

Which Short Tandem Repeat Polymorphisms are Required for Identification? Lessons from Complicated Kinship Cases

Jürgen Henke, Lotte Henke

Institut für Blutgruppenforschung, Köln, Germany

Abstract This paper presents 5 examples of complicated deficient parentage cases, which were sufficiently resolved by extensive DNA typing using short tandem repeat (STR) and restriction fragment length polymorphisms (RFLPs). The latter have greatly contributed to the solution of deficiency cases, although their application is only feasible, if high molecular weight DNA and time are in abundance. This apart, RFLP technique is available in a few laboratories only, and its extinction can be expected in medium term. This development will pose a problem unless more highly polymorphic STR systems are at the service of forensic genetic laboratories. The required "new" additional STR polymorphisms must be able to fully replace RFLPs in terms of their respective information content. STR loci of this quality are e.g. ACTBP2 (SE33), D5S2360, and gonosomal loci. Moreover, the newly introduced STR kit "Humantype Chimera" is considered valuable from this point of view.

Identification of human remains by means of reverse parentage testing is prone to technical and statistical problems (1-5, and Alonso A, National Institute of Toxicology and Forensic Science, Madrid, Spain, personal communication).

The technical problems may lie in (a) degraded DNA, (b) limited number of polymorphisms available, and (c) lack of appropriate experience, whereas statistical problems could be attributed to (a) complex hypotheses, and (b) neutral a priori probabilities smaller than 0.5 (6-11).

Extensive DNA profiling in reverse parentage testing may well serve as a training field for the approach to the forensic genetic handling of mass identification. For the time being, and if high molecular weight DNA as well as time are abundant, these parentage cases can be treated by employing batteries of short tandem repeat (STR) loci along with restriction fragment length (RFLP) minisatellite polymorphisms. The latter have greatly contributed to the solution of deficiency

cases. It is, however, foreseeable that the forensic use of RFLPs will soon be terminated and the question must be addressed which STRs could be well suited to fill the then evolving gap of information. Adequate STR loci are e.g. ACTBP2, (SE33, ref. 12), D5S2360 (13), X/Y-chromosomal loci, as well as the newly introduced loci of the "Humantype Chimera" kit. This paper aims at presenting data: (a) regarding the solution of 5 complicated parentage cases, and (b) elaborations on the usefulness of "new," little known loci.

Materials and Methods

Parentage Casework

The following situations were brought to our attention: (a) motherless case, with 2 STR mutations (21,885), (b) grandmother case (20,365), (c) half sibling case (21,547), (d) full sibling case (21,809), and (e) inconclusive full sibling case (20,535).

Loci Analyzed

Hinf I polymorphisms of loci D1S7 (MS1), D2S44 (YNH24), D5S110 (LH1), D7S21 (MS31), D7S22 (G3), D12S11 (MS43), and D16S309 (MS205) were employed using established, standard protocols. The listed probes and molecular weight markers (MW 100) are currently distributed by Tepnel Diagnostics Ltd., Abingdon, UK.

Autosomal STR polymorphism kits SGM-Plus (Applied Biosystems, Foster City, CA, USA), PowerPlex 16 (Promega Madison, WI, USA), and FFFL (Promega) were utilized according to the respective manufacturer's recommendations.

Typing of autosomal STR loci ACTBP2 and D5S2360 was carried out in duplex reactions along with published primers for locus FGA in order to avoid sample mix-up (12-14).

Five X-chromosomal loci (DXS6789, STR-X1, DXS8377, DXS101, and HPRTB) were typed in a pentaplex assay (15).

The autosomal STR kit "Humantype Chimera" is designed to amplify 12 loci (D7S1517, D3S1744, D12S391, D2S1360, D6S474, D8S1132, D5S2500, D18S51, D21S2055, D10S2325, SE33, and D4S2366) (Biotype AG, Dresden, Germany).

