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Case Report

Analysis of a Paternity Case in which the Alleged Father was Deceased: Single Locus Mismatch

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Abstract We performed a paternity test without the alleged father, who was deceased, and we found a mismatch in one of the alleles of the autosomal short tandem repeat (STR) locus D3S1358 in the illegitimate daughter. DNA genotypes of the dead alleged father were estimated from the DNA genotypes of his wife and their four children. The ABO genotype, the DXS10011 locus and the calculations using the 12 STR loci and the D1S80 locus, but not the STR locus D3S1358 suggested that there was a high probability that the dead alleged father was in fact the actual biological father. The genotype of locus D3S1358 of the dead alleged father, the illegitimate daughter's mother and the illegitimate daughter was 15 / 17, 15 / 17 and 15 / 18, respectively. PCR and sequencing after TA cloning of allele 17 of one of the children who had only a homozygote of allele 17, of allele 17 of the illegitimate daughter's mother and of allele 18 of the illegitimate daughter revealed that allele 18 which was considered to be a mutated allele had one more repeat of (GATA) within the first repeat region.

Key words: Paternity test, STR, D3S1358 locus, Single locus mismatch, Mutation, Slippage

Introduction

Short tandem repeats (STRs) are highly polymorphic in both their sequence and their length, with the result that this property has been exploited for paternity tests. However, one of the limitations of STRs is that the mutation rates in microsatellites are very high compared to other polymorphic markers. For this reason, some cases of a single locus mismatch have been reported among affirmed paternity cases.

We performed a paternity test without the alleged father who was deceased and we found a single locus mismatch in the useful autosomal STR locus D3S1358[1)2)].

Materials and Methods

An illegitimate daughter hoped to confirm that a deceased man was her father by DNA profiling. Blood samples were obtained from the wife of the dead alleged father and from their four children, as well as from the illegitimate daughter and her own mother. After DNA extraction using the QIAamp DNA Mini Kit (QIAGEN), we performed PCR using the AmpFlSTR Profiler Plus™ PCR Amplification Kit and the COfiler™ PCR Amplification Kit (Applied Biosystems) which were usually used for personal identification in a field of forensic science because of high heter-

ozygosity rate and a lot of allele numbers. PCR products were run on the ABI PRISM 310 DNA Sequencer. DNA analysis was performed by GeneScan and Genotyper Software. D1S80 typing and ABO genotype were also analyzed. TA cloning of the mismatch allele was performed in the illegitimate daughter, her own mother and one of the children of the alleged father who had clearly inherited the allele from the father.

Results and Discussion

The results of STRs, D1S80 and ABO genotype of the 7 individuals are shown in Table 1. We estimated the DNA types of the dead alleged father based on the findings from the legitimate children and their mother. After that, we examined whether or not the illegitimate daughter had one of the alleles of the dead alleged father, and we found that she did indeed have one of the alleles of the dead alleged father in all loci except for the STR locus D3S1358 (Table 1). Accordingly, we also analyzed the DXS10011 locus which is one of the X chromosome-specific STR loci and which has a particularly high ability of helping with identification[3]. The illegitimate daughter had the same allele, which had a peak at 239.05 − 239.28 bp by ABI 310 analysis using POP6, as the two legitimate female children of the alleged father. This same allele was inherited from the alleged father by all three daughters and the frequency of its appearance is reported as being 0.0483. The probability of paternity as calculated by using the 12 STR loci and the D1S80 locus, but not the D3S1358 locus was between 0.999999954 and 0.999999978. The ABO genotype and these results suggest that there was a high probability that the dead alleged father was in fact the biological father of the illegitimate daughter.

The genotype of locus D3S1358 of the dead alleged father and the illegitimate daughter's mother was 15 / 17 and 15 / 17, respectively. The expected genotype of the

Table 1 Results of DNA analysis of all members concerned in the paternity test and DNA types of the dead alleged father as estimated from four legitimate children and their mother. Examination to show whether the illegitimate daughter had one of the alleles of the dead alleged father or not. Underlined alleles denote the alleles which were inherited from the dead alleged father.
IM, illegitimate daughter's mother; ID, illegitimate daughter; AF, dead alleged father; LM, legitimate children's mother (wife); C1 (son), C2 (son), C3 (daughter), and C4 (daughter), legitimate children, respectively.

