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A D19S433 Primer Binding Site Mutation and the Frequency in Japanese of the Silent Allele It Causes

ABSTRACT: Short tandem repeat studies are powerful tools for parentage analysis and for identification of missing persons, victims of murder, and victims of mass fatalities when reference samples are unavailable. The primer in the Identifiler® kit failed to amplify an allele at the D19S433 locus, producing a silent (''null'') allele. The causal mutation is a base change (G>A) 32 nucleotides downstream from the 3′ end of the AAGG repeats. The silent alleles are problematical in parentage analysis because when transmitted, they can cause a parent–child inconsistency that is unrelated to Mendelian genetics. The inconsistency is sometimes termed an ''apparent opposite homozygosity'' and it produces false evidence of nonparentage. Alternative primers were designed to amplify the D19S433 locus alleles and they detect the silent allele. Frequencies of the (no longer) silent allele were determined to be 0.0114 in 176 people from Shizuoka (Honshu) and 0.0128 in 156 people from Okinawa.

KEYWORDS: forensic science, DNA typing, allele-specific polymerase chain reaction, short tandem repeat, paternity testing, silent allele, D19S433

Short tandem repeat (STR) analysis is a powerful tool for including or excluding an individual as a possible source of a biological sample, and for parentage analysis. It can identify murder victims, victims of mass fatality, and missing persons even when a direct reference sample from the individual is unavailable (1–3). Since November 2006, the AmpFℓSTR® Profiler® kit (4) has been replaced by the AmpFℓSTR® Identifiler® kit (Applied Biosystems, Foster City, CA) (5,6) for analyzing casework samples in 47 forensic science laboratories attached to the Prefectural Police Headquarters across Japan. The Identifiler® kit contains six autosomal STR loci, D2S1338, D8S1179, D16S539, D18S51, D19S433, and D21S11, in addition to the nine autosomal STR loci included in the Profiler® kit. The Identifiler® kit enables simultaneous amplification and typing of the 15 STR loci. In parentage analysis, we found several cases in which there was a single inconsistency in the Mendelian inheritance pattern at D19S433. The cases were referred to us by biologists at local forensic science laboratories. In all cases, a putative parent and a child appeared to be homozygous for different alleles at D19S433 (e.g., the genotype of a putative parent was 12, 12 and that of the child was 14, 14). If a silent allele that cannot be amplified using the Identifiler® kit is present, the inconsistency can be explained by inheritance of the silent allele by the child from the biological parent. Single inconsistencies have been observed at other loci included in the Identifiler® kit, but their frequencies were much lower than D19S433. Furthermore, either or both a putative parent and child were heterozygotes in most cases, suggesting that a possible cause of the inconsistency was not a silent allele but a germline mutation in the repeat number. Therefore, it was anticipated that the frequency of a silent allele at D19S433 might be much higher in the Japanese population, compared to other loci. We report here the cause of the D19S433 silent allele and its frequency in two distant regions of Japan. A method is described for detection of the silent allele and we discuss the impact of the D19S433 silent allele on human identification and parentage analysis.

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Materials and Methods

DNA Samples

Blood samples were collected at random from 176 individuals in Shizuoka and 156 in Okinawa regions. DNA was isolated from 200 μL of whole blood and eluted with 200 μL of TE buffer by MagNA Pure LC (Roche Diagnostics, Mannheim, Germany) or BioRobot® EZ1 (QIAGEN, Hilden, Germany), both of which use magnetic silica beads.

Direct Sequencing

DNA fragments containing the D19S433 repeat regions were amplified using the primer set #1, WILD or SILENT (Table 1). PCR product sizes shown in Table 1 include the 3′ adenine added by the template-independent activity of DNA *Taq* polymerase (7). Each primer set, WILD and SILENT, contained an allele-specific reverse primer. An allele of interest could be amplified selectively from a heterozygous sample of wild-type and silent alleles using the primer set WILD or SILENT. PCR reactions were performed in a total volume of 50 μL containing 50 pmol for each primer, 10 ng genomic DNA, 5 U AmpliTaq Gold® DNA polymerase (Applied Biosystems) and 1× GoldST*R Buffer (Promega, Madison, WI). Thermal cycling was performed using a GeneAmp®

TABLE 1—*Sequences of primers designed for direct sequencing and STR typing with information on primers in the Identifiler® kit.*

Primer Set ID	Primer ID	Sequence*	Label	PCR Product Size (bp)†
No. 1	1fwd	ATTTCTAAGGCTGGGTGAGGTG		
	1rev	TTAAGGAACAGGTGGTGTTGGTT		464
WILD	1fwd	ATTTCTAAGGCTGGGTGAGGTG		
	wild_rev	GGTGCACCCATTACCCGAA		403
SILENT	1fwd	ATTTCTAAGGCTGGGTGAGGTG		
	silent_rev	ATTGGTGCACCCATTACCTG		406
COM	2fwd	GCTGCAAAAAGCTATAATTGTACCA		
	com_rev	GATATTTTGGTGCACCCATTACC	6FAM™	183
ASP	2fwd	GCTGCAAAAAGCTATAATTGTACCA	PET®	
	wild_rev	GGTGCACCCATTACCCGAA		175
	silent_rev	ATTGGTGCACCCATTACCTG		178
Identifiler kit		Unpublished by the manufacturer	NED™	126

*Allele-differentiating nucleotide is underlined in primer set WILD, SILENT, and ASP.
†PCR product size for the allele with 14 repeats ([AAGG][TAGG][AAGG]$_{12}$) at the D19S433 marker.

