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

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ABSTRACT: Six red blood cell (RBC) antigen systems, coupled with human lymphocyte antigen (HLA) phenotyping, were used to establish paternity on 28 mother/child/alleged-father trios. Samples were subsequently examined using the deoxyribonucleic acid (DNA) fingerprinting test with the multilocus Jeffreys DNA probes 33.6 and 33.15. In 27 of 28 paternity cases, the DNA fingerprinting test results supported and enhanced the results of RBC and HLA typing by resolving disputed paternity cases conclusively. One discrepancy between conventional serological methods and DNA analysis is discussed.

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

Testing for multiple polymorphic genetic marker systems, such as red blood cell (RBC) antigens and human lymphocyte antigens (HLA), has been used traditionally for paternity establishment [*1–7*]. The combined use of RBC and HLA typing results has a mean exclusion capability (that is, the probability of excluding a non-father from paternity prior to testing) of approximately 91 to 97% [*8*]. Although results from conventional testing may exclude an alleged father, these tests can only provide a statistical estimate of paternal inclusion.

Deoxyribonucleic acid (DNA) fingerprinting analysis, using restriction endonucleases and two multilocus DNA probes developed by Jeffreys et al. [*9–11*] and Gill et al. [*12*], permits direct examination of human DNA in the form of an individual-specific band

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pattern that is genetically determined. The power of these probes to detect multiple hypervariable regions within an individual's DNA allows the paternity tester to exclude non-fathers (except for identical twins) for a given paternity case trio. Below we describe a comparative study between traditional serological methodologies and the DNA fingerprinting test to evaluate the application of DNA analysis to paternity determinations.

Materials and Methods

Antisera Testing

Blood samples from 28 mother/child/alleged-father paternity-case trios were collected and analyzed using six RBC systems and HLA at the Blood Center of Southeastern Wisconsin, Milwaukee, Wisconsin. Red cell agglutination tests for the ABO, Rh, MNSs, Kell, Duffy, and Kidd blood groups were performed using standard serological methods [*13*]. Serologic typing of 16 A-locus and 31 B-locus HLA antigens was performed using the standard microlymphocytotoxicity test [*14*] with commercially available antisera.

In each case in which an alleged father could not be directly excluded, a probability of paternity value was determined according to published methods [*15*]. Briefly, the frequency with which the alleged father could produce a gamete with all the obligatory paternal alleles is compared with the frequency with which those alleles could be expected to occur in a single gamete of a random male of the same race. This value is commonly expressed as a percentage, using a modification of Bayes theorem [*16*].

DNA Analysis

The DNA fingerprinting test for all paternity cases was performed at Cellmark Diagnostics, the authors' company, essentially as described by Jeffreys et al. [*9,10*]. DNA was extracted from 700-μL aliquots of whole blood with phenol/chloroform, after which it was precipitated using ethanol. An amount ranging from 1 to 10 μg of DNA was digested with restriction endonuclease HinfI (New England Biolabs, Beverly, Massachusetts). The resulting fragments of DNA were fractionated by size, using agarose gel electrophoresis on two separate gels. The DNA fragments were transferred by Southern blotting [*17*] to nylon membranes (Hybond, Amersham, Arlington Heights, Illinois). The DNA probes were prepared from the human minisatellites 33.6 and 33.15 [*9*] and were labeled with phosphorus-32 (^{32}P) *d*-guanosine triphosphate (*d*GTP) (New England Nuclear/DuPont, Boston, Massachusetts) using a specific primer by a modification of the method of Feinberg and Vogelstein [*18*]. The hybridization conditions were a modification of the conditions used by Jeffreys et al. [*9,10*]. The DNA band patterns were made visible by autoradiography (Lightning Screens, DuPont, Boston, Massachusetts).

DNA Results Analysis

The DNA test results were analyzed by simultaneously comparing the DNA banding patterns from the mother, child, and alleged father on each autoradiograph (Fig. 1). For probe 33.6, bands in the molecular weight (MW) range of 3 to 12 kilobases (kb) were scored. For probe 33.15, all bands with a MW between 3 and 25 kb were scored.