All STR analyses were carried out on ABI310, Genetic Analyzers (Applied Biosystems).

Results

Motherless Case with 2 Mutations (Case 21,885)

A 38 year old woman was claiming not to be the daughter of her legal father.

Twenty years after her mother's death, she filed a law suit and demanded a DNA test.

The typing program consisted of 7 minisatellite loci, 23 STR loci (including ACTBP2 and D5S2360), as well as 5 ChrX loci. Two autosomal STR loci showed "exclusions." An excerpt from the typing results is compiled in Table 1.

Altogether 35 DNA polymorphisms were analyzed; 2 of them showing exceptions from Mendelian inheritance. Because the exclusions were based on one-repeat differences, the occurrence of a double mutation was taken into consideration.

The problem can be addressed by two statistic approaches.

Table 1. Excerpt from typing results obtained in a motherless case*

Locus	Alleles in	
	child	alleged father
D21S11	30.2/ 31	28/30
HumvWA	18/20	17/19
ACTBP2	19/27.2	15/19
D5S2360	22.2/24.2	21.2/24.2
STRX-1	14.3/15	14.3
DXS6789	20/22	20
DXS101	18/27	18
HUMHPRTP	12/14	14
DXS8377	46/50	50

*Matching restriction fragments/alleles are marked in bold.

The approach 1 had 2 hypotheses: (a) legal father is biological father versus (b) legal father is excluded from paternity and also from close kinship.

Given the neutral *a priori* of 0.5 and by assuming two mutations (16,17), the 30 autosomal loci would deliver a Paternity Index (PI) of 2354522903 which equals the *a posteriori* probability of $W = 99.99999995\%$.

The approach 2 had 3 hypotheses: (a) legal father is biological father, (b) legal father is excluded from paternity, but is close kin (uncle, e.g.), and (c) legal father is excluded from paternity and also from close kinship.

Given the neutral *a priori* of 0.33, the *a posteriori* probabilities would be: (a) $W = 83.04\%$, (b) $W = 16.96\%$, and (c) $W = 0.00\%$.

This calculation indicates that it was 4 times more likely that we were dealing with a double mutation case rather than a close relative case.

Grandmother Case (Case 20,365)

A young girl claimed to be the daughter of a man who was deceased and thus unavailable for testing. However, the man's mother was available and was therefore included in the test as alleged grandmother.

In the course of our initial typing, a match at minisatellite locus D12S11 (MS43A) was observed, leading us to the suspicion of kinship. After the "full round" of 7 minisatellite loci and 23 (autosomal) STR loci, the situation appeared to be a different one, because the D12S11 match remained the only one among the minisatellites and the overall *a posteriori* probability plunged to $W = 0.02\%$. Finally, the decision was made to use ChrX markers, because we were interested to learn which information they would provide. The typing results are compiled in Table 2.

Table 2. ChrX typing results in a grandmother case*

Locus	Alleles found in		
	child	mother	alleged grandmother
STRX-1	3/15	13/15	14.3/15
DXS6789	20/ 21	20/22	20/22
DXS101	18 /26	21/26	23/26
HUMHPRTB	11 /14	12/14	13/14
DXS8377	46 /51	49/51	48/49

*Matching restriction fragments/alleles are marked in bold.

The paternal ChrX alleles must be present in the grandmother. Since 4 out of 5 were absent, kinship could be ruled out.

Half-Sibling Case (Case 21,547)

In 1983, 1986, and 1987, three newborns were released for incognito adoption. In 2004, one of the "orphans" investigated the identity of her mother. In the course of these investigations, the child welfare office revealed to her the possible existence of two more siblings. After informed consent was obtained, the identities of the other two "orphans" and their respective families were disclosed. The two young women and the young man then requested a private DNA test in order to find evidence that they really have the same mother. This test was carried out by employing 7 minisatellite, 23 autosomal STR, and 5 ChrX polymorphisms. An excerpt of relevant typing results is given in Table 3.

