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Use of Deoxyribonucleic Acid (DNA) Fingerprints for Identity Determination: Comparison with Traditional Paternity Testing Methods—Part I

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ABSTRACT: A study involving comparison of the deoxyribonucleic acid (DNA) fingerprinting test with traditional methods used for paternity testing is presented. Samples from 191 cases were tested for a series of blood group and polymorphic protein markers. DNA "fingerprints" were then obtained for all samples using the multilocus probes 33.6 and 33.15. The results of DNA fingerprinting correlated well with those of traditional methods and proved to be informative in cases where traditional methods yielded inconclusive or insufficient results.

KEYWORDS: pathology and biology, deoxyribonucleic acid (DNA), paternity, genetic testing

The genetic marker systems traditionally used in disputed parentage testing include red cell antigens, red cell enzymes, serum proteins, and, more recently, human lymphocyte antigens (HLA), [*1*]. These markers can serve to exclude a falsely accused individual or yield a probability that the individual in question is the biological parent. Recent advances in recombinant deoxyribonucleic acid (DNA) technology have provided forensic serologists with alternatives to traditional testing [*2*]. The use of DNA probes has made it possible to establish identity with the same certainty as that possible for a classical fingerprint [*3–5*]. This report describes a comparative study which examines the reliability and usefulness of DNA fingerprinting in the determination of paternity.

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Material and Methods

Preliminary Testing

Blood samples from 191 mother/child/alleged-father trios were analyzed using electrophoretic and red cell agglutination methods to detect genetic markers in six red cell antigen systems, four red cell enzyme systems, and seven serum protein systems [*6*]. The presence in the child of an allele which was absent in the mother and in the alleged father constituted an exclusion of paternity. A paternity index (PI) value was calculated in cases where no exclusion was evident. In simple terms, the PI value is the ratio of the probability that the alleged father is the true biological father versus the probability that a random male fathered the child. The PI value is based on the frequency with which the obligatory paternal alleles could be produced in a single gamete in the alleged father in comparison with that frequency in a single gamete of a random man of the same racial population [*7*].

DNA Methodology

DNA was extracted from the white blood cells in the sample [*8*] and was digested using Hinf I restriction endonuclease (New England Biolabs, Beverly, Massachusetts). Duplicate samples of the restricted DNA were electrophoresed on separate agarose gels for approximately 20 h [*3–5*]. The lambda Hind III marker (Bethesda Research Laboratories, Bethesda, Maryland) was used to monitor migration. The double-stranded DNA was then denatured and transferred by a Southern blot [*9*] technique to a nylon membrane (Hybond, Amersham, Arlington Heights, Illinois). The probe preparation and hybridization conditions were as previously described [*3–5*]. Following hybridization with phosphorus-32 (^{32}P) radioactively labeled *d*-guanosine triphosphate (*d*GTP) (New England Nuclear/DuPont, Boston, Massachusetts) 33.6 and 33.15 multilocus DNA probes [*3*], the membrane was placed in contact with X-ray film for a sufficient period of time to allow development of an autoradiograph (Lightning Screens, DuPont, Boston, Massachusetts).

Interpretation of Autoradiographs

Using both 33.6 and 33.15 multilocus probes yields two sets of DNA fingerprints, consisting of approximately 15 to 20 resolvable bands in the 3 to 12-kilobase (kb) and 3 to 25-kb molecular-weight ranges, respectively. The samples are loaded on gels so that the child's DNA pattern is between those of the mother and the alleged father [*3–5*]. Since half of the child's DNA comes from each parent, all nonmaternal bands present in the child (with the exception of a mutation) must be derived from the biological father [*3–5,10,11*]. The probability of an individual, other than the biological father, sharing all nonmaternal bands with the child can be calculated by x^n, where x is the frequency of band sharing between unrelated individuals [*3,5*], and n is the number of shared child/alleged-father bands.

Results

Autoradiographs obtained for two mother/child/alleged-father trios are shown in Fig. 1. All of the bands in the child's DNA fingerprint in Fig. 1*a* are matched by bands in the mother's and alleged father's fingerprints, indicating that the alleged father is the father of the child. In Fig. 1*b*, however, there are clearly a number of nonmaternal bands in the child's DNA fingerprint which are not matched by bands in the alleged father's fingerprint, indicating nonpaternity.