	IM	ID	AF	LM(wife)	C1(son)	C2(son)	C3(daughter)	C4(daughter)
D3S1358	15, 17	15, 18*	15, 17	15, 17	15, 15	15, 17	15, 17	17, 17
vWA	16, 17	17, 17	14, 17 or 16, 17	14, 16	14, 17	14, 16	16, 17	16, 17
FGA	22, 23	22, 22	22, 24	20, 21	21, 22	21, 24	21, 22	21, 24
D8S1179	13, 13	12, 13	12, 13	10, 14	10, 13	12, 14	13, 14	10, 12
D21S11	29, 29	29, 31	30, 31	29, 30	30, 30	29, 30	30, 31	30, 30
D18S51	12, 15	12, 15	11, 15	15, 25	15, 15	11, 25	11, 25	11, 25
D5S818	10, 11	11, 14	12, 14	12, 13	12, 12	12, 12	12, 13	12, 14
D13S317	8, 8	8, 8	8, 9	8, 8	8, 9	8, 8	8, 8	8, 9
D7S820	9, 13	10, 13	10, −	11, 12	10, 12	10, 12	10, 12	10, 11
D16S539	11, 12	10, 12	9, 10	9, 12	9, 9	9, 12	9, 9	10, 12
TH01	7, 9	9, 9.3	6, 9.3	7, 8	6, 8	6, 7	8, 9.3	8, 9.3
TPOX	8, 11	8, 9	8, 9 or 9, 11	8, 11	8, 11	8, 9	8, 9	9, 11
CSF1PO	11, 13	11, 12	12, 15	13, 14	14, 15	14, 15	12, 14	13, 15
D1S80	16-31	16-18	18-31	28-31	31-31	18-31	18-28	31-31
ABO genotype	O^AO^G	AO^G	AO^G	AO^A	AA	AA	AO^G	AO^G

*Mismatch allele between the alleged father and illegitimate daughter

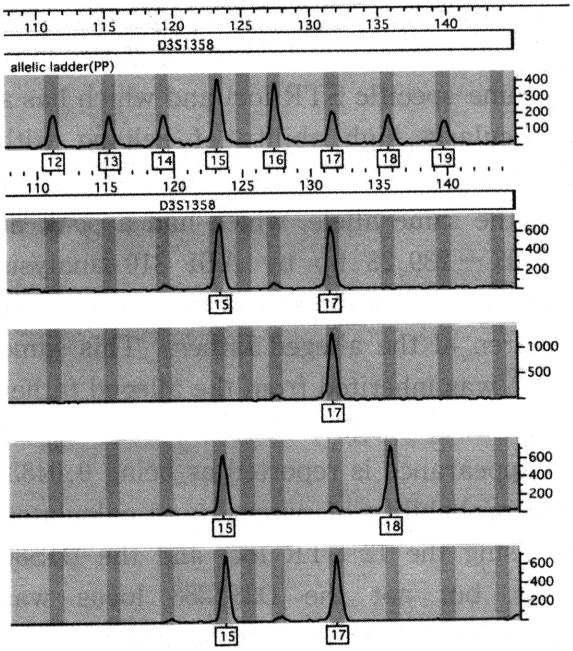

Fig. 1 Profiles of D3S1358 locus of alleged father's wife, legitimate daughter C4, illegitimate daughter and her mother in Genotyper view after GeneScan analysis.
Five berths show allelic ladder, alleged father's wife, legitimate daughter C4, illegitimate daughter and her mother from the upper beath, respectively.