The alleles ACTBP2 30.2 and D5S2360 26.3 had frequencies of ~5% and ~1%, respectively, and the alleles of the boy's maternal X chromosome "haplotype" were observed in both alleged half-sisters.

The possibility that the three children had the same mother and father could be ruled out. The *a posteriori* probability of being half-siblings, however

Table 3. Relevant typing results observed in a half-sibling-case*

Locus	Alleles found in child		
	1	2	3
D1S7	9.65 /3.64	9.65 /4.91	5.90/1.43
D7S21	8.68 /4.28	8.68 /5.22	6.63/5.10
D12S11	6.88/ 4.94	4.94 /4.63	10.6/8.29
D7S22	6.57/ 5.02	6.21/ 5.02	8.04/1.69
D2S44	3.51/	7.08 /2.53	7.08 /4.26
D16S309	3.94 /1.93	3.94 /3.14	2.71/2.15
D5S110	4.63/2.69	4.87 /3.52	5.20/ 4.87
ACTBP2	16/	30.2 /	23.2/ 30.2
D5S2360	23.2/26	22/ 26.3	22.2/ 26.3
STRX-1	13.3/ 14	12.3/13.3	14
DXS6789	21 /22	20/ 21	21
DXS101	18 /26	24/25	18
HUMHPRTB	13 /14	12/14	13
DXS8377	46 /48	46 /47	46

*Matching restriction fragments/alleles are marked in bold.

was $W > 99.9\%$ in comparison with the hypothesis that there was no kinship at all.

Full-Sibling-Case (Case 21,809)

A girl sued a man of unknown place of residence of being her biological father.

Some years earlier, the same man accepted her 10 year old sister as his biological daughter. In other words, the two girls had the same mother and the court wanted us to find evidence that the girls also had the same father.

Relevant typing results are presented in Table 4.

Table 4. Relevant typing results observed in a full-sibling-case*

Locus	Alleles found in		
	claiming girl	mother	legal daughter
D1S7	5.41/ 2.16	9.47/5.41	5.41/ 2.16
D7S21	7.38/5.88	7.38/3.94	6.88/3.94
D12S11	9.34/ 8.34	9.34/4.65	8.34 /4.65
D7S22	7.11 /1.70	7.01/1.70	7.11 /7.01
D2S44	3.76/2.44	4.30/3.76	4.30/4.02
D16S309	2.76/2.06	2.91/2.76	4.17/2.76
D5S110	5.06 /3.73	4.09/3.73	5.06 /3.73
STRX-1	12.3 /14.3	14/14.3	12.3 /14.3
DXS6789	20 /22	22	20 /22
DXS101	22 /29	19/29	22 /29
HUMHPRTB	12	12/14	12 /14
DXS8377	46/ 54	44/46	44/ 54

*Matching restriction fragments/alleles are marked in bold.

In 4 out of 7 minisatellite systems, the two girls shared the respective paternal fragments and obviously they carried the same paternal X chromosomal haplotype. Along with 17 autosomal STR typing results there was an *a posteriori* probability of 99.87% in favor of full-siblingship as opposed to 0.13% for half-siblingship.

Inconclusive Full-Siblingship (Case 20,535)

Due to rumors in the family, a brother and his sister wanted to learn whether they had the same father. Other family members were not available. Although the clients were instructed that this is the worst case scenario, they insisted to have the test done. The typing results are given in Table 5.

Although 7 autosomal minisatellite loci, 27 autosomal STR loci and also HLA-A*, -B*, -DRB1*, and -DQB1* alleles were typed, the situation remained unclear to us.

We are convinced that the two people were related to each other and their statement of having the same mother could not be ruled out.

