human genome. Determination of DNA fragment sizes was accomplished with a computer assisted digitizing system. Co-migration of paternally derived DNA fragments in the child and alleged father indicated possible paternity, and a paternity index (P_I) was calculated from allele frequency tables accumulated for each polymorphic locus. The cumulative power of exclusion with these four probes, for American Blacks and Caucasians, was on average 99.5% and the mean paternity index more than 3600.

Introduction

Genetically inherited differences among individuals can be visualized at the DNA level as restriction fragment length polymorphisms (RFLPs)(1,2). The most polymorphic DNA regions are those containing short tandem repeats of a short DNA sequence (3). The present report discusses the use of a combination of four such DNA probes to determine biological paternity in disputed parentage cases.

Materials and Methods

DNA Purification and Probes

High molecular weight DNA was isolated from 1 ml of peripheral blood by standard procedures (2) from samples sent to Lifecodes Corp. for paternity determinations. Five micrograms of each DNA sample was digested twice with a five-fold excess of Pst 1 (Bethesda Research Laboratories) restriction endonuclease and size separated by electrophoresis in an 0.9% agarose gel. After transfer to a nylon membrane and hybridization with ^{32}P-labelled recombinant DNA probes, the filters were washed and exposed to X-ray film (2).

Four DNA probes were used for this study: pAC061 (this probe detects the major hypervariable region of the D14S1 locus), pAC222 (clone derived from pLM0.8)(2), pAC255, and pAC256. Each probe recognizes an independent single locus. Each DNA probe was isolated as a lambda clone from a total human genomic library and following subcloning, propagated as an insert in the cloning vector Bluescribe (Promega). All probes were used as purified inserts. DNA probes were labelled using a random oligo priming reaction (4).

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Pst 1 digested DNA samples from paternity cases were loaded on agarose gels with mother, child, alleged father, and a mixture of child and alleged father flanked by size standards composed of bacteriophage lambda and phi X174 DNAs cut with various endonucleases. A total of three gels were generated for each case by two individuals. The gels were hybridized with different combinations of probes to provide duplication and allele assignment in cases where DNA probe alleles might overlap.

Paternity Determination and Calculation of the P_I.

The size of the bands detected with each DNA probe was determined by comparing the relative mobility of the restriction fragments to that of DNA size standards. To ensure accuracy in size determination a fitting procedure described by Elder and Southern (5) was incorporated into a computer program that determines the best fit for the standards.

A database of allele frequencies, for American Blacks and Caucasians, has been established with each of the four DNA probes and used in the P_I calculations. The frequency of a particular DNA band size, co-migrating between father and child, was derived from these databases, taking into account the standard deviation in band size determination and resolution of the gel (manuscript in preparation).

Results and Discussion

The average amount of high molecular weight DNA isolated from blood samples was about 50 micrograms. Each sample was processed by two individuals independently. After digestion, each sample was ethanol precipitated to concentrate the sample and redigested. Approximately 0.5 ug of each digestion was evaluated for completeness of digestion by electrophoresis. The diagnostic gels were prepared for maximal resolution in the size range from 2.0 kb to 20.0 kb. After transfer to a nylon membrane, the filters were hybridized with combinations of two probes which recognize highly polymorphic loci (Table 1). The size range of the bands generated with pAC255 and pAC256 did not overlap and they could be unambiguously assigned to their respective locus (Fig 1A). Probe pAC255 hybridized to DNA fragments within the size range of 7.0 kb to 25.0 kb while the probe pAC256 annealed to fragments within the range of 2.0 to 5.0 kb. The alleles recognized with pAC061 and pAC222 ranged in size from 3.0 kb to 25.0 kb and 1.6 to 5.0 kb respectively (Fig 1B). In the example presented in this figure the P_I was 4376 and the probability of paternity (W) 99.98%. To resolve potential overlap for this last combination of probes and as a duplication of sample processing, a third blot was hybridized to pAC061 and pAC255 (results not shown). The analysis of the results of these three blots allows the unambiguous assignment of the alleles corresponding to each polymorphic locus. This system also allows the duplication of testing by having duplicate results on probes pAC061 and pAC255.

Table 1. Probes used for the analysis of Pst 1 digested DNA: number of alleles and median P_I.

Probe	Number of Alleles	Median P_I
pAC061	>80	13.14
pAC222	15	2.24
pAC255	>80	13.39
pAC256	>30	9.23
	Combined P_I: 3638	

With a combination of probes such as the one listed above the median P_I was in 50% of cases, greater than 3600. This corresponds to a probability of paternity (W) greater than 99.97%, using a 0.5 prior probability. In all paternity cases where the 4 DNA probes included the alleged father, the P_I was greater than 100.

An exclusion was indicated when the child contained DNA bands not present in the alleged father. This was confirmed by fractionating in a single lane an equal mixture of DNA from the child and the alleged father. Two bands were considered of different size if they did not co-migrate in this mixture lane. All cases of exclusion, of an alleged father, were observed with at least 2 of the 4 probes used for testing. The highest P_I obtained in cases of exclusion, with the probe(s) that did not exclude was 49.3. The exclusion probability, or the efficiency of the system in eliminating falsely accused fathers was calculated for each probe using the allele frequency database and the formula described for RFLPs by Ito et al. (6)(Table 2). Therefore the analysis of paternity cases with these 4 probes is expected to exclude approximately 99.5% of falsely accused males.

Table 2. Average power of exclusion with four hypervariable loci.

	DNA probes				
	pAC061	pAC222	pAC255	pAC256	Total
Ethnicity					
Blacks	0.77	0.56	0.83	0.81	0.9967
Caucasians	0.58	0.44	0.88	0.79	0.9940

In conclusion, the use of DNA probes that detect highly polymorphic loci is an extremely powerful system for the analysis of cases of disputed paternity.

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Figure Legends

Figure 1. Example of DNA-Print for a paternity inclusion.

DNA digested with Pst 1 was hybridized with 4 DNA probes recognizing hypervariable loci. Lane 1, DNA from the mother. Lane 2, DNA from the child. Lane 3, DNA from the alleged father. Lane 4, Mixture of DNA from the child and alleged father. (A). Pattern with probes pAC255 and pAC256. (B). Pattern with probes pAC061 and pAC222.

Figure 1.