Biomedical Science Letters 2022, 28(4): 327~333
https://doi.org/10.15616/BSL.2022.28.4.327
eISSN : 2288-7415

Brief Communication

A Case of Single-step Mutations at Two Short Tandem Repeat loci (D13S317 and DXS10148) among Three Generations of a Korean Family

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The DNA profiling of short tandem repeat (STR) markers is a powerful tool for forensic identification and forensic paternity testing. However, STR loci are susceptible to mutation that cause mismatches between parents and children when paternity is tested. Herein, we examined paternity disputes with 23 autosomal STR loci using two commercial human identification kits and revealed successive mismatches at the D13S317 locus across three generations of a Korean family. Additionally, we investigated 12 X-chromosomal STRs and discovered an inconsistency at the DXS10148 locus between the father and daughter of the same Korean family. Furthermore, we confirmed STR genotypes at the D13S317 and DXS10148 loci of the family using sequencing analysis. Consequently, we identified a successive single-step mutation at the D13S317 locus and one single-step mutation at the DXS10148 locus in three generations of the Korean family. Therefore, this case study may be useful for interpreting and understanding forensic paternity tests.

Key Words: Short tandem repeat (STR), DNA profiling, Paternity testing, Single-step mutation, Korean family

Microsatellites, also known as short tandem repeats (STRs), are widely used as genetic markers for human identification (HID) in forensic science and are applied in paternity testing (Lander et al., 2001; Lu et al., 2012). More than one million STR loci are estimated to exist in the human genome and are considered one of the most variable regions of DNA sequences in the human genome (Weber, 1990). Therefore, STR profiling is a powerful tool in paternity testing and forensic identification (Singh Negi et al., 2006). However, STR loci are susceptible to mutations, and three mechanisms have been suggested for these (Fan and Chu, 2007) with the main contributor being replication slippage (Levinson and Gutman, 1987; Schlotterer and Tautz, 1992). These STR mutations cause allelic inconsistency among families and misinterpretation in forensic paternity tests. Therefore, uniparental markers, such as X-chromosomal STRs (X-STRs), Y-chromosomal STRs, or hypervariable regions (HV1 and HV2) of mitochondrial DNA, were additionally analyzed to supplement kinship establishment (Singh Negi et al., 2006; Dumache et al., 2018). Although the proportion of single-step mutations is likely overestimated (Slooten and Ricciardi, 2013), most STR mutations correspond to single-step mutations that result in the gain or loss of a single repeat unit (Weber and Wong, 1993; Junge et al., 2006), and no consecutive single-step mutations have been reported in the same STR locus in Korean families.

This study analyzed 23 autosomal STR (A-STR) loci and 12 X-STR loci for the Korean family that already affirmed their kinship relationship. We present a case of successive single-step mutations of the D13S317 locus across the three

Received: October 6, 2022 / Revised: December 5, 2022 / Accepted: December 12, 2022

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generations and one single-step mutation at the DXS10148 locus between parents and child (daughter) of the family.

The genomic DNA of the family comprised of grandfather, grandmother, father, mother, and child was extracted from buccal samples stored on Whatman FTA cards using a QIAamp DNA Micro Kit (Qiagen, NRW, Germany) according to the manufacturer's protocol. And all participants provided written informed consent for participation. Extracted genomic DNA was quantified using a 7500 Real-Time PCR instrument (Applied Biosystems, CA, USA) and the Quantifiler™ Trio DNA Quantification Kit (Thermo Fisher Scientific, MA, USA). The use of samples and analytical procedures were approved by the Institutional Review Board (IRB) of the National Forensic Service (*Approval No. 906-211221-BR-002-02, 906-211221-BR-001-03*).

STR profiling for 23 A-STR loci was performed using two commercial HID kits, GlobalFiler™ PCR Amplification Kit (Thermo Fisher Scientific) and PowerPlex® Fusion System (Promega, WI, USA), according to the manufacturer's instructions. Because the mother and her child (daughter) were female, 12 X-STR loci were profiled using the Argus X-12 QS Kit (Qiagen) according to the manufacturer's instructions. All amplification reactions were performed on GeneAmp PCR system 9700 (Thermo Fisher Scientific). Subsequently, capillary electrophoresis (CE) was conducted on an Applied Biosystems 3,500 xL Genetic Analyzer using a 36 cm capillary and POP-4 polymer (Thermo Fisher Scientific), and the data were analyzed with GeneMapper ID-X v1.4 software (Thermo Fisher Scientific).

