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Case report

A STR mutation in a heteropaternal twin case

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Abstract

A heteropaternal male twin case with two men being alleged fathers was investigated as requested by the Court. Up to 37 PCR-based polymorphic DNA systems were studied in this case which was complicated by a paternal ACTBP2 mutation detected in one twin. This is the first report on a STR mutation in a double paternity case where both biological fathers were indisputably identified. The STR systems enable the resolution of these complex genetic relationships even in a case where a mutation in one STR locus was encountered.

Keywords: Paternity investigation; Heteropaternal twins; Minisatellites; Short tandem repeats; ACTBP2 mutation

1. Introduction

Dizygous twins may arise by fertilisation of two ova at the same (superfecundation) or at different menstrual cycles (superfoetation). Sexual intercourse of a woman during a polyovulatory period with at least two men may thus lead to superfecundation with the resulting twins having two different biological fathers.

Paternity investigation of male twins was performed as requested by a Portuguese Court. In the first stage, only one alleged father (Af1) was investigated and the results were somewhat puzzling—Af1 seemed to match only one child (Ch1) while several genetic inconsistencies existed to the other child (Ch2). Relative to child 1 there also existed two possible genetic inconsistencies—a second-order genetic inconsistency in Duffy (data not shown) and a possible first order genetic inconsistency in ACTBP2 (Table 1). The second alleged father (Af2) was then studied. Biostatistical evaluation finally lead to two matching fathers, i.e. Af1 matching Ch1 and Af2 matching Ch2, elucidating a rare case of heteropaternal twins arisen by superfecundation [1–8].

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2. Materials and methods

2.1. DNA typing

Genomic DNA was extracted from blood stains by the Chelex method [9]. Polymarker and HLA-DQA1 were detected by reverse dot-blot (Perkin-Elmer), YNH24 and MS 43A were analysed using the RFLP technique with probes, the PCR-based STR loci were analysed by automated fluorescent detection (ALF DNA Sequencer, Pharmacia and ABI Prism 310 DNA Sequencer, Perkin-Elmer/Applied Biosystems). References for the DNA systems studied are given in Table 1 [10–24].

2.2. ACTBP2 sequencing

Sequencing of all ACTBP2 alleles was performed using the Taq Cycle sequencing kit and an ABI Prism 373A DNA Sequencer (Perkin-Elmer/Applied Biosystems).

3. Results and discussion

The DNA typing was performed with respect to classical polymarker loci, minisatellites and microsatellites. Due to

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Table 1
Paternity investigation results and paternity index values[a]

System	Alleged father 1 (Af1)	Twin 1 (Ch1)	Mother (M)	Twin 2 (Ch2)	Alleged father 2 (Af2)	Exclusion between	Reference
HLA-DQA1	4/4	4/4	4/4	1.2/4	1.2/1.2	Af1/Ch2 Af2/Ch1	[10]
LDLR	BB	BB	AB	AB	AA	Af2/Ch1	[10]
GYPA	AA	AB	BB	BB	AB	Af1/Ch2	[10]
HBGG	AB	AA	AA	AA	AA		[10]
D7S8	AB	AA	AA	AA	AA		[10]
Gc	CC	AC	AC	AC	AC		[10]
D1S80	24/31	22/24	18/22	22/22	18/22	Af1/Ch2 Af2/Ch1	[11]
TH01	9/9.3	6/9.3	6/7	6/7	6/7	Af1/Ch2 Af2/Ch1	[12]
FES/FPS	11/13	11/13	12/13	12/12	12/12	Af1/Ch2 Af2/Ch1	[12]
VWA	16/17	16/16	16/16	15/16	15/17	Af1/Ch2 Af2/Ch1	[11]
F13A1	6/14	5/14	4/5	5/5	5/5	Af1/Ch2 Af2/Ch1	[11]
D19S253	8/12	12/12	7/12	7/12	12/12		[13]
D18S51	13/16	13/17	12/17				[13]
D12S391	15/21	15/24	20/24	17/20	16/17	Af1/Ch2 Af2/Ch1	[14]
FGA	23/24	24/24	23/24	23/23	22/23	Af2/Ch1	[15]
APOAI1	287.32	286.79	251.56/287.89				[16]
D21S11	61/67	61/63	63/63				[15]
YNH24	3286/3466	3306/3825	3829/4411				[17]
MS43A	5067/10046	5052/7565	7519/10189				[17]
Paternity index		Af1/Ch1/M 1,249,999					
ACTBP2	20/23.2	17/19	13.2/17	17/31.2	22/31.2	Af1/Ch2 Af2/Ch1	[18,19]
Paternity index		Af1/Ch1/M 9700		Af2/Ch2/M 11,111,110			
D8S1132	17/24	17/18	18/19	18/22		Af1/Ch2	[20]
D1S1656	15.3/16.3	15.3/15.3	15/15.3	15.3/16.3			[21]
D3S1358	14/16	14/16	16/16	16/16			[15]
TPOX	8/8	8/11	8/11	8/8			[22]
CSF1PO	12/12	10/12	10/12	11/12		Af1/Ch2	[22]
D5S818	11/11	11/14	12/14	11/14			[15]
D13S317	8/12	8/12	8/11	11/12			[15]
D7S820	9/11	8/9	8/9	8/10		Af1/Ch2	[15]
Paternity index		Af1/Ch1/M 12,499,999					
DYS19	15	15		15			[23,24]
DYS390	24	24		24			[23,24]
DYS391	11	11		11			[23,24]
DYS392	13	13		14		Af1/Ch2	[23,24]
DYS393	12	12		13		Af1/Ch2	[23,24]
DYS389	13/29	13/29		13/29			[23,24]
DXYS156	X7/Y12	X7/Y12	X7/X7	X7/Y12			[23,24]
Paternity index		Af1/Ch1/M 1,249,999,999					

