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Chakraborty Report on DNA Typing Involving Richard Eberling, Sam Sheppard, and Marilyn Sheppard.

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REPORT ON THE DNA TYPING WORK DONE IN THE CASE INVOLVING RICHARD EBERLING, SAM SHEPPARD, AND MARILYN SHEPPARD

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Background:

1). I received the following materials from Attorney Terry Gilbert and Indianapolis-Marion County Forensic Services Agency, requesting me to offer an opinion on the statistical strength of the DNA evidence gathered in the context of the above-mentioned case:

(i) Reports on the DNA analyses (Polymarkers and HLA-DQA1 analysis) from the Indianapolis-Marion County Forensic Services Agency (dated February 3, 1997 and April 21, 1999);

(ii) Xerox copies of PCR result sheets from the same laboratory; and

(iii) A report of Dr. Mitchell Holland (dated October 15, 1999).

2). Through my prior reviews of work of the Indianapolis-Marion County Forensic Services Agency (hereafter called FSA), I am also familiar with their Standard Operating Procedure (SOP) for DNA analysis (including PCR analyses), and further, I have familiarity of DNA typing population databases generated that are relevant for the loci typed in this case.

3). Upon review of these materials (mentioned above), I also performed my own independent statistical analyses to determine the statistical weight of the conclusions reached by the Laboratory in their reports on this case. Further, these analyses also allow me to examine the scientific validity of some of the statements made by Dr. Holland in his report dated 15 October 1999.

4). Thus, the comments made in the following paragraphs are based on review, examination and (statistical) analyses of the materials mentioned above, on which I applied my general experience and expertise in this field of research (see my publication list for foundations of the later).

Conclusions:

5). The PCR techniques of DNA typing (for polymarkers and HLA-DQA1) consist of scientifically valid and generally accepted methods and these have been correctly employed in this case.

6). The conclusions reached in this case are based on scientifically valid and generally accepted methods.

7). The summary results of genetic typing at the HLA-DQA1 and polymarker (LDLR, GYPA, HBGG, D7S8 and GC) loci, conducted by the FSA laboratory, may be represented by the following table (Table 1).

Table 1. DNA Profiles of the relevant items tested

Locus	Item/Source							
	Richard Eberling	Sam Sheppard	Marilyn Sheppard	Stain closet	Wood chip	Trouser blood	Vag. sp.	Swab frac.
				#1-C	#3	#b-3-b-1	A-59-1	A-59-2
HLA-DQA1	-	-	1.1	1.1	1.1	-	1.1	-
	-	1.2	-	(1.2)	(1.2)	1.2	(1.2)	1.2
	-	1.3	1.3	1.3	-	-	1.3	-
	-	-	-	-	2	2	2	2
	-	-	-	-	3	3	3	3
	4.1	-	-	4.1	4.1	4.1	4.1	4.1
LDLR	-	A	A	A	A	A	A	A
	B	B	B	B	B	B	B	B
GYPA	A	-	A	A	A	A	A	A
	B	B	-	B	B	B	B	B
HBGG	A	A	-	A	A	A	A	A
	B	-	B	B	B	B	B	B
	-	-	-	-	C	C	C	C
D7S8	A	-	A	A	A	A	A	A
	B	B	-	B	B	B	B	B
GC	A	-	-	A	A	A	A	A
	B	B	B	B	B	B	B	B
	-	C	C	C	C	C	C	C

Note: Alleles not directly observed, but whose presence cannot be ruled out, are shown in parentheses (only applicable for the HLA-DQA1 locus typing).

From these DNA profile comparisons, the following conclusions may be reached:

(i) DNA extracted from the sample on the wood chip (item #3) is a mixture of DNA from at least two persons since it indicates the presence of four alleles (and possibly a fifth allele as well) at the HLA-DQA1 locus and three alleles each at the HBGG and GC loci. Richard Eberling cannot be excluded as a possible contributor of this mixed DNA sample. In contrast, Marilyn Sheppard as well as Sam Sheppard are both excluded as part contributors of this mixture DNA.