For sequencing analysis of the STR loci D13S317 and

Table 1. Genotypes of the family for 23 autosomal STR loci

No.	Locus	Grandfather	Grandmother	Mother	Father	Child (daughter)
1	D3S1358	16, 18	16, 16	16, 16	16, 16	16, 16
2	vWA	14, 17	18, 18	14, 18	16, 16	16, 18
3	D16S539	9, 11	9, 13	9, 11	12, 12	11, 12
4	CSF1PO	11, 12	10, 12	12, 12	11, 12	12, 12
5	TPOX	8, 9	8, 11	8, 9	8, 8	8, 8
6	D8S1179	13, 14	12, 15	13, 15	10, 13	13, 13
7	D21S11	30, 30	30, 30	30, 30	28.2, 30	30, 30
8	D18S51	14, 17	14, 22	14, 17	13, 15	13, 14
9	D2S441	12, 14	10, 11	10, 12	10, 14	10, 10
10	D19S433	13, 15.2	11, 14.2	14.2, 15.2	14.2, 16.2	14.2, 14.2
11	TH01	9.3, 10	6, 9	9, 9.3	9, 9	9, 9.3
12	FGA	21, 24	26, 27	24, 26	22, 25	22, 24
13	D22S1045	16, 17	16, 16	16, 16	16, 17	16, 17
14	D5S818	9, 12	13, 13	9, 13	10, 11	9, 11
15	**D13S317**	**8, 12**	**10, 13**	**10, 13[a]**	**8, 11**	**11, 14[b]**
16	D7S820	10, 11	11, 13	11, 13	11, 12	11, 13
17	D10S1248	14, 15	13, 15	13, 14	13, 14	14, 14
18	D1S1656	12, 13	15, 17.3	13, 15	15, 17	13, 15
19	D12S391	17, 20	17, 18	17, 20	18, 18	17, 18
20	D2S1338	23, 24	19, 20	19, 24	18, 18	18, 19
21	Penta E	10, 17	11, 11	10, 11	11, 12	10, 11
22	Penta D	12, 13	10, 12	12, 13	9, 11	11, 12
23	SE33	24.2, 31.2	17, 18	17, 24.2	28.2, 31.2	17, 31.2
	Ameolgenin	XY	XX	XX	XY	XX

[a]The mutated allele that is mismatched with the grandfather, [b]The mutated allele that is mismatched with the mother

Table 2. Genotypes of the family for 12 X-chromosomal STR loci

No.	Locus	Grandfather	Grandmother	Mother	Father	Child (daughter)
1	DXS10103	16	16, 17	16, 17	18	17, 18
2	DXS8378	11	10, 10	10, 11	10	10, 11
3	DXS7132	14	15, 17	14, 17	15	14, 15
4	DXS10134	42.3	34, 37	34, 42.3	36	34, 36
5	DXS10074	16	16, 17	16, 17	16	16
6	DXS10101	28	30, 32	28, 30	30.2	30, 30.2
7	DXS10135	19	18, 20	19, 20	20	19, 20
8	DXS7423	15	14, 15	15, 15	15	15, 15
9	DXS10146	29	23, 29	29, 29	34.3	29, 34.3
10	DXS10079	20	18, 19	19, 20	20	20, 20
11	**DXS10148**	**28.1**	**23.1, 26.1**	**26.1, 28.1**	**30.1**	**28.1, 29.1**[a]
12	HPRTB	13	13, 13	13, 13	13	13, 13

[a]The mutated allele that is mismatched with the father

DXS10148, samples were amplified in a total volume of 25 μL using the primers selected from the literature (Singh Negi et al., 2006; Hundertmark et al., 2008; Gomes et al., 2016) (Supplementary Table 1). The PCR mixture contained 1 ng of DNA template, 2.5 U of AmpliTaq Gold polymerase (Thermo Fisher Scientific), 2.5 μL of Gold ST*R 10X buffer (Promega), 1 μL of each primer (10 pmol), and distilled water. Amplification was performed on a GeneAmp PCR system 9700 (Thermo Fisher Scientific) under the following conditions: 96℃ for 15 min; 35 cycles of 94℃ for 20s, 56℃ for 30s, and 72℃ for 1 min; and a final extension at 72℃ for 7 min. All amplified PCR products were purified by adding 10 μL of ExoSAP-IT™ (Thermo Fisher Scientific) at 37℃ for 30 min and 80℃ for 20 min. Purified PCR products were TA cloned and sequenced by Bioneer Inc. (Daejeon, Korea).