illegitimate daughter would therefore have been 15 / 15, 17 / 17 or 15 / 17, but it was in fact 15 / 18 (Fig. 1). These results show an allele mismatch in locus D3S1358 in the illegitimate daughter. It was considered that the mismatch allele 18 of the illegitimate daughter was derived from allele 17 of the father or the mother. However, both her mother and the alleged father had the same D3S1358 genotype. Furthermore, the legitimate children's mother also had the same genotype. One of the legitimate children (C4 in Tables 1 and 2) had only a homozygote of allele 17 at locus D3S1358. PCR and sequencing after TA cloning of allele 17 of C4 showed only one allele with the structure $(GATA)_{13}(GACA)_2(GATA)_2$. This result indicates that the dead alleged father had this same allele. Sequencing of all the clones of allele 17 of the illegitimate daughter's mother and all the clones of allele 18 of the illegitimate daughter also revealed the same structure $(GATA)_{13}(GACA)_2(GATA)_2$ and $(GATA)_{14}(GACA)_2(GATA)_2$, respectively. Allele 18 which was considered to be a mutated allele had one more repeat of (GATA) within the first repeat region (Fig. 2). However, it was impossible to identify that whether this mutated allele had derived from the dead alleged father or from the mother because they both shared the same sequence in this locus.

It has been considered that this mutation occurs during meiosis and it has been obser-

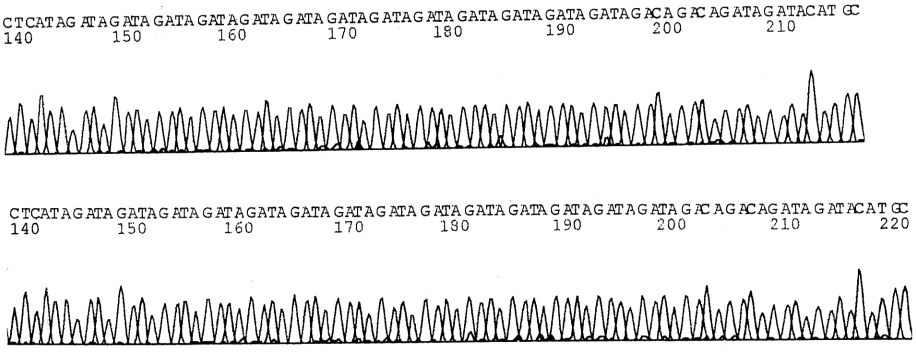

Fig. 2 Sequence electropherogram of the locus D3S1358 of clones of allele 17 (upper) and 18 (under) from legitimate daughter C4 (upper) and the illegitimate daughter (lower), respectively.

ved to have a higher probability in fathers than in mothers. It is also reported that the mutation rate of D3S1358 locus is higher in paternal meioses than in maternal meioses, with a six-fold frequency based on the mutations observed at STR loci in the course of paternity testing. Therefore it can perhaps be considered that allele 18 of the illegitimate daughter had been derived from the dead alleged father.

It is known that STRs are prone to mutation for replication slippage[4]. The mutation rate of D3S1358 is reported to be 0.13 %. Therefore a single locus mismatch in a paternity test is not regarded as a negation of the parent-child relationship. A high probability of paternity based on other findings besides the mutated allele is also important. Results of additional tests which have a high ability of helping with identification, for example, Y chromosome-specific STR in males or X chromosome-specific STR in females, should also point toward a high probability of paternity in such cases.

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(和文抄録)

擬父死亡の親子鑑定例：一座位のみの不一致

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擬父が既に死亡している親子鑑定を実施した．妻と4人の嫡出子のDNA型から推定した擬父の型と妻でない女性を母とした非嫡出子の12種類のSTR型においてD3S1358座位のみで不一致を認めた．ABO式血液型の遺伝子型とX-STR型のDXS10011座位およびD3S1358座位を除く他の座位におけるDNA型による父権肯定確率は擬父がこの非嫡出子の生物学的父親であることを示していた．D3S1358座位における遺伝子型は擬父，非嫡出子の母共に15 / 17であったが，非嫡出子は15 / 18であった．擬父の対立遺伝子17が遺伝していると考えられる娘と非嫡出子およびその母のD3S1358座位のクローニングおよびシークエンスにより，非嫡出子の対立遺伝子18は(GATA)の繰り返しが一回多い突然変異であることが判明した．