(ii) DNA from the bloodstain from Sam Sheppard's trousers (Item # b-3-b-1) is also a mixture of DNA from at least two contributors (as it indicates the presence of four alleles at the HLA-DQA1 locus and three

alleles each at the HBGG and GC loci). Richard Eberling cannot be excluded as a part contributor of this mixture DNA. In contrast, Marilyn Sheppard as well as Sam Sheppard are excluded as part contributors of this mixture DNA.

(iii) The DNA profile of item # 1-C (stain from bedroom closet) is also a mixture of DNA from at least two contributors, since it exhibits three (and possibly four) alleles at the HLA-DQA1 locus, and three alleles at the GC locus. Neither Marilyn Sheppard nor Richard Eberling can be excluded as part contributors of this mixture DNA. Further, since the 1.2 allele of the HLA-DQA1 locus is not directly seen in the DNA of item # 1-C, the mixture DNA of this item is consistent of being originated from Marilyn Sheppard and Richard Eberling. Since the presence of the allele 1.2 of the HLA-DQA1 locus in the mixture DNA of item # 1-C cannot be ruled out, Sam Sheppard cannot be definitely excluded as a part contributor of this mixed DNA evidence. However, DNA from Sam Sheppard and Marilyn Sheppard together do not explain all of the alleles found in the mixture DNA of item # 1-C (because of the presence of the 4.1 allele of the HLA-DQA1 and A allele of the GC loci in this sample).

(iv) The DNA from the vaginal smear sperm fraction (item # A-59-2) is also a mixture of DNA from at least two sources (since four alleles are seen in it at the HLA-DQA1 locus and three alleles at each of the HBGG and GC loci). Marilyn Sheppard and Sam Sheppard are excluded as part contributors of this DNA. All alleles (at the six loci typed) of the DNA from Richard Eberling are present in this mixture DNA and hence, he cannot be excluded as a part contributor of this mixture DNA. DNA from Richard Eberling together with one or more unknown sources has to be considered to explain all alleles of this mixture DNA.

(v) The DNA extracted from the vaginal smear sperm fraction (item # A-59-1) is also a mixture of DNA from at least three donors (because of the presence of five and possibly six alleles at the HLA-DQA1 locus and three alleles at the HBGG and GC loci). Neither Richard Eberling nor Marilyn Sheppard can be excluded as part contributors of DNA of this mixed DNA sample. Since the possible presence of the 1.2 allele of the HLA-DQA1 locus cannot be ruled out in this mixture (although it is not directly observed), Sam Sheppard cannot be definitely excluded as a part contributor. Nonetheless, any combination of DNA from the known subjects tested does not explain all alleles of this mixture DNA (since the alleles 2 and 3 of the HLA-DQA1 locus and the allele C of the HBGG locus that are observed in the mixture are not present in any of the known persons tested).

8). In summary, the evidentiary items #3 (wood chip), b-3-b-1 (bloodstain from Sam Sheppard's trousers), A-59-1 and A-59-2 (vaginal smears sperm fraction) as well as item #1-C (stain from the bedroom closet) are all DNA of mixed origin (from at least two donors). However, they exhibit two distinct characteristics, since, the first four of them (Items #3, b-3-b-1, A-59-1 and A-59-2) each shows presence of alleles foreign to any of the known persons tested (alleles 2 and 3 of the HLA-DQA1 locus and allele C of the HBGG locus). At least another person, other than the ones tested, would have to be a part contributor of the DNA of these mixture samples. In contrast, from the alleles directly observed in the item #1-C (stain from the bedroom closet), it can be postulated that DNA from Marilyn Sheppard and Richard Eberling can explain all of the alleles directly observed in the mixture DNA of this item.