STR profiling for 23 A-STR loci of the family that already confirmed their kinship relationship was conducted, and consecutive mismatches at the D13S317 locus in the mother and her child (daughter) were observed (Table 1). Furthermore, in 12 X-STR profiling, conducted to gain more genetic information, we discovered an inconsistency at the DXS10148 locus in the daughter (Table 2). The alleles for the D13S317 locus in the grandfather, grandmother, mother, father, and child (daughter) were 8/12, 10/13, 10/13, 8/11, and 11/14, respectively (Fig. 1). We confirmed that the same DNA profiles were obtained with both kits, the GlobalFiler™ PCR Amplification Kit and PowerPlex® Fusion System. The alleles observed at the DXS10148 locus in the grandfather, grandmother, mother, father, and child (daughter) were 28.1, 23.1/26.1, 26.1/28.1, 30.1, and 28.1/29.1, respectively (Fig. 2). Sequencing was performed to confirm the STR genotypes. The resulting allele sequences were identical to the CE length on both STR loci, D13S317 and DXS10148, considering the sequence variation at the D13S317 locus (Table 3) (Gettings et al., 2015).

STR mutations cause discrepancies between parents and children, making it difficult to establish kinship relationships. However, some kinship analyses do not exclude paternity if one or two STR loci are mismatched between parents and their children (Dumache et al., 2018). In such cases, lineage markers (e.g., X-STRs, Y-chromosomal STRs, or mitochondrial DNA) can be crucial for demonstrating genetic evidence when assessing kinship relationships (Quiroz-Mercado et al., 2017).

This study identified a successive single-step mutation of the D13S317 locus across three generations of the Korean family that conclusively established a kinship relationship. Moreover, as a result of X-STR analysis to compensate for the genetic information, we discovered a complete match on all alleles between grandparents and the mother, while there was a mismatch in locus DXS10148 between parents

