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CASE REPORT

Paternity Testing in Case of Brother-sister Incest

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We performed a paternity test in a case of incest between brother and sister. DNA from blood samples of the alleged parents and their two children was obtained with Chelex DNA extraction method and quantified with Applied Biosystems QuantiBlot® quantitation kit. Polymerase chain reaction (PCR) amplification of DNA samples was performed with AmpFlSTR SGM Plus™ PCR amplification kit and GenePrint® PowerPlex™ PCR amplification kit. The amplified products were separated and detected by using the Perkin Elmer's ABI PRISM™ 310 Genetic Analyser. DNA and data analysis of 17 loci and Amelogenin confirmed the suspicion of brother-sister incest. Since both children had inherited all of the obligate alleles from the alleged father, we could confirm with certainty of 99.999999% that the oldest brother in the family was the biological father of both children. Calculated data showed that even in a case of brother-sister incest, paternity could be proved by the analysis of Amelogenin and 17 DNA loci.

Key words: *DNA fingerprinting; forensic medicine; gentic markers; incest; paternity*

DNA paternity testing, a highly accurate analysis of the genetic profiles of the mother, child, and alleged father, is based on the fact that the child inherits half of its DNA pattern from the mother and half from the father according to the Mendel's laws of inheritance (1). In the beginning, the molecular analysis of parentage relied on blood antigen systems, but the method was limited by the invariability of the loci analyzed (1). With the discovery of highly variable microsatellites, DNA-based paternity testing has become the main method for establishing parentage. Microsatellites are defined as tandem arrays of short stretches of nucleotides repeated between 15 and 30 times. They are widely dispersed in the human genome and form a significant proportion of the growing family of repetitive DNA sequences. Due to their ubiquity, polymerase chain reaction (PCR) typability, Mendelian co-dominant inheritance, and extreme polymorphism, microsatellites have assumed very important role as markers in paternity testing (2). Microsatellite alleles conform to Hardy-Weinberg criteria and Mendelian laws of inheritance (2-4). Tri-, tetra-, and pentanucleotide short tandem repeats (STRs) have been reported to amplify more reliably, and with fewer artefactual bands during PCR amplification. Microsatelites, especially tetra- and pentanucleotide STRs, are therefore excellent candidates for DNA identification systems in forensic analyses (2). The true power of DNA testing lies in the polymorphism of the individual loci and the number of loci tested (5).

Here we present a case of suspected incest between a brother and a sister, which was confirmed with DNA-based paternity testing using 17 loci and Amelogenin locus.

Case Report

In a family with seven children, four brothers and three sisters, one of the sisters reported her suspicion of incest between her oldest brother and one of the sisters. The sister suspected of incest has two children: a girl, who is now four years old, and a boy of two. Police ran a complete criminal investigation and blood samples were taken from the mother, both children, and alleged father (the oldest brother) for DNA analysis.

DNA was extracted from a blood spot of approximately 3 mm^2 in diameter, dried on FTA filter-paper, by using Chelex method (6), and quantified with QuantiBlot® quantitation kit (Applied Biosystems, Foster City, CA, USA) (7). PCR amplification was performed with AmpFlSTR SGM Plus™ PCR amplification kit (Applied Biosystems) and GenePrint® PowerPlex™ PCR amplification kit (Promega Corporation, Madison, WI, USA), and GeneAmp® PCR Systems 9700 (Applied Biosystems). The amplified products were separated and detected on the ABI PRISM™ 310

Genetic Analyser using GenoTyper 2.5.2 software (Applied Biosystems).