Thus, for assessing the statistical strength of the DNA evidence of this case, the following table (Table 2) can be helpful:

Table 2. Possible scenarios to explain the DNA mixtures in evidentiary samples

Item	Alleles present: Locus	Alleles	Scenarios
#1-C	HLA-DQA1	1.1, 1.3, 4.1 (may be 1.2)	(i) RE + MS explains all alleles directly observed;
	LDLR	A, B	(ii) MS + SS can not explain the mixture (since HLA-DQA1 4.1 allele and GC-A allele would remain unexplained);
	GYPA	A, B	
	HBGG	A, B	
	D7S8	A, B	(iii) SS would be excluded as a part contributor if the possibility of the presence of HLA-DQA1 1.2 allele is ruled out in the mixture;
	GC	A, B, C	
			(iv) Other scenarios are: MS + one or more UN; RE + one or more unknown; 2 or more UN;
			(v) No direct evidence of any contributors other than the known persons tested.
#3	HLA-DQA1	1.1, 2, 3, 4.1 (may be 1.2)	(i) RE + at least two UN can explain all alleles directly observed;
	LDLR	A, B	(ii) Neither MS nor SS can be part contributors of this DNA mixture.
	GYPA	A, B	
	HBGG	A, B, C	
	D7S8	A, B	
	GC	A, B, C	
#b-3-b-1	HLA-DQA1	1.2, 2, 3, 4.1	(i) RE + at least two UN can explain all alleles observed in the mixture;
	LDLR	A, B	(ii) Neither MS nor SS can be part contributors of this DNA mixture.
	GYPA	A, B	
	HBGG	A, B, C	
	D7S8	A, B	
	GC	A, B, C	
#A-59-1	HLA-DQA1	1.1, 1.3, 2, 3, 4.1, (may be 1.2)	(i) RE + at least two UN can explain all alleles observed in the mixture;
	LDLR	A, B	(ii) Also, MS and SS can not be excluded as part-contributors of this DNA mixture. However, any combination of mixtures of DNA from MS, SS and RE cannot explain all alleles seen.
	GYPA	A, B	
	HBGG	A, B, C	
	D7S8	A, B	
	GC	A, B, C	
#A-59-2	HLA-DQA1	1.2, 2, 3, 4.1	(i) RE + at least one UN can explain all contributors of this DNA mixture.
	LDLR	A, B	(ii) Neither MS nor SS can be part contributors of this DNA mixture.
	GYPA	A, B	
	HBGG	A, B, C	
	D7S8	A, B	
	GC	A, B, C	

Note: SS = Sam Sheppard; MS = Marilyn Sheppard; RE = Richard Eberling; UN = Unknown.

9. Statistical strength of DNA mixture evidence is generally assessed by answering two types of questions, for which two different approaches are to be adopted. First, since in all of the above evidentiary samples, their DNA profiles indicate that certain known persons tested cannot be excluded as part contributors of the DNA mixture, we ask the question: How often a random person would remain unexcluded? Generally, the lower this probability is, the stronger is the evidence that a certain (unexcluded) person could indeed be a part contributor. The computation of this probability, obviously, does not require the knowledge of the DNA profile of the tested person (who was, or was not, excluded). The NRC (1994) report explains the computation of the exclusion probability, which relies on the total frequencies of all alleles seen in the mixture. The following table (Table 3) lists these exclusion probabilities for the four evidence samples (items #1-C, #3, #b-3-b-1, #A-59-1, and #A-59-2) in which allele frequencies for four different population samples are used. The allele frequencies are taken from the Perkin Elmer product brochure (of HLA-DQA1 and Polymarker test kits) that I have personally validated for forensic computations.

Table 3. Exclusion probabilities (in %) for being part-contributors in the evidence samples of DNA mixtures

Item	Locus	Exclusion Probability (in %) for			
		US Caucasians	African-Americans	US-Hispanics	Japanese
#1-C	HLA-DQA1	13.32	9.18	19.62	25.91
		(30.80)	(39.94)	(32.83)	(39.31)
	LDLR	0.00	0.00	0.00	0.00
	GYPA	0.00	0.00	0.00	0.00
	HBGG	0.02	11.09	2.03	0.00
	D7S8	0.00	0.00	0.00	0.00
	GC	0.00	0.00	0.00	0.00
	Combined	13.34	9.25	19.79	25.91
		(30.81)	(46.60)	(32.97)	(39.31)
#3	HLA-DQA1	1.02	1.99	2.66	8.70
		(8.47)	(22.09)	(8.58)	(17.06)
	LDLR	0.00	0.00	0.00	0.00
	GYPA	0.00	0.00	0.00	0.00
	HBGG	0.00	0.00	0.00	0.00
	D7S8	0.00	0.00	0.00	0.00
	GC	0.00	0.00	0.00	0.00
	Combined	1.02	1.99	2.66	8.70
		(8.47)	(22.09)	(8.58)	(17.06)
#b-3-b-1	HLA-DQA1	6.71	7.08	7.18	7.18
	LDLR	0.00	0.00	0.00	0.00
	GYPA	0.00	0.00	0.00	0.00
	HBGG	0.00	0.00	0.00	0.00
	D7S8	0.00	0.00	0.00	0.00
	GC	0.00	0.00	0.00	0.00
	Combined	6.71	7.08	7.18	7.18