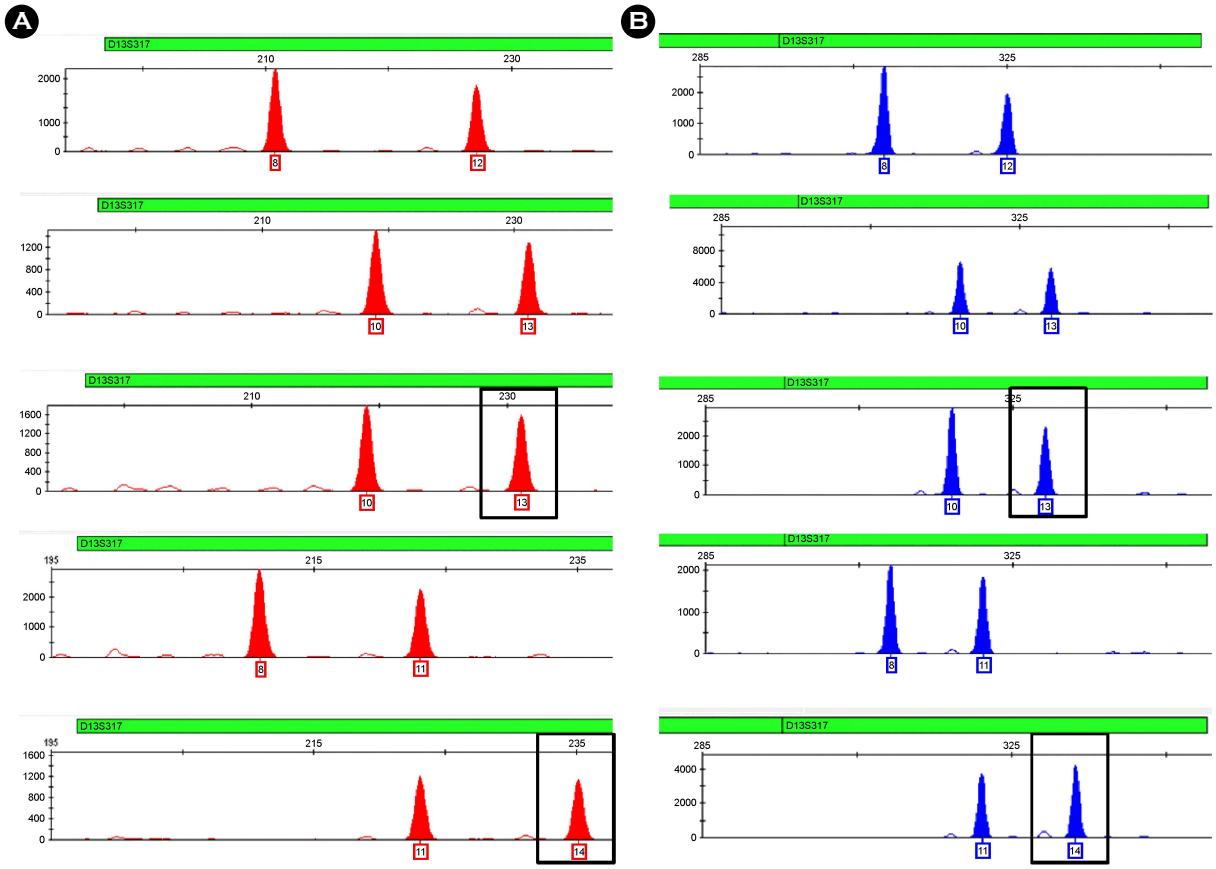

Fig. 1. Electropherograms showing results at the D13S317 locus of the family. (A) Profile obtained with GlobalFiler™ PCR Amplification Kit. (B) Profile obtained with the PowerPlex® Fusion System. Mutated alleles are shown in boxes. From top to bottom: grandfather, grandmother, mother, father, and child (daughter).

Table 3. Allele sequences of D13S317 and DXS10148 from the family

Locus	Sample	Allele	Repeat structure
D13S317	Grandfather	8	$(TATC)_8(AATC)_2(ATCT)_3$
		12	$(TATC)_{13}(AATC)(ATCT)_3$
	Grandmother	10	$(TATC)_{11}(AATC)(ATCT)_3$
		13	$(TATC)_{14}(AATC)(ATCT)_3$
	Mother	10	$(TATC)_{11}(AATC)(ATCT)_3$
		13	$(TATC)_{14}(AATC)(ATCT)_3$
	Father	8	$(TATC)_8(AATC)_2(ATCT)_3$
		11	$(TATC)_{12}(AATC)(ATCT)_3$
	Child	11	$(TATC)_{12}(AATC)(ATCT)_3$
		14	$(TATC)_{15}(AATC)(ATCT)_3$
DXS10148	Mother	26.1	$(GGAA)_4(AAGA)_{17}A(AAAG)_3N_8(AAGG)_2$
		28.1	$(GGAA)_4(AAGA)_{17}A(AAGA)(AAAG)_4N_8(AAGG)_2$
	Father	30.1	$(GGAA)_4(AAGA)_{19}A(AAGA)(AAAG)_4N_8(AAGG)_2$
	Child	28.1	$(GGAA)_4(AAGA)_{17}A(AAGA)(AAAG)_4N_8(AAGG)_2$
		29.1	$(GGAA)_4(AAGA)_{18}A(AAGA)(AAAG)_4N_8(AAGG)_2$