The maternal bands in the child's DNA fingerprint were identified. The remaining bands in the child must have come from the biological father. The analysis then determined whether or not the alleged father (AF) shared the paternal bands in the child's band pattern. When sharing was observed between the child's paternal bands and bands from the alleged father's DNA pattern, paternity was established. If the alleged father did not share the vast majority of the child's paternal bands, he was excluded as the

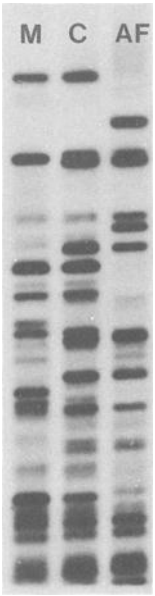

FIG. 1—*The DNA banding patterns from this mother* (M), *child* (C) *and alleged father* (AF) *trio were produced using probe 33.15. In this example, all nonmaternal bands seen in the child are found in the father.*

biological father. A small number of unassigned bands (matching neither the mother nor the father) between a child and the true biological father was observed, as was expected in some cases because of mutation in these hypervariable regions [*9,19,20*].

The DNA banding patterns resulting from each multilocus probe were independently analyzed and served as a check on each other. The chance of an unrelated individual sharing the same number of bands with the child as the true father has been determined to be 0.25^n, where the random band-sharing frequency is equal to 0.25, and n equals the number of paternal bands shared with the alleged father [*9,10,21*]. The calculations also take into account the probability of an unassigned band occurring as a result of mutation, based on mutation rates determined by Jeffreys and co-workers [*9,19,20*].

Results

As shown in Table 1, of the 28 cases tested, the combined red cell antigen and HLA analyses could not exclude paternity in 19 cases. In these cases, a probability of paternity value was calculated [*15*]. These probabilities ranged from 94.0 to 99.9%, assuming a 0.5 prior probability. In 4 of the 19 (21%) cases with inclusions, additional testing for red cell enzyme and serum protein markers was needed to reach a probability of paternity value greater than or equal to 95%. DNA fingerprinting analysis established paternity conclusively in the same 19 cases. Using both DNA probes 33.6 and 33.15, the number of bands that were shared between a child and an alleged father to whom paternity had been assigned ranged from 12 to 29, with a mean value of 20 (Table 2). Statistically, the probability of a child sharing 20 bands with an unrelated man (in the absence of any mutant bands) is 0.25^{20} or approximately 1 in 1 trillion [*9,21*]. The same probability calculated for the case of 20 shared bands and 1 mismatched mutant band is 1 in 6.8×10^{10}.

TABLE 1—*Summary of paternity testing results.*

Result	Number of Cases
Paternity excluded	
Total cases excluded	
DNA fingerprinting	8
HLA typing	8
RBC typing	8
Rh	3
Rh + MNSs	1
Nonexclusion/paternity established	
Total cases established	19
DNA fingerprinting	19
HLA and RBC typing	19
Exclusion by HLA/paternity established by DNA fingerprinting[a]	1
Total cases	28

[a]See text for an explanation of this discrepancy.

TABLE 2—*Summary of DNA fingerprinting test results.*

	Number of Bands Shared				
	Child's Bands[a]	M/C[b]	AF/C[c]	AF/M/C[d]	Unidentified Paternal-Specific Bands[e]
		EXCLUSION DATA			
Average (8 total cases)	41	18	8	5	10
		INCLUSION DATA			
Average (19 total cases)	47	19	20	8	<1

[a]The average number of bands scored in a child's DNA fingerprint using DNA probes 33.6 and 33.15. All the averages are based on the number of cases listed.
[b]The average number of bands shared exclusively between the mother and child.
[c]The average number of bands shared exclusively between the alleged father and the child.
[d]The average number of bands shared simultaneously by the alleged father, mother, and child.
[e]The total number of unassigned paternal-specific bands.

