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A Mixed DNA Profile Controversy: Corrigendum

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Abstract

DNA has been playing a crucial role in identifying or exonerating potential suspects. While single source DNA traces face interpretational challenges, the evaluation of a forensic DNA mixture trace faces much greater challenges especially with increased allele sharing and homozygosity. The present report describes a challenging case where eight, related, potential persons of interest (POI) related could not be excluded in a simulated mixed DNA analysis. Even though relevant frequency datasets and an inbreeding coefficient were considered, and a semi-continuous expert DNA mixture analysis software was used, the DNA results still did not support ground truth. The present case sheds light on the effect of allele sharing and homozygosity on the evaluation of DNA mixtures especially in consanguineous and endogamous populations. Recommendations as to DNA mixture analysis were issued for local forensic uses and for other similar populations.

Keywords: Forensic DNA • Mixed traces • Mixture software • Inbreeding • Likelihood ratio

Introduction

Traces involving DNA mixtures are frequently encountered in forensic casework [1]. Such traces originate from two or more contributors [2]. Interpretation of mixed traces is facilitated when 1. The amount of amplified DNA from all contributors is sufficient and above the analytical threshold, 2. The ratio of DNA contributed by each source is reflected on the peak heights and consequently the possible genotypes of major and minor contributors may be determined [3], 3. There is no degradation and allele drop out, 4. No artifacts and allele drop in and 5. The contributors are unrelated and have few shared alleles. But since the situation of forensic cases is rarely ideal and the likelihood in which it is not possible to distinguish the alleles of the different contributors does exist, several models have been suggested for the interpretation of mixed DNA profiles [4].

The present report describes a challenging case where eight POIs could not be excluded from a mixed DNA trace and highlights the fact that semi-continuous expert DNA mixture software might not be sufficient in populations with increased relatedness even if inbreeding coefficient is considered, when major pieces of information such as peak heights and interpretation strategies (such as conditioning) that allow better specificity and sensitivity in mixture cases are not accounted to.

Case Note

The present case is a mixed DNA trace. Profiling was performed with 23 STR systems by combining two multiplex STR kits: PowerPlex®16 HS, the PowerPlex® ESI 17 (Promega Corporation; Madison, WI, USA) (Table 1).

Systems D3S1358, D5S818, TH01, D21S11, TPOX, D7S820, D2S1338, Penta D and D1S1656 (highlighted in blue) and systems D8S1179, SE33 and D12S391 (highlighted in yellow) show that at least two individuals contributed to this trace.

Only two alleles per locus appear in systems D19S433, vWA, D13S317, FGA, D16S539, D18S51, CSF1PO, Penta E, D22S1045 and D2S441 (highlighted in green), which is potentially due to homozygosity and allele sharing [5] characteristics that are frequent in the Lebanese population due increased inbreeding in the community under study [6-8]. Allele drop out could be a further reason to whether only two or more profiles contributed to the mixture obtained from the trace.

Eight POIs are to be considered, if all other non-DNA evidence relevant to the allegation failed to provide an alibi [9]. Before assigning the number of contributors (NOC) to the mixture and formulating any proposition for interpretation, all eight DNA profiles showed a *complete* match with the mixed DNA trace (Table 2).

Statistical interpretation was performed based on the semi-continuous model using the DNA mixture analysis expert software LRmix studio, under the following proposition $LR_{iu/uu}$.

where,

H_{iu}: DNA comes from P_i and an unknown unrelated person *U*.

H_{uu}: DNA comes from two unknown people *U*, *U* unrelated to P_i or each other.

The allele frequencies of the Lebanese population were considered, and the analysis was repeated under the following situations: with and without the inbreeding coefficient Θ that reflects the rate of inbreeding in the Lebanese population 0.01.

If the NOC is limited to two contributors only and drop in is not accounted to, the obtained LR for POI 1 was 0 due to system D12S391. The obtained Likelihood ratios for the other POIs ranged between 10^{18} and 10^{22} (Table 2). The statistical results confirmed the inclusion of seven POIs and none of the POIs could be excluded by any of the 23 tested loci.

The number of systems was increased to 28 by adding the systems of the PCR CS7 kit (Promega Corporation; Madison, WI, USA) to the previously used PowerPlex®16 HS, PowerPlex® ESI 17 and PowerPlex® kits.

By increasing the number of tested loci, three POIs were still not excluded (Table 3). The statistical results supported the proposition that the POIs had contributed to the mixture rather than an unknown person for three POIs and none of the individuals could be excluded by any of the 28 tested loci.

Efforts in the recent decade have been made to assess the degree of

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Table 1. Profile of the mixed trace.

Locus	Trace
D3S1358	15 17 18
D19S433	13 16
D8S1179	8 12 13 15
D5S818	11 13 14
TH01	7 9 10
vWA	14 20
D21S11	29 30 30.2
D13S317	10 12
TPOX	8 10 11
FGA	22 23
D7S820	9 10 12
D16S539	11 12
D18S51	12 19
CSF1PO	11 12
D2S1338	18 19 20
Penta E	5 12
Penta D	8 11 12
SE33	19 25.2 28.2 29.2
D22S1045	11 15
D1S1656	12 16 17
D10S1248	12 14
D2S441	11 14
D12S391	19 21 22 23

Table 2. Profiles of all eight POI and their relevant LRs.