N8=AAGGAAAG

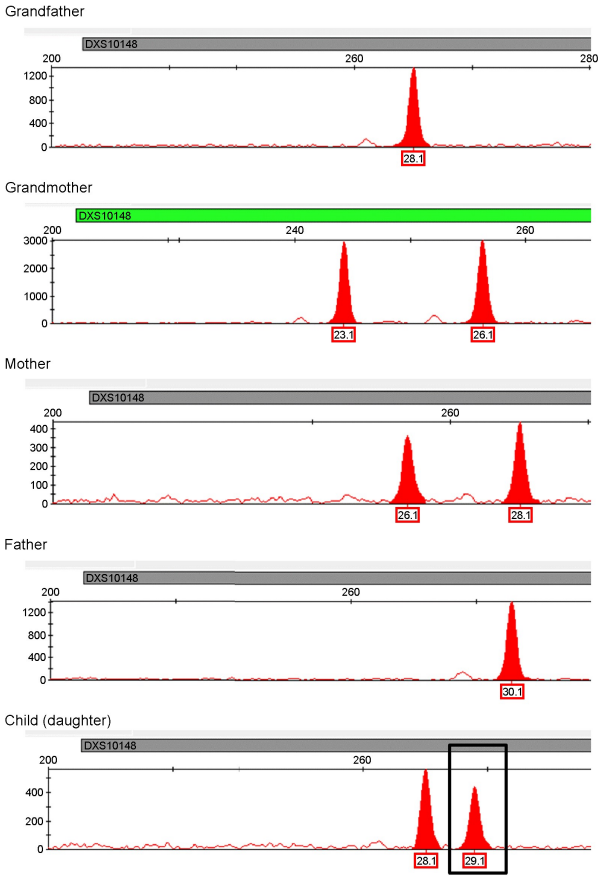

Fig. 2. Electropherograms of genotypes at the DXS10148 locus of the family, obtained using Argus X-12 QS kits. Mutated allele is shown in boxes.

and their child (daughter). Subsequently, we verified STR genotypes by TA cloning and sequencing.

It is known that paternal mutations are five to six times more frequent than maternal mutations during paternal meiosis (Ellegren, 2000; Junge et al., 2006; Muller et al., 2010), and previous studies have shown that single-step mutations are the most common STR mutations, followed by double-step mutations and extremely rare multistep mutations (Weber and Wong, 1993; Brinkmann et al., 1998). Therefore, based on STR profiling, the observed alleles at locus D13S317 of the grandfather, the grandmother, and child (mother) were 8/12, 10/13, and 10/13 (Table 1), indicating a paternally transmitted single-step mutation. The alleles for the D13S317 locus in the father and the child (daughter) were 8/11 and 11/14, respectively (Table 1), which suggests a maternally transmitted single-step mutation. In addition, the alleles of the DXS10148 locus in the mother, father, and child (daughter) were 26.1/28.1, 30.1, and 28.1/29.1, respectively (Table 2), implying a paternally transmitted single-step mutation.

Sequencing of the STR loci to verify the STR repeat motifs revealed that sequences of DXS10148 were identical to the CE length, while some sequences of D13S317 were not consistent with the CE length. D13S317 is a $(TATC)_n$ tetranucleotide repeat on the long arm of chromosome 13. The sequences of allele 8 for D13S317 indicated $(TATC)_8$, but alleles 10, 11, 12, 13, and 14 indicated $(TATC)_{11}$, $(TATC)_{12}$, $(TATC)_{13}$, $(TATC)_{14}$, and $(TATC)_{15}$, respectively due to the nearby flanking-region variant (*rs9546005*) (Table 3). *rs9546005* was found to be common in the Korean population (minor allele frequency; 0.2 in the Korean Genome Project), and this would make it appear as if there is one additional repeat sequence, whereas length-based methods would not count these additional repeats. High-throughput sequencing has made great strides in forensic analysis, and it has disclosed the actual variation of the STR loci (Borsting and Morling, 2015), such as complex and compound STRs (Dalsgaard et al., 2014; Gelardi et al., 2014; Scheible et al., 2014). However, length-based analysis is usually considered sufficient for HID in forensic science (Gettings et al., 2015).

In conclusion, we identified a successive single-step mutation at the D13S317 locus and one single-step mutation at the DXS10148 locus among three generations of a Korean family. Although additional studies are necessary to investigate paternity disputes in extended Korean families, the present case study may be useful for interpreting and understanding forensic paternity tests.

ACKNOWLEDGEMENT

This work was supported by the National Forensic Service (*NFS2022DNA02*), Ministry of the Interior and Safety, Republic of Korea.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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https://doi.org/10.15616/BSL.2022.28.4.327

Cite this article as: Youn BJ, Lee K, Kim CH. A Case of Single-step Mutations at Two Short Tandem Repeat loci (D13S317 and DXS10148) among Three Generations of a Korean Family. Biomedical Science Letters. 2022. 28: 327-333.

Supplementary Table 1. Primer information for sequencing anaylsis

Locus	Sequences (5'-3')
D13S317	
Forward	TGGG ATGG GTTG CTGG ACAT GG
Reverse	AAAA CATA TTCA GAGA GCTT GAA
DXS10148	
Forward	CCTG CATG ACAG AGGG AGA
Reverse	GCAC ATGT ACCC CTGA ACCT