Combined red cell antigen and HLA analysis excluded paternity in 8 of the 28 cases. Direct exclusions were observed in the HLA system for all 8 cases. In 3 of the cases, exclusions were also observed in one or two red cell antigen systems. DNA analysis excluded paternity in the same 8 cases. The number of bands in the child's DNA fingerprint pattern that could not be assigned to either the mother or the alleged father ranged from 5 to 17, with a mean value of 10 (Table 2).

In one case, combined red cell antigen and HLA analysis resulted in an exclusion, whereas paternity was established for this same case by the DNA fingerprinting test. An investigation indicated that a misidentification had occurred in the transfer of samples between the two laboratories (that is, the two laboratories had examined different samples). The only way to resolve the discrepancy in this case would have been to repeat testing. However, the lack of remaining samples precluded resolution of this discrepancy by repeat testing.

Discussion

Paternity testing using multiple genetic marker systems is well recognized in the medical and legal communities. The use of RBC and HLA typing combined can provide an average exclusion capability of approximately 91 to 97% [*1–8*]. Although such results are completely informative for exclusion, traditional serological testing is subject to limitations in cases where an alleged father cannot be directly excluded. The fact that a chance still exists for a random male in the population to possess the same marker phenotype as the true biological father means that it has not been possible to prove paternity positively, regardless of the number of genetic markers examined. Chimera et al. [*22*] note that a significant number of cases exist in which the probability of paternity remains low even after extensive testing, and that the availability of sufficient polymorphic markers to resolve these ambiguous cases has become problematic.

The usefulness of the multilocus probes 33.6 and 33.15 for establishing paternity lies in their ability to detect simultaneously many highly variable genetic loci in human DNA [*9–12*]. This discriminatory power, coupled with the fact that the DNA fragments detected by these probes exhibit stable Mendelian inheritance patterns [*9–11,20,23*], permits the exclusion of non-fathers (except identical twins) for a given paternity trio [*9–10*].

The purpose of the present study was to compare paternity test results from conventional methods, such as red cell antigen and HLA typing, with results obtained from DNA fingerprinting analysis on 28 cases of disputed paternity. In 27 of the 28 cases, the DNA fingerprinting test supported the paternity test results obtained by traditional blood group phenotyping. In 8 of the 28 cases, the alleged fathers were excluded by both conventional testing and DNA fingerprinting. In 19 cases, the alleged fathers were not excluded by conventional methods and a probability of paternity was calculated using standard Bayesian techniques [*15*]. Although the results from DNA fingerprinting analysis concurred with these findings, classical probability of paternity values are not necessary when using the DNA fingerprinting technique. Since the standard methods have been criticized both statistically and legally [*24–28*], the ability of the DNA fingerprinting test to eliminate the reliance on these methods (particularly the prior probability assumption) is one advantage of this new test.

Aside from its specificity, the DNA fingerprinting test has a number of other advantages. The DNA fingerprinting test provides more exclusion power than the six red cell antigen and the human lymphocyte antigen tests combined. In addition, DNA results may be obtained from very small samples. The DNA molecule is very stable and can often be tested from a sample that is months—or even years—old [*12,23,29,30*]. If necessary, several analyses could be done from a single tube of blood. The small sample requirements of the DNA test also facilitate paternity establishment in newborns, as sufficient blood samples for testing can be obtained by a simple heel stick.

The ability to conclude that paternity is established [*9–11*], without having to calculate the standard probability of paternity value, means that supplemental genetic testing will not have to be performed, as it is when inclusionary statistics fall below judicial standards. This occurred in 4 of the 28 cases (4 out of 19 inclusions) in this study. The results indicate that DNA fingerprinting using DNA probes 33.6 and 33.15 is as reliable as currently accepted serological paternity tests and has been able to resolve disputed paternity cases. These studies demonstrate that DNA fingerprinting is a powerful and highly valuable tool in parentage testing.

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