A Comparative Paternity Study: DNA vs Traditional Testing

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INTRODUCTION

We have analyzed 145 paternity trios using both traditional serology (six red cell systems and the HLA system), and RFLP analysis with three single-locus VNTR probes -- D14S13, D2S44, and D17S79 (Lifecodes Corporation). This study was done to evaluate the benefit of using DNA testing in association with our traditional battery of tests. Specifically, we wished to (1) determine if DNA testing corroborated exclusions found by traditional testing (2) determine whether additional exclusions would be found by DNA analysis (3) evaluate the strength of the genetic evidence provided by DNA testing in cases where no exclusion was found. The results of this study indicate that DNA analysis is a very useful additional system for distinguishing non-excluded non-fathers from "true" fathers, and for providing convincing evidence in favor of paternity in cases of non-exclusion.

MATERIALS AND METHODS

Blood samples from paternity trios were typed for the following blood group systems: ABO (A,B,O); Rh (D,C,c,E,e); MNSs (M,N,S,s); Kell (K,k); Duffy (Fy^a, Fy^b); Kidd (Jk^a,Jk^b) and HLA (16 A-locus, 31 B-locus, and 8 C-locus antigens). All serological assays were performed in duplicate, according to standard protocols.

DNA analysis was performed using the LifePrint^{TM} system (Lifecodes Corporation, Valhalla, NY). DNA isolated from peripheral blood was digested with the restriction enzyme PstI. Approximately one microgram of the restricted DNA was electrophoresed through a 0.9% agarose gel and transferred to nylon membranes. Following overnight hybridization with ^{32}P-labelled probes (D2S44, D14S13, and/or D17S79) the membranes were washed and exposed to X-ray film. The resulting autoradiograms were analyzed using a digitizing pad (Scriptel Corporation, Columbus, Ohio) and software developed by Lifecodes Corp. to determine allele sizes.

The Paternity Index (PI) and Probability of Paternity (W) were calculated according to standard methods (Walker, 1983). A conservative method of determining the Y value was used, in which all allele frequencies within 3 standard deviations (± 1.8%) from the measured size of the obligate gene were summed.

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr

RESULTS AND DISCUSSION

Table 1 presents the results obtained when DNA analysis was used in addition to our standard battery of tests. All direct exclusions obtained by standard testing were confirmed by DNA analysis (exclusion by at least 2 probes). Out of the 133 cases in which the alleged father was not excluded by traditional testing, 8 exclusions were found by DNA testing. Four of these DNA exclusions had one or more indirect red cell exclusions (Table 2). Of 33 cases involving non-excluded men with an initial PI of less than 20, 15% were excluded by DNA analysis. DNA exclusions were also found in 3 of the 48 cases with an initial PI of 20-49. No exclusions were observed in any case with an initial PI of greater than 50, supporting the contention that most alleged fathers having Paternity Indices of 50 or more are probably true fathers.

TABLE 1: A COMPARISON OF TRADITIONAL TESTING AND DNA TESTING IN 145 PATERNITY CASES

INITIAL PI	INITIAL W	# CASES	# EXCLUSIONS WITH DNA	% EXCLUSION WITH DNA
0	0	12	12	100%
0-1	0-50%	6	3	50%
1-9	50-90%	11	2	18%
10-19	90-95%	16	0	0%
20-49	95-98%	48	3	6%
50-99	98-99%	37	0	0%
99 or >	99% or >	15	0	0%
		145	20	13.8%

TABLE 2: EIGHT CASES IN WHICH ALLEGED FATHERS WERE EXCLUDED BY SUBSEQUENT DNA TESTING

RACE	INITIAL PI	INITIAL W	INDIRECT RBC EX.	# OF PROBES EX. THE AF
C	<0.01		KIDD & MNSs	2
C	0.07		Rh	2
B	0.08		Rh	3
C	1.7	62.96%		3
C	6	85.71%	Duffy	2
C	26	96.30%		2
B	28	96.55%		3
B	48	97.96%		2

The Power of Exclusion (PE) for the three probes used in this study have been calculated using the formula described by Ito et al. (1985) and reported elsewhere as 99.8% for Caucasians and Blacks (Balazs et al., 1989). We have empirically determined the Power of Exclusion of these probes by creating novel "trios" composed of the mother and child from one paternity case and an alleged father from a different, unrelated case. Since we assume that the new "alleged father" is not the biological father of the child, this analysis provides a measure of the frequency with which each probe would exclude a falsely accused man. We considered that the "alleged father" was excluded if his allele(s) differed from the obligate band by more than 2%. Using this approach, D14S13, D2S44, and D17S79 were found to have a combined Power of Exclusion of approximately 98% in Caucasians and 99% in Blacks.

These observed values are lower than those calculated using the Ito formula, which uses allele frequencies to derive the Power of Exclusion. Nevertheless, these probes have a high combined Power of Exclusion, and thus provide a very powerful tool for paternity determination.

In addition to increasing the Power of Exclusion of our battery of tests, DNA analysis provided very useful evidence in support of paternity in those cases where no exclusion was found. The median Paternity Index observed in our laboratory was 145 for Caucasians and 404 for Blacks, when all three probes were used. These values are 10 to 12 fold lower than those previously reported (Balazs et al., op cit) reflecting the fact that we have used a more conservative means of calculating the PI than that used by these authors. Even so, our data indicate that DNA typing using these three probes can provide strong evidence in support of paternity when no exclusion is found.

CONCLUSION

We have examined the effect of using DNA analysis in addition to the standard battery of genetic tests currently used in our laboratory for paternity testing. We find that the data obtained from DNA typing are generally in agreement with the results of traditional testing, and that all exclusions found with our standard tests were confirmed by DNA analysis. In addition, 9.9% of men included by red cell and HLA testing with PIs of less than 50 were excluded by DNA analysis, reflecting the high combined power of exclusion of the three probes used in this study. In those cases where no exclusion was found with any of the tests used, DNA typing provided strong additional genetic evidence in support of paternity. Thus, we conclude that the use of these three probes in addition to standard testing provided a powerful means of separating "true" fathers from falsely accused men.

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