Table 5. Typing results observed in a questioned full-siblingship case*

Locus	Alleles found in	
	men	women
D1S7	17.45/14.05	9.97/4.17
D7S21	6.90/ 6.65	6.65 /5.06
D12S12	10.04 /6.78	10.04 /
D7S22	3.25/3.07	12.33/ 3.25
D2S44	4.70/1.57	3.10/ 1.57
D16S309	3.76/2.73	3.05/2.64
D5S110	4.91/4.03	5.52/3.66
APOAII	287 bp/ Variant	283bp/ Variant
ACTBP2	27.2/ 29.2	17.3/ 29.2
D5S2360	22.2 /	22.2 /26

*Matching restriction fragments/alleles are marked in bold.

Table 6. Compilation of Humantype Chimera short tandem repeat (STR) loci*

STR Locus	Alleles	Heterozygosity	Distance to locus
D18S51	7-33	0.95	/
ACTBP2	4.2-49	0.95	/
D2S1360	19-31	0.88	180 cM to 1338
D3S1744	13-22	0.84	~150 cM to 1358
D5S2500	9-18	0.87	52 cM to 818
D7S1517	16-28	0.92	30 cM to 820
D8S1132	13.1-27	0.93	22 cM to 1179
D10S2325	6-21	0.87	/
D12S391	15-28	0.91	8 cM to vWA
D21S2055	16.1-37	0.90	20 cM to D21S11; distance to Penta D unknown

*According to ref. 19.

The statistic approach resulted in an a *posteriori* probability of ~88% for full sibs versus ~12% for half sibs. However, because we did not observe at least one identical genotype among the 11 highly polymorphic systems, we remained reluctant to accept the statistic approach.

Usefulness of "New" Loci

ACTBP2 (SE33). Among the little known loci, *ACTBP2* is the best known one (12,18). Because this locus is part of the German 8-loci-DNA database, it was included in at least the following kits: genRES MPX-2 LF (SERAC, Bad Homburg, Germany), genRES MPX-3 SE (SERAC, Bad Homburg, Germany), PowerPlex ES (Promega, Madison, WI, USA), AmpF/STR SEfiler (Applied Biosystems, Foster City, CA, USA), and Humantype Chimera (Biotype AG, Dresden, Germany).

Approximately 40 alleles, ranging from 4.2-49 repeats contribute to the heterozygosity of ~95%, making this locus a very powerful tool in forensic genetics.

D5S2360. *D5S2360* is indeed very little known and to our knowledge it is not part of any commercially available kit (13). Thirty-three alleles ranging in size from 17.2-38 repeats contribute to a heterozygosity of ~92.5%. The power of discrimination was calculated to be ~98%. The majority of alleles show frequencies less than 6%. We employ this locus in special cases, in a duplex assay together with primers for locus FGA (14).

Humantype Chimera

The kit contains primers for 10 autosomal STR loci. With 8 of them, we were not familiar until now (Table 6)

Discussion

In case no. 5 we were reluctant to subscribe the results to the statistical interpretation, because we failed to observe an identical genotype in at least one highly polymorphic locus.

Apart from this case, the compilation of cases has demonstrated that even highly complicated questions can be answered trustworthily, if a large battery of polymorphisms, as well as appropriate DNA samples, is available. However, in the case of a mass screening like the tsunami disaster, these individual approaches to resolve cases must regrettably fail because of organizational obstacles. The "new" STR loci appear to be promising for the substitution of RFLP loci, but still a great deal of work (evaluation and validation studies) is waiting to be done.

Acknowledgements

We acknowledge S. Goldmann (Ulm) and M. Stenersen (Oslo) for HLA and *APOAII* typing; respectively, and B. Rolf (Munich) for providing us with preliminary Humantype Chimera data, and M.P. Baur (Bonn) for assistance with statistical interpretations. Neither the authors nor the Institute have financial interest in any of the companies mentioned in the article.

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Received: May 2, 2005

Accepted: June 2, 2005

Correspondence to:

Juergen Henke
Institut für Blutgruppenforschung
Hohenzollernring 57
50672 Koeln, Germany
D49221253037@t-online.de