[a] Paternity indices are based on calculations using the preceding systems above the respective index value and including all preceding calculations.

Table 2
ACTBP2 allele sequences from mother, alleged father 1 and the dizygotic twins

		5′-Flanking region	Repeat region
Mother (M)	13.2	$(AAAG)_3$	$(AAAG)_{14}$
	17	$(AAAG)_3$ AG	$(AAAG)_{17}$
Twin 1 (Ch1)	17	$(AAAG)_3$ AG	$(AAAG)_{17}$
	19	$(AAAG)_3$ AG	$(AAAG)_{19}$
Alleged father 1 (Af1)	20	$(AAAG)_3$ AG	$(AAAG)_{20}$
	23.2	$(AAAG)_3$ AG	$(AAAG)_{11}$ AAAAAG $(AAAG)_{11}$
Twin 2 (Ch2)	17	$(AAAG)_3$ AG	$(AAAG)_{17}$
	31.2	$(AAAG)_3$ AG	$(AAAG)_{14}$ AAAAAG $(AAAG)_{16}$

their high discrimination power short tandem repeats are today's method of choice for forensic individualisation purposes [25].

The genotypes of the loci analysed for the mother, the two alleged fathers and the twins are given in Table 1.

3.1. Analysis of alleged father 1 (Af1)

While Af1 showed 14 genetic inconsistencies towards Ch2, leading to a Ch2 paternity exclusion, only a single genetic inconsistency to Ch1 at the ACTBP2 locus was observed (Table 1). We have therefore analysed this case assuming a mutation at this locus [26].

In addition to the length polymorphism based on the number of repeats, an enormous number of sequence variants has been observed at the ACTBP2 locus [18,19]. We have sequenced all the ACTBP2 alleles and observed a regular 5′-flanking region (Table 2), except for ACTBP2 allele 13.2 of the mother, representing an AG loss, which has been previously found by Rolf et al. [19].

Alleles 19 (Ch1) and 20 (Af1) both show simple repeat structures, respectively, with 19 and 20 AAAG repeats in the repetitive region and no variation in the flanking region. There is only one repeat unit difference between these two alleles. Losses or gains of single repeat units are observed in most microsatellite mutations events [27,28]. The mutation rate of the ACTBP2 locus was determined to be 7×10^{-3} [26], which is very high compared to other loci (e.g. mutation rate of TH01 is 4×10^{-5} [29]). Our biostatistical calculation for the inclusion of the mutation [16,30] at the ACTBP2 locus made, indeed, use of a mutation rate of 7×10^{-3}.

Excluding the ACTBP2 locus from the statistical analysis, a high paternity probability was observed (Table 1), while the inclusion of the ACTBP2 locus led to a lower paternity probability value. We therefore analysed, in this special case, additional autosomal and gonosomal STRs (Table 1).

Concerning Y-chromosomal loci, six Y-loci and the XY locus DXYS156 were statistically evaluated using their haplotype frequencies. Since the haplotype present in Af1 has been observed twice in the Caucasian Y-STR database (based on a European population sample of 4115 minimal haplotypes; URL:http://ystr.charite.de), which does not include DXYS156, we have very conservatively assumed a frequency of 1% for this haplotype.

Inclusion of the Y-STR loci strongly supported paternity over non-paternity (paternity index = 1,249,999,999 and paternity probability = 99.99999992%, based on the assumption of a 0.5 a priori probability). Therefore, it is beyond reasonable doubt that Af1 is the biological father of Ch1. Also, a paternal one-step mutation at the ACTBP2 locus was proven, which resulted in a tetrameric repeat unit loss in an uninterrupted regular allele (Table 2).

3.2. Analysis of alleged father 2 (Af2)

While Af2 showed 10 genetic inconsistencies towards Ch1 leading to a Ch1 paternity exclusion, there was no genetic inconsistencies relative to Ch2 (Table 1) and the final paternity probability reaches 99.999991% (paternity index = 11,111,110, based on the assumption of a 0.5 a priori probability). Therefore, it can be concluded beyond reasonable doubt that Af2 is the biological father of Ch2.

This is the first case of a STR mutation in heteropaternal twins where both fathers were intensively studied by PCR-based DNA polymorphisms. Microsatellites as performed by most forensic laboratories have been found to enable the resolution of complex genetic relationships even in double paternal twin cases where mutations can be encountered.

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