Locus	Trace	POI 1	POI 2	POI 3	POI 4	POI 5	POI 6	POI 7	POI 8
D3S1358	15 17 18	17 18	15 17	17 18	15 18	15 17	15 17	15 17	15 17
D19S433	13 16	13 13	13 16	13 16	13 16	13 13	13 16	13 16	13 16
D8S1179	8 12 13 15	12 13	12 15	12 13	8 12	12 15	8 15	8 13	12 15
D5S818	11 13 14	11 14	11 13	13 14	11 13	11 13	11 11	11 14	11 14
TH01	7 9 10	9 9	9 10	7 10	9 10	9 9	9 10	9 10	9 10
vWA	14 20	20 20	14 20	14 20	14 20	14 20	20 20	20 20	14 14
D21S11	29 30 30.2	29 30	29 30	30 30.2	30 30	29 30	29 30	29 30	29 30
D13S317	10 12	10 12	12 12	10 12	10 12	12 12	12 12	12 12	10 12
TPOX	8 10 11	10 11	8 10	8 11	8 10	10 10	8 10	8 11	8 10
FGA	22 23	22 23	22 22	23 23	22 23	22 23	22 23	22 23	22 23
D7S820	9 10 12	10 12	10 10	9 10	9 10	9 12	10 12	10 12	9 10
D16S539	11 12	11 11	11 11	11 12	11 11	11 11	11 11	11 11	11 11
D18S51	12 19	12 12	12 19	12 12	12 12	12 19	12 19	12 19	12 19
CSF1PO	11 12	11 11	12 12	11 12	11 12	11 12	11 12	11 11	11 12
D2S1338	18 19 20	19 20	19 20	18 19	20 20	19 20	19 20	19 20	19 20
Penta E	5 12	5 12	5 12	5 12	5 5	5 12	5 12	5 12	5 12
Penta D	8 11 12	8 11	11 11	8 11	8 11	11 12	11 12	11 12	8 11
SE33	19 25.2 28.2 29.2	19 25.2	19 25.2	28.2 29.2	25.2 28.2	25.2 29.2	19 25.2	28.2 29.2	25.2 29.2
D22S1045	11 15	11 11	11 15	11 15	11 15	11 15	11 15	11 15	11 11
D1S1656	12 16 17	12 17	12 17	16 17	12 17	16 17	12 16	12 16	12 17
D10S1248	12 14	12 12	12 14	14 14	12 14	14 14	12 14	12 14	12 12
D2S441	11 14	11 14	11 14	11 14	14 14	11 14	11 14	11 14	11 14
D12S391	19 21 22 23	**19 19**	19 22	22 23	19 22	21 22	19 21	19 21	19 22
$LR_{iu/uu}$		-	2×10^{19}	3.2×10^{22}	6.4×10^{22}	1.4×10^{18}	4.4×10^{20}	2×10^{21}	2.9×10^{22}
$LR_{iu/uu}$ with θ		-	8.8×10^{12}	3.5×10^{16}	1.7×10^{15}	6.3×10^{12}	8.9×10^{13}	3.2×10^{14}	3.1×10^{15}

uncertainty in the analysis of STR profiles, in particular the mixed DNA profiles. In undertaking such a study, it was important to know the true contributors of the DNA mixture before matching it against the database.

517 samples were collected randomly from Lebanese villages of different religious backgrounds. Profiling was performed with three different profile sizes: 15, 23 and 28 STR systems using three multiplex STR kits: PowerPlex® 16 HS, the PowerPlex® ESI 17 and the PowerPlex® CS7 (Promega Corporation; Madison, WI, USA). The obtained profiles were used to simulate DNA mixtures of two contributors. Each of the 517 profiles was then probed against the electronically simulated two-contributor mixtures (the same was done with each of the 15, 23 and 28 profile sizes). Mixture analysis was performed, and Likelihood Ratios were generated whenever false inclusions were detected.

In the present case, profiles of individuals #3 and #6 were the true contributors of the mixture.

Table 3a. Profiles of all adventitious matches that could not be excluded from the mixed trace with 28-locus profile with their relevant LR.