- continued

Table 3. Exclusion probabilities in % (Continued)

Item	Locus	Exclusion Probability (in %) for			
		US Caucasians	African-Americans	US-Hispanics	Japanese
#A-59-1	HLA-DQA1	0.08	0.69	1.21	0.35
		(4.25)	(16.97)	(5.71)	(3.13)
	LDLR	0.00	0.00	0.00	0.00
	GYPA	0.00	0.00	0.00	0.00
	HBGG	0.00	0.00	0.00	0.00
	D7S8	0.00	0.00	0.00	0.00
	GC	0.00	0.00	0.00	0.00
	Combined	0.08	0.69	1.21	0.35
		(4.25)	(16.97)	(5.71)	(3.13)
#A-59-2	HLA-DQA1	6.71	7.08	7.18	7.18
	LDLR	0.00	0.00	0.00	0.00
	GYPA	0.00	0.00	0.00	0.00
	HBGG	0.00	0.00	0.00	0.00
	D7S8	0.00	0.00	0.00	0.00
	GC	0.00	0.00	0.00	0.00
	Combined	6.71	7.08	7.18	7.18

Note: The estimated exclusion probabilities shown in parentheses are the ones in which the 1.2 allele of the HLA-DQA1 locus are excluded, since in the mixture its presence is only indircetly inferred in the respective evidentiary samples. Consequently, the combined exclusion probabilities are also shown with as well as without (in parentheses) including the 1.2 allele at the HLA-DQA1 locus.

10. These computations clearly show that for all of the evidentiary samples, the exclusion probabilities are at best modest. In other words, one may conclude that, by chance, a random person, of any ethnicity, could remain unexcluded as a part-contributor of any of these evidence sample DNA mixtures. Two considerations must be recalled before interpreting these calculations any further. First, the modest exclusion probability (for being part-contributors) in DNA mixtures is an inherent limitation of DNA markers that exhibit only a few variant alleles. Since there are only seven possible alleles at the HLA-DQA1 locus, and 2 (for LDLR, GYPA and D7S8) or 3 (for HBGG and GC) alleles at each of the five polymarker loci, the modest exclusion probability estimates are quite natural. Second, and more importantly, even when a person is not excluded, he/she alone may not explain all alleles in the mixture. Therefore, a second step of computation is needed for DNA mixture analysis, for which the logic (generally accepted in the scientific community) is discussed in the recent NRC (1996) report (see also Weir BS, Triggs CM, Startling L, Stowell LI, Walsh KAJ, Buckleton J (1997) Interpreting DNA mixtures. Jour of Forensic Sciences 42:213-222). This entails comparisons of the probability of finding the mixture profiles under various scenarios of the origin of the mixtures. In this particular case, as suggested earlier, only for the evidence item #1-C (stain from the bedroom closet), we have a notion of (postulated known) origin that might explain all alleles of the mixture. For all other mixture samples (items #3, #b-3-b-

1, #A-59-1 and #A-59-2), contributor(s) have to include person(s) not tested by the FSA laboratory. Therefore, the following table (Table 4) presents the probabilities of observing the mixture profile observed in the sample #1-C under some relevant scenarios (of the origin of the mixture), so that they may be contrasted to evaluate which scenario explains the mixture with a greater chance (i.e., the ratio of such probabilities ia called the likelihood ratio).