Parentage analysis is based on the fact that each allele of a child matches one allele of each parent. The parentage is established on the basis of exclusion, since there is no possible way to absolutely prove that there are no other man or woman in the world who could be the father or mother of that particular child (5). The relative chance of the alleged father *vs* an unrelated individual in the population to transmit the obligate allele is called Paternity Index (PI). If the father is homozygous or has both obligate alleles, his likelihood of transmitting the obligate allele is $x = 1.0$ (2/2). If the alleged father has only one copy of the obligate allele or one of the two obligate alleles, his likelihood of transmitting the obligate allele is $x = 0.5$ (1/2). The PI for a given locus is the likelihood that the alleged father transmitted the allele divided by the frequency of the obligate allele(s). Thus, PI will be either 1/p or 0.5/p. If there are two obligate alleles as determined by the types of the mother and child, *p* can be calculated according to the formula $p = p_1 + p_2$, whereby PI is $1/(p_1 + p_2)$ or $0.5/(p_1 + p_2)$ (8). Multiplication of all individual likelihood ratios generates Combined Paternity Index (CPI) (9): $CPI = PI_1x...xPI_{17}$. In Child 1, CPI was 1.1225×10^{10}. The power of a paternity test can be determined by the calculation of the Random Man Not Excluded (RMNE). The RMNE is the proportion of the population that could contribute all obligate alleles and therefore cannot be excluded. RMNE was calculated by using the formula RMNE = 1-(1-p)2. The Combined Random Man Not Excluded (CRMNE) is analogous to CPI, ie, it is the product of individual values: CRMNE = RMNE1x...x RMNE17 (9). Thus, CRMNE for Child 1 in our case was 1.4548 x 10-10. Reciprocal of this number is (1-CRMNE) is Power of Exclusion (PE). The probability of paternity (PP) is the probability that the alleged father is the bio-

Table 1. Complete paternity examination of Child 1 in the case of brother-sister incest*

STR loci	Mother	Child 1	Obligate allele	Alleged father	p1(Y)	RMNE1	X	PI1
Penta E	12,17	**7**,12	**7**	**7**,17	0,1476*	0.2734	0.5	3.3875
D18S51	12,17	**12**,17	**12**	**12**,12	0.1214	0.3967	0.5	2.2391
			17		0.1019			
D21S11	29,30.2	**29**,30.2	**29**	**29**,30	0.1942	0.9387	0.5	2.0194
			30.2		0.0534			
TH01	9,9.3	9,**9**	**9**	**9**,9.3	0.2129	0.3805	0.5	2.3485
D3S1358	15,18	**14**,15	**14**	**14**,17	0.0693	0.1338	0.5	7.215
FGA	22,26	**25**,26	**25**	**25**,26	0.0874	0.1672	0.5	5.7208
TPOX	8,8	8,**8**	**8**	8,**8**	0.5446	0.7926	1	1.8362
D8S1179	10,13	10,**10**	**10**	**10**,14	0.0728	0.1403	0.5	6.8681
vWA	17,17	**16**,17	**16**	16,**16**	0.1961	0.3537	1	5.0994
Amelogenin	X, X	X,X		X,Y				
Penta D	9,12	12,**13**	**13**	9,**13**	0.1*	0.19	0.5	5.0
CSF1PO	12,15	12,**12**	**12**	11,**12**	0.3564	0.5858	0.5	1.4029
D16S539	9,9	9,**9**	**9**	**9**,12	0.0891	0.1703	0.5	5.6117
D7S820	9,10	9,**11**	**11**	10,**11**	0.1931	0.3489	0.5	2.5893
D13S317	8,11	8, **12**	**12**	11,**12**	0.2379	0.4192	0.5	2.1017
D5S818	11,14	14,**14**	**14**	11,**14**	0.00952*	0.0189	0.5	52.5210
D2S1338	17,19	19,**20**	**20**	17,**20**	0.145*	0.269	0.5	3.4483
D19S433	12,13	13,**15**	**15**	12,**15**	0.165*	0.3028	0.5	3.0303

*Abbreviations: STR – short tandem repeat; p(Y) – allele frequency for each allele or obligate allele based on Croatian database (10), indicating the frequencies taken from Ivan Vučetić Forensic Science Center DNA laboratory database; RMNE – random man not excluded; X – likelihood that the alleged father can transmit the obligate allele, determined by the father's type (1/CRMNE); PI – paternity index.