Locus	Trace	POI 2	POI 3	POI 4	POI 5	POI 6	POI 7	POI 8
D3S1358	15 17 18	15 17	17 18	15 18	15 17	15 17	15 17	15 17
D19S433	13 16	13 16	13 16	13 16	13 13	13 16	13 16	13 16
D8S1179	8 12 13 15	12 15	12 13	8 12	12 15	8 15	8 13	12 15
D5S818	11 13 14	11 13	13 14	11 13	11 13	11 11	11 14	11 14
TH01	7 9 10	9 10	7 10	9 10	9 9	9 10	9 10	9 10
vWA	14 20	14 20	14 20	14 20	14 20	20 20	20 20	14 14
D21S11	29 30 30.2	29 30	30 30.2	30 30	29 30	29 30	29 30	29 30
D13S317	10 12	12 12	10 12	10 12	12 12	12 12	12 12	10 12
TPOX	8 10 11	8 10	8 11	8 10	10 10	8 10	8 11	8 10
FGA	22 23	22 22	23 23	22 23	22 23	22 23	22 23	22 23
D7S820	9 10 12	10 10	9 10	9 10	9 12	10 12	10 12	9 10
D16S539	11 12	11 11	11 12	11 11	11 11	11 11	11 11	11 11
D18S51	12 19	12 19	12 12	12 12	12 19	12 19	12 19	12 19
CSF1PO	11 12	12 12	11 12	11 12	11 12	11 12	11 11	11 12
D2S1338	18 19 20	19 20	18 19	20 20	19 20	19 20	19 20	19 20
Penta E	5 12	5 12	5 12	5 5	5 12	5 12	5 12	5 12
Penta D	8 11 12	11 11	8 11	8 11	11 12	11 12	11 12	8 11
SE33	19 25.2 28.2 29.2	19 25.2	28.2 29.2	25.2 28.2	25.2 29.2	19 25.2	28.2 29.2	25.2 29.2
D22S1045	11 15	11 15	11 15	11 15	11 15	11 15	11 15	11 11
D1S1656	12 16 17	12 17	16 17	12 17	16 17	12 16	12 16	12 17
D10S1248	12 14	12 14	14 14	12 14	14 14	12 14	12 14	12 12
D2S441	11 14	11 14	11 14	14 14	11 14	11 14	11 14	11 14
D12S391	19 21 22 23	19 22	22 23	19 22	21 22	19 21	19 21	19 22
LPL	9 10	10 10	9 9	9 10	9 9	10 10	9 10	10 10
F13B	7 8 10	8 8	7 10	8 **9**	8 8	8 10	**9** 10	8 **9**
FESFPS	10 11 12	10 12	11 11	10 11	10 12	10 12	10 12	11 12
F13A01	5 6	5 6	5 5	5 6	5 **12**	6 6	6 6	6 6
Penta C	11 11	11 11	11 11	11 **14**	11 **14**	11 11	11 11	11 11
$LR_{iu/uu}$		2.9×10^{20}	2.2×10^{25}	*	*	1.2×10^{21}	*	*
$LR_{iu/uu}$ with $\theta=0.01$		2×10^{17}	1.2×10^{22}	*	*	5.8×10^{17}	*	*

*The following POIs are excluded by at least one system at 28 loci. They no longer show complete match with the mixture, their LRs is equal to 0 if drop in and drop out were both set to 0. However, if drop out is set to 0.1 and drop in is set to 0.05 then their LRs will be as follows (Table 3b).

Table 3b. LRs of POI if drop out set to 0.1 and drop in set to 0.05

	POI 4	POI 5	POI 7	POI 8
$LR_{iu/uu}$	1.6×10^{22}	3.4×10^{17}	7.6×10^{21}	2.1×10^{22}
$LR_{iu/uu}$ with $\theta=0.01$	2.5×10^{15}	4.8×10^{12}	5×10^{15}	1.9×10^{16}

DNA mixture analysis software (based on peak designation only) yielded LR values of 2×10^{19}, 6.4×10^{22}, 1.4×10^{18}, 2×10^{21} and 2.9×10^{22} for POIs 2, 4, 5, 7 and 8, respectively with 23 locus profiles that supported the propositions that they contributed to the mixture rather than an unrelated person. In some cases, the LRs were larger than the LR values of the true contributors. Since we do have background information on these POIs we intended to consider the below hypothesis:

H_{iu}: DNA comes from P_i and an unknown person *U*.

H_{uu}: DNA comes from an unknown individual *U* and *R* who is a relative to P_i.

And the LRs were as follows:

LR2u/ur=4.9×10^6, LR4u/ur=1.2×10^6, LR5u/ur=5.0×10^5, LR7u/ur=5.3×10^6, LR8u/ur=6.6×10^6

The only situation where ground truth was supported by the LR values is when we considered conditioning and used continuous model PG software.

Discussion and Conclusion

While increasing the number of STRs successfully helped excluding individuals 4, 5, 7 and 8, it strongly supported the false inclusion of individual 2 with a higher LR value, thus results supporting the proposition that was not ground truth (however, in this case both propositions were false, as the ground truth involved a close relative).

Interpreting DNA mixtures is among the most challenging forensic casework and cases like the one presented may be encountered in inbred populations. Equally important to using the relevant allele frequency dataset [10,11] and taking into account the inbreeding coefficient, is the choice of the DNA mixture analysis software and of the propositions. As apparent in this work, DNA mixture analysis software that uses semi-continuous models (i.e. do not account to peak heights) can falsely support the inclusion of non-contributors.

Presenting the DNA evidence without statistics renders the DNA evidence inadmissible. However, these findings challenge the admissibility of semi-continuous DNA mixture statistics and raise the attentiveness to forensic DNA mixture inclusive conclusions when dealing with communities with high-level of inbreeding. To reduce the rate of false inclusions, information based on peak heights and the use of conditioning profiles [12], whenever possible, are better alternatives [13].

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