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Two fathers for twin sisters

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Abstract

When a mother gives birth to twins, it is assumed that whether or not identical, they share the same father. In our laboratory, we recently had a case involving little twin sisters Anne and Caroline and a putative father Mr. Y. We started by using conventional serological tests. They showed that the two little girls were not identical twins (systems MNSs, Duffy and HLA) but more importantly Mr. Y was excluded as the father of Caroline and an inconclusive result was obtained for Anne. We decided to solve the matter using STR DNA analysis. Surprisingly, whilst confirming the exclusion of Mr. Y as the father of Caroline, our data showed clearly that Mr. Y is indeed Anne's father. Although documented, this kind of situation is rather rare. Biological analyses are more and more frequently used to solve paternity disputes. This casework is an illustration that DNA analysis is the method of choice in comparison to other haematological techniques in parentage testing.

Keywords: Twin; STR-DNA analysis; Parentage testing

1. Introduction

Until recently, parentage testing was traditionally performed using serological tests on blood samples. We present a case where the serological analysis showed its limits and where DNA analysis came to the rescue.

History of the case: We received a commission from a judge to solve a paternity dispute involving two young twin sisters Anne and Caroline whose legal father (Mr. Y) had a

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Table 1
Serological testing for red blood cell surface antigens and for HLA AB antigens

Systems	Mother	Child 1: Anne	Child 2: Caroline	Alleged father
ABO	O	A1	A1	A1
Rhésus	DCe	DCce	DCce	DCce
MNS s	MNs	NSs	Ms	MNS
Kell/cellano	k	k	k	k
Duffy	Fy (a+b−)	Fy (a+b+)	Fy (a+b−)	Fy (a+b+)
Kidd	Jk (a+b+)	Jk (a+b+)	Jk (a+b+)	Jk (a+b−)
P	P1	P1	P1	P2
HLA AB	A_2; A_3	A_2; A_3	A_2; A_3	A_3; A_{31}
	B_{18}; B_{62}	B_7 ; B_{18}	B_{62} ; B_{49}	B_7; B_{38}

Phenotype results.

Table 2
Serologic testing for red blood cell surface antigens and for HLA AB antigens

Systems	Mother	Child 1: Anne	Child 2: Caroline	Alleged father
ABO	O/O	A1/O	A1/O	A1/A1
				A1/A2
				A1/O
Rhésus	R1/R1	R1/r	R1/r	R1/r
	R1/R′	R1/Ro	R1/Ro	R1/Ro
		Ro/R′	Ro/R′	Ro/R′
MNSs	Ms/Ns	Ns/NS	Ms/Ms	MS/NS
Kell/cellano	k/k	k/k	k/k	k/k
Duffy	Fy^a/Fy^a	Fy^a/Fy^b	Fy^a/Fy^a	Fy^a/Fy^b
Kidd	Jk^a/Jk^b	Jk^a/Jk^b	Jk^a/Jk^b	Jk^a/Jk^a
P	P1/P2	P1/P2	P1/P2	P2/P2
	P1/P1	P1/P1	P1/P1	
HLA AB	A_2 B_{18}/A_3 B_{62} or	A_2 B_7/ A_3 B_{18} or	A_2 B_{62}/A_3 B_{49} or	A_3B_7/A_{31} B_{38} or
	A_3 B_{18}/A_2 B_{62}	A_3 B_7/A_2 B_{18}	A_3 B_{62}/A_2 B_{49}	A_{31} B_7/A_3 B_{38}

Genotypes results.

Table 3
Identification of paternal allele for children Anne and Caroline

Systems	Anne		Caroline	
	Paternal allele	Alleged father	Paternal allele	Alleged father
ABO	A1	No exclusion	A1	No exclusion
Rhésus	r or Ro	No exclusion	R or Ro	No exclusion
MNSs	NS	No exclusion	MS	Exclusion
Kell/cellano	k	No exclusion	k	No exclusion
Duffy	Fy^b	No exclusion	Fy^a	No exclusion
Kidd	Jk^a or Jk^b	No exclusion	Jk^a or Jk^b	No exclusion
P	P1 or P2	No exclusion	P1 or P2	No exclusion
HLA AB	A_3 B_7 or	No exclusion	A_3 B_{49} or	Exclusion
	A_2 B_7	Exclusion	A_2 B_{18}	Exclusion

doubt about his biological fatherhood. The blood analysis using serological markers was able to show that one of the little girls (Caroline) was not his biological daughter but left us with uncertainty regarding the case of little Anne. In order to resolve this problem, we decided to type the blood samples using DNA.

