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Paternity Testing by Using Erythrocyte Enzyme Esterase D

The recently discovered erythrocyte enzyme system esterase D (EsD) [*1*] has been found to be a reliable genetic marker in population studies. It is presumed to be transmitted as an autosomal codominant allele with two common variants, EsD^1 and EsD^2 [*1*], and one rare variant EsD^3 [*2,3*]. Gene frequencies for the EsD^1 allele ranged from 0.649 to 0.945 in the twelve populations reviewed by Welch and Lee [*4*].

The potential usefulness of EsD in forensic medicine has been suggested by Welch [*5*]. In March 1975 we added phenotyping of EsD to the genetic markers we routinely test in cases of disputed paternity. The following report describes the results of EsD testing in 206 paternity cases.

Materials and Methods

Paternity cases used in the study were limited to Caucasians residing in the state of Minnesota. Specimens were tested for the erythrocyte antigens A-B-O, Rh, M-N-S-s, and K; the serum proteins group-specific component (Gc), transferrin (Tf), haptoglobin (Hp), ceruloplasmin (Cp), gamma globulins Gm (a, z, x, f; g, b^1, b^5, c^3, c^5, s, t) and Km(1); and the erythrocyte enzymes adenylate kinase (AK), adenosine deaminase (ADA), 6-phosphogluconate dehydrogenase (6-PGD), acid phosphatase (acP), phosphoglucomutase (PGM_1), and esterase D (EsD).

Erythrocytes used for enzyme phenotyping were washed three times in physiological saline, diluted 1:1 in distilled water, mixed on a vortex, and frozen at −20°C. Clear hemolysates were obtained by spinning specimens at high speed to eliminate stroma. Erythrocyte enzymes ADA, acP, and EsD were stabilized by adding 2-mercaptoethanol (2-ME) to specimens not tested within 5 days. One drop of 0.1% 2-ME was added to three drops of hemolysate and incubated for 30 min at room temperature immediately before electrophoresis.

Electrophoresis on 6-mm starch slabs (12 g/100 ml) and staining (4-methylumbelliferyl acetate) of EsD was performed with a pH 5.9 citrate phosphate buffer system [*1*]. Esterase D and acP were phenotyped on the same gel. A 1:60 dilution of the chamber buffer instead of a 1:100 dilution permitted simultaneous electrophoresis of AK, ADA, 6PGD, acP, and EsD.

Results

Phenotyping of 506 Minnesota Caucasians (Table 1) enabled the gene frequencies for

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TABLE 1—*Esterase D phenotypes.*

	Observed		Expected	
Type	no.	%	no.	%
1-1	422	83.40	420.03	83.01
2-1	78	15.41	81.97	16.20
2-2	6	1.19	4.00	0.79
Total	506	100.00	506.00	100.00

the EsD^1 and EsD^2 alleles to be determined. The frequency for EsD^1 was 0.9111 and for EsD^2, 0.0889. Statistical analysis for the EsD^1 allele gave a chi-square result of 1.2015 and a probability value of $0.30>P>0.20$. The Minnesota Caucasian population demonstrated gene frequencies similar to those reported in Western Europeans [*1,2,4-6*]. Mendelian inheritance of EsD was further documented by means of mother-child phenotypic combinations (Table 2).

Thirty-nine of 206 alleged fathers were excluded by the battery of tests used (Table 3). Although there were no cases in which the alleged father was excluded only by means of EsD, 5 of 39 observed exclusions were corroborated by EsD exclusions. The exclusion probability P for EsD is $P = 0.0744$, which when combined with the P values of the other genetic systems in this population yielded a cumulative P of 0.8914.

Discussion

A battery of tests using 16 genetic marker systems was found to exclude 39 alleged fathers in 206 paternity cases, 5 by means of EsD phenotyping. The observed exclusions were lower than in the study conducted by Welch [*5*], which included 10 EsD exclusions among the 50 of 156 alleged fathers who were excluded. The cumulative exclusion probabilities of the two studies did not differ significantly; therefore, the variance in observed exclusion rates probably represents differences in nongenetic factors such as legal and social pressures affecting who is charged with paternity and why a suit is being filed.

One factor we found to significantly alter the exclusion rate was documented in our earlier study [*7*], which demonstrated a fourfold increase in exclusions of husbands compared to nonhusbands. In the present study we observed only a twofold increase, with fewer husbands constituting the paternity population. If these factors do in fact affect the number of observed exclusions it would help explain why the exclusion rate of the present study is no greater than our earlier study in spite of a 6% increase in the cumulative exclusion probability with the addition of K, Gm, Km, and EsD.

Although family and population studies have established the genetic transmittance of EsD, and all the exclusions in this study were direct, exclusions based upon a single "indirect" exclusion of EsD should be regarded with caution. In contrast to such genetic systems as acP [*8*], GPT [*9*], and ADA [*10*], genetic amorphs and methods for biochemically quantitating EsD have not been documented.

Esterase D appears to be a reliable exclusion determinant which meets the criteria required for paternity testing: it is polymorphic, follows Mendelian inheritance patterns, is stable upon storage, and the methods produce clear and unambiguous differentiation of the known genetic variants. Added time and expense in phenotyping for EsD is negligible because of the ability to simultaneously electrophorese EsD with other useful systems.

TABLE 2—*Mother/child combinations of EsD polymorphism.*

	Children Type 1-1		Children Type 2-1		Children Type 2-2		Children, Total	
Mother's Type	Observed	Expected	Observed	Expected	Observed	Expected	Observed	Expected
1-1	179	174.71	20	17.05	0	...	199	191.76
2-1	17	17.05	13	18.71	1	1.66	31	37.42
2-2	0	...	1	1.66	0	0.16	1	1.82
Total	196	191.76	34	37.42	1	1.82	231	231.00

TABLE 3—*Exclusion probabilities* P *and distribution of exclusions observed among 39 falsely accused males.*

System	Independent System Exclusions				Individuals Excluded
	P Value	Direct[a]	Indirect[b]	Total	
M-N-S-s	0.3112	9	5	14	...
Rh	0.2767	15	2	17	...
A-B-O	0.1537	10	0	10	29
Kell	0.0430	1	0	1	...
Gm	0.2201	3	4	7	...
Hp	0.1845	0	2	2	...
Gc	0.1618	1	0	1	...
Km	0.0613	2	1	3	11
Cp	0.0123	1	0	1	...
Tf	0.0018	0	0	0	...
acP	0.2231	4	1	5	...
PGM	0.1541	3	2	5	...
EsD	0.0744	5	0	5	...
ADA	0.0516	2	1	3	16
AK	0.0429	0	1	1	...
6PGD	0.0146	1	0	1	...
Total	0.8916	57	19	76	39

[a]Class 1 or direct exclusion is defined as the presence in the child of a marker absent in both putative parents.

[b]Class 2 or indirect exclusion is defined as the absence of an expected characteristic in the child when the putative father is presumed homozygous.

Summary

A total of 206 paternity cases were tested for the erythrocyte enzyme esterase D (EsD) along with the 15 genetic marker systems routinely phenotyped. Of 39 observed exclusions of falsely accused males, 5 were exonerated by EsD. Reliability and ease of testing indicates that EsD is a useful exclusion determinant.

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