Table 2. Complete paternity examination of Child 2 in the case of brother-sister incest*

STR loci	Mother	Child 2	Obligate allele	Alleged father	p2(Y)	RMNE2	X	PI2
Penta E	12,17	**7**,17	**7**	**7**,17	0.1476*	0.2734	0.5	3.3875
D18S51	12,17	12,**12**	**12**	**12**,12	0.1214	0.2281	1	8.2372
D21S11	29,30.2	**29**,30.2	**29**	**29**,30	0.1942	0.4339	0.5	2.0194
			30.2		0.0534			
TH01	9,9.3	9,**9**	**9**	**9**,9.3	0.2129	0.3805	0.5	2.3485
D3S1358	15,18	15,**17**	**17**	14,**17**	0.1683	0.3083	0.5	2.9709
FGA	22,26	26,**26**	**26**	25,**26**	0.0388	0.0761	0.5	12.8866
TPOX	8,8	8,**8**	**8**	8,**8**	0.5446	0.7926	1	1.8362
D8S1179	10,13	13,**14**	**14**	10,**14**	0.2573	0.4484	0.5	1.9433
vWA	17,17	**16**,17	**16**	16,**16**	0.1961	0.3537	1	5.0994
Amelogenin	X,X	X,Y		X,Y				
Penta D	9,12	12,**13**	**13**	9,**13**	0.1*	0.19	0.5	5.0
CSF1PO	12,15	12,**12**	**12**	11,**12**	0.3564	0.5858	0.5	1.4029
D16S539	9,9	9,**12**	**12**	9,**12**	0.2723	0.4705	0.5	1.8362
D7S820	9,10	9,**11**	**11**	10,**11**	0.1931	0.3489	0.5	2.5893
D13S317	8,11	8,**11**	**8**	**11**,12	0.0922	0.7199	0.5	1.0620
			11		0.3786			
D5S818	11,14	11,14	**11**	**11**,**14**	0.3447	0.5829	1	2.8231
			14		0.00952*			
D2S1338	17,19	19,**20**	**20**	17,**20**	0.145*	0.2689	0.5	3.4483
D19S433	12,13	12,**12**	**12**	**12**,15	0.083*	0.1591	0.5	6.0241

*Abbreviations: STR – short tandem repeat; p(Y) – allele frequency for each allele or obligate allele based on Croatian database (10), indicating the frequencies taken from Ivan Vučetić Forensic Science Center DNA laboratory database; RMNE – random man not excluded; X – likelihood that the alleged father can transmit the obligate allele, determined by the father's type (1/CRMNE); PI – paternity index.

logical father of the child: PP = 1/1 + (1/CPI) (9). PP in Child 1 in our case was 99.999999991%. Regarding RMNE, there was a 99.999999% chance of excluding falsely the alleged father. In other words, the alleged father was approximately 6,873,797,086 times as likely to be the father to Child 1 as an unrelated Croatian male (Table 1).

For Child 2 in our case, CPI was 1.9148 x 10^8, and CRMNE was 9,9603 x 10^{-9}, which yielded a 99.9999995% probability of paternity. Regarding RMNE, there was a 99.999999% chance of excluding falsely the alleged father, because he was approximately 100,398,582 times as likely to be the father to Child 2 as an unrelated Croatian male (Table 2).

This analysis of results showed that both children had inherited all of the obligate alleles from the alleged father.

Discussion

The basic function of parentage testing is to exclude the maximum number of individuals that could be biological fathers of the child in question. This is done by identifying the obligate allele in the child and determining if the alleged father also carries this allele (1). The obligate allele is the allele which a child obligatorily inherits from the biological father. If an alleged father is not excluded, the weight of this evidence can be calculated. Since progenitors are closely related, it is very likely that the alleles inherited from the mother are included in the father's DNA profile. Therefore, we used 17 loci and Amelogenin for paternity testing. DNA typing results revealed that the oldest brother in the family was the biological father of both children. Calculated data showed that even in a case of brother-sister incest, paternity could be proven with a high degree of certainty by analysis of 17 DNA loci and Amelogenin.

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