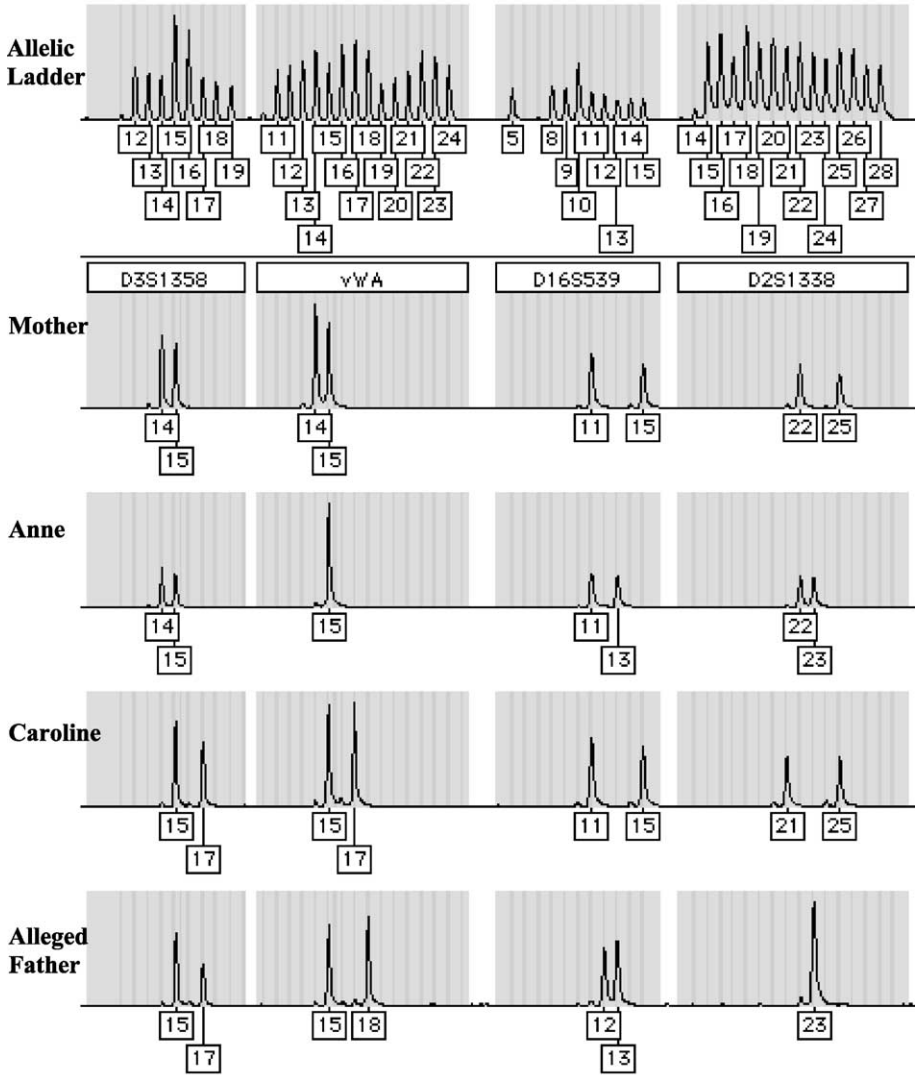

Fig. 1. STR analysis of DNA extracted from blood samples of the mother, the twin sisters (Anne and Caroline) and the alleged father.

2. Material and methods

2.1. DNA analysis

DNA was extracted from blood samples of the mother, the two children and Mr. Y using the QiaAmp DNA extraction kit (QIAGEN). Amplifications were then performed with the SGM+ kit (Applied Biosystems). The different fragments obtained were electrophoresed on an ABI 377 platform.

3. Results

3.1. Serological markers

We present in Table 1 the various phenotypes obtained using seven different systems. The MNSs, Duffy and HLA AB loci showed us that Anne and Caroline were not identical twins. From all the phenotypes we deduced the possible genotypes of each individual (Table 2). This allowed us to identify maternal and paternal alleles for Anne and Caroline (Table 3). Comparison with Mr. Y's genotypes unambiguously excluded him as Caroline's biological father (results of MNSs and HLA systems). However, Anne's case was far more difficult to resolve as only one marker possibly excluded Mr. Y as the father (i.e. the HLA AB was inconclusive).

3.2. DNA analysis

Fig. 1 shows an example of the results.

Mr. Y was excluded as being the father of Caroline in the vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51 and FGA loci, therefore confirming previous results from the serological analysis.

Surprisingly, Mr. Y was shown to be Anne's father for all tested loci. We calculated a combined paternity index (CPI) of 219,397 (Table 4). This corresponds to a probability of paternity (W) equal to 99.9995% (using a prior odds of 50%).

Table 4
Calculation of paternity index for child Anne

Locus	Mother	Anne	Paternal allele	Alleged father	Frequency	PI
D3S1358	14–15	14–15	14 or 15	15–17	0.064 or 0.263	1.52905199
vWA	14–15	15–15	15	15–18	0.076	6.57894737
D16S539	11–15	11–13	13	12–13	0.186	2.68817204
D2S1338	22–25	22–23	23	23–23	0.076	13.15789474
D8S1179	13–13	13–13	13	11–13	0.39	1.28205128
D21S11	27–32.2	27–29	29	29–29	0.225	4.44444444
D18S51	12–21	12–18	18	14–18	0.093	5.37634409
D19S433	13–15	13–14	14	14–15	0.297	1.68350168
TH01	6–9.3	6–7	7	6–7	0.123	4.06504065
FGA	18–21	21–21	21	19–21	0.17	2.94117647

4. Conclusion

We analysed a case of double ovulation leading to a double fertilisation. Even if one considers a double ovulation to be quite common, the odds for having each ovum fertilised by sperm from a different partner are quite exceptional. Although documented [1–3], this situation must be quite rare (even though one can think that it is underestimated keeping in mind that the DNA technology is recent and that the vast majority of twins are not tested). Also the rarity of such a situation depends not only on whether tests are done but also on social behaviour. From this example, it is clear that routine DNA STR analyses superceded other haematological techniques to unravel the truth of a rather exceptional matter.

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