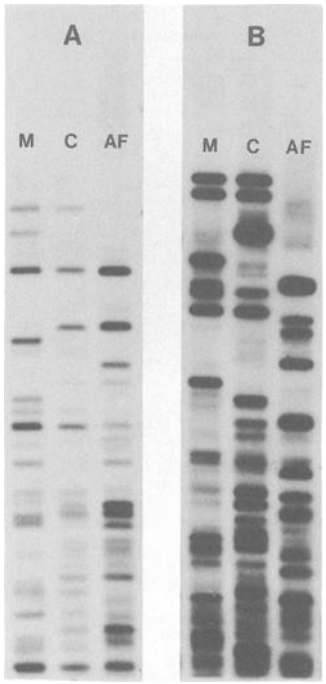

FIG. 1—*DNA fingerprints using probe 33.15, showing two mother* (M), *child* (C), *and alleged-father* (AF) *trios. In Panel* A *the alleged father is the father of the child, and in Panel* B *the alleged father is excluded from being the father.*

The results of the 191 cases tested for red cell antigens and polymorphic protein markers were compared with results for the same cases tested by DNA fingerprinting, as shown in Table 1. In 49 of the cases, paternity was excluded by red cell and protein phenotyping. In all of these 49 cases, DNA fingerprinting established nonpaternity. The average number of unshared (not derived from the mother or the alleged father) bands in these cases was 11. In 139 cases, the PI values obtained by red cell and protein phenotyping ranged from 3 to 1.3×10^6. The authors concluded in each of these cases that there is a high probability that the alleged father is the true father. DNA fingerprinting established paternity in the same 139 cases. The average number of shared child/alleged-father bands was 16. For the purpose of this study, a conservative band-share frequency of 0.25 was used [*3*].[7] With 16 shared bands and no mutations, the probability of an individual other than the true biological father sharing all bands is 0.25^{16}, or 1 in 4 billion. The number of unassigned child's bands present in these cases ranged from 0 to 2, which is consistent with the reported mutation rates seen with the 33.6 and 33.15 probes [*5,12*].

In three cases, red cell and protein phenotyping resulted in a single indirect exclusion with PI values ranging from 0.75 to 10. Indirect exclusions result when a child does not inherit a genetic marker that should have been received from a parent who is believed to be homozygous for that marker [*13*]. Often an indirect exclusion occurs because that

[7]Smith, J. C., et al., "Highly Polymorphic Minisatellite DNA Probes: Further Evaluation for Individual Indentification and Paternity Testing," *Journal of the Forensic Science Society*, Vol. 30, No. 1, 1990, pp. 3–18.

TABLE 1—*Comparison of results from traditional methods for paternity testing and DNA fingerprinting, by number of cases.*

	Red Cell and Protein Phenotypes	DNA Fingerprinting
PI value		
<9	6	NA
9 to 19	1	
19 to 95	34	
>95	98	
Total	139	
Paternity established		139
Paternity excluded	49	52
Inconclusive (indirect exclusion)	3	0

parent possesses a rare (or silent) allele that can only be detected by a specialized reagent, which frequently is unavailable [*13*]. Because of the significant possibility of error with a single indirect exclusion, most laboratories like to find exclusion in at least two different genetic marker systems before excluding parentage [*14,15*]. In the three cases mentioned, the DNA fingerprinting test definitively excluded paternity. The average number of unassigned paternal specific bands in these cases was 13.

Conclusions

These results demonstrate that the DNA fingerprinting test for paternity establishment compares favorably with red cell and protein phenotyping. Moreover, the uniqueness of DNA fingerprints can prove to be advantageous in cases where traditional methods provide insufficient (a PI value of 3 corresponds to only a 75% probability of paternity) or inconclusive results. Because of the discrimination power of the multilocus probes (33.6 and 33.15), theoretically everyone but the biological father will be excluded from paternity [*3,5*]. Therefore, the DNA fingerprinting test obviates the need to calculate a probability of paternity value by the traditional Bayesian methods. This single technique (using two probes) can more effectively prove paternal identity than the several individual genetic tests employed in this study. This investigation and others [*3–5,16*] demonstrate that DNA probe methodology is a powerful tool for determination of paternity in disputed cases. The test should also be equally useful in other forensic science cases involving paternity analysis, such as infanticide, incest, and rape cases involving impregnation of the victim.

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