Table 4. Probabilities for observing the mixed DNA profile of item #1-C under different DNA mixture scenarios

Item	Scenario	Probability of finding the mixture profile for			
		US Caucasians	Afr.-Americans	US-Hispanics	Japanese
#1-C with HLA-DQA1 1.2 allele + 5 PM loci					
	1) RE+MS+SS	1 in 1	1 in 1	1 in 1	1 in 1
	2) RE+MS+UN	1 in 5	1 in 6	1 in 9	1 in 10
	3) RE+UN+SS	1 in 6	1 in 14	1 in 10	1 in 17
	4) UN+MS+SS	1 in 10	1 in 58	1 in 13	1 in 31
	5) RE+UN+UN	1 in 54	1 in 534	1 in 197	1 in 208
	6) UN+MS+UN	1 in 20	1 in 144	1 in 56	1 in 129
	7) UN+UN+SS	1 in 21	1 in 256	1 in 50	1 in 147
	8) UN+UN+UN	1 in 121	1 in 2816	1 in 418	1 i n 775
	9) UN+ SS	1 in 69	1 in 790	1 in 116	1 in 375
	10) UN+MS	1 in 74	1 in 295	1 in 179	1 in 466
	11) UN+UN	1 in 529	1 in 8827	1 in 1340	1 in 2388
#1-C without HLA-DQA1 1.2 allele + 5 PM loci					
	1) RE+MS	1 in 1	1 in 1	1 in 1	1 in 1
	2) RE+UN	1 in 80	1 in 1,087	1 in 187	1 in 214
	3) UN+MS	1 in 42	1 in 352	1 in 80	1 in 164
	4) UN+UN	1 in 452	1 in 15,780	1 in 817	1 in 1510
	5) RE+UN+UN	1 in 79	1 in 1,987	1 in 173	1 in 253
	6) UN+MS+UN	1 in 53	1 in 1,257	1 in 98	1 in 172
	7) UN+UN+UN	1 in 500	1 in 43,215	1 in 980	1 in 1925

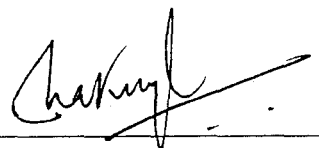
NOTE: RE=Richard Eberling; MS=Marilyn Sheppard; SS=Sam Sheppard; UN=Unknown

These computations show that the mixed DNA profile of the item #1-C is best explained under the scenario that it has DNA from Richard Eberling and Marilyn Sheppard. In order that the mixed DNA also consists of DNA of Sam Sheppard, we have to include the 1.2 allele of the HLA-DQA1 locus (whose absence cannot be ruled out in a DNA mixture, even when it is not directly observed).

11. In aggregate, these statistical computations indicate that in spite of the modest chances of excluding any random person in the DNA mixtures observed in the five evidentiary samples (items #1-C, #3, #b-3-b-1, #A-59-1 and #A=59-2), it is significant that Marilyn Sheppard is excluded as a part-contributor of three of these DNA mixtures (items #3, #b-3-b-1 and #A-59-2) and Sam Sheppard is definitely excluded for two of these samples (items #3 and #b-3-b-1). In addition, Sam Sheppard's possible inclusion in the samples #1-C and #A-59-1 is due to the inherent limitation of the HLA-DQA1 typing method. In sharp contrast, Richard Eberling was not

excluded as a part-contributor in any of the five DNA mixtures. Furthermore, the chance of finding a DNA mixture as observed in the sample #1-C is best explained when it is considered to be a mixture of DNA from Richard Eberling and Marilyn Sheppard. Sam Sheppard's inclusion in this mixture cannot be definitely asserted by these tests.

12. Dr. Holland, in his report dated October 15, 1999, attempted a statistical assessment of these test results by computing the power of discrimination (in a table on page 2 of his report) and exclusion probability (in a table on page 3 of his report). The concept of power of discrimination is relevant for DNA profiles that are of single donor origin, and its application to DNA mixture analysis is not meaningful, since no statement with regard to the number of contributor(s), known or unknown, is made in his computations. Likewise, his exclusion probability estimates, even if they are correct, are only an initial step of interpretation of DNA mixtures (as stated earlier). Since, exclusion probability estimates do not make use of the observed data as to which of the known persons explain part (or whole) of the mixture profiles, such computations cannot judge which scenarios of mixtures explain the observed data with the greatest chance.



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