PCR system 9700 (Applied Biosystems): an initial denaturation step of 11 min at 95°C, 32 cycles consisting of 1 min at 94°C, 1 min at 65°C, and 1 min at 72°C, then a final extension step of 10 min at 72°C. The ABI PRISM® BigDye® Terminator version 1.1 cycle sequencing kit and an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems) were used for direct DNA sequencing according to the manufacturer's instructions. Sequencing primers were the same as the PCR primers.

Amplification for STR Analysis

The 15 STR loci of CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, TH01, TPOX, and vWA were co-amplified using an AmpFℓSTR® Identifiler® PCR Amplification Kit (Applied Biosystems) following the manufacturer's recommendations. The D19S433 locus alone was amplified using the primer sets COM or ASP (Table 1). The reverse primer of the COM and the forward primer of the ASP were labeled with the fluorescent dyes 6FAM™ and PET® (Applied Biosystems), respectively. The ASP contained two allele-specific reverse primers. PCR reactions were performed in a total volume of 25 μL containing ~1 ng genomic DNA, 2.5 U AmpliTaq Gold® DNA polymerase (Applied Biosystems), and 4 pmol each primer for the COM or 8 pmol each primer for the ASP in 1× GoldST*R Buffer (Promega) or AmpFℓSTR® PCR Reaction Mix (Applied Biosystems). Thermal cycling was performed in a GeneAmp® PCR system 9700 (Applied Biosystems): with an initial denaturation step of 11 min at 95°C, 28 cycles consisting of 1 min at 94°C, 1 min at 59°C, and 1 min at 72°C, then a final extension step of 60 min at 60°C. Conditions were identical to the thermal cycling for the Identifiler® kit.

Electrophoresis and Typing

The amplified products were separated by electrophoresis on a 3130*xl* Genetic Analyzer (Applied Biosystems) according to the manufacturer's recommended protocols. The GeneScan™ 600 LIZ® Size Standard (Applied Biosystems) was used for sizing DNA fragments. The sample run data were analyzed together with an allelic ladder and positive and negative controls using GENEMAPPER® *ID* Software version 3.2.1 (Applied Biosystems). An allelic ladder was prepared for each primer set (COM and ASP) and contained alleles 14 and 15 amplified from the wild-type.

Data Analysis

Gene frequencies and expected and observed heterozygosity values were calculated, and the exact test of the Hardy–Weinberg equilibrium (1 000 000 steps in Markov chain) was performed using ARLEQUIN software version 3.11 (8).

Results

Individuals were collected who showed a single inconsistency at D19S433 in parentage analysis, presumably caused by transmission of a silent allele to an offspring. It was hypothesized that frequency of the D19S433 silent allele might be relatively high in Japanese individuals. One of the D19S433 primers is labeled with fluorescent dye NED™, and allele sizes are 101–148 bp (5). We carefully reviewed electropherogram profiles of DNA samples using the Identifiler® kit, which were analyzed previously for a population study. These DNA samples were extracted from blood drawn from a cubital vein, and were not likely to be degraded and contaminated with substances that affect PCR efficiency of STR loci. One of the vWA primers is also labeled with NED™ and allele sizes are similar to that of D19S433, namely 151–203 bp (5). Peak heights of D19S433 and vWA were compared within the samples. In most samples, the total peak height (if a sample was heterozygous, the peak height was the sum of two peaks) of D19S433 was nearly equal to that of vWA, which indicated that PCR efficiencies of the two loci were nearly equal for analyzing nondegraded and noncontaminated samples (Fig. 1, samples A–C). However, there was one sample which appeared homozygous (i.e., monoallelic) for D19S433, but amplified only half as efficiently as the vWA locus. That suggested presence of the D19S433 silent allele (Fig. 1, sample D). Direct sequencing analysis of sample D was performed using the primer set #1. As a control, a sample heterozygous for D19S433 also was analyzed. Sequencing the forward strand of the PCR products generated from sample D, and comparison with the control DNA sequence, as well as GenBank reference sequence (AC008507.11), detected a point mutation (guanine to adenine) at 32 nucleotides downstream of the 3′ end of the AAGG repeats (named as the G32A mutation) (Fig. 2). No mutation was detected in the 5′ flanking region of the D19S433 repeat region by sequencing the reverse strand. D19S433 is a tetranucleotide repeat marker. The amplicon size generated using the Identifiler® primers and the length of the repeat region was 126 bp including the 3′ adenine (7) and 56 bp for allele 14, respectively. Therefore, the 5′ end of both the Identifiler® forward and reverse primers must locate within 69

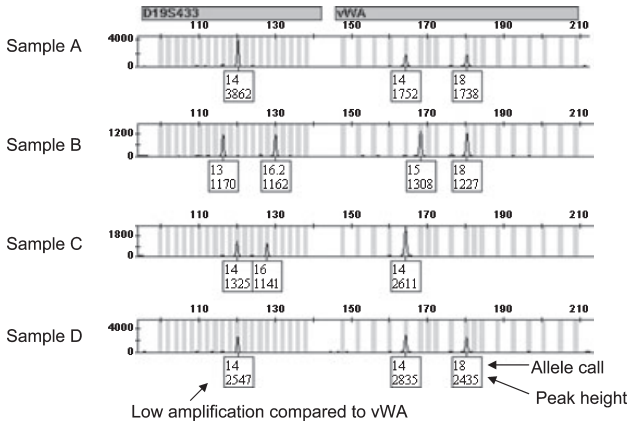

FIG. 1—*Peak height comparison between D19S433 and vWA in Identifiler® kit electropherogram profiles.*

nucleotides upstream or downstream from the repeat region. As G32A was the only mutation found in these ranges, we concluded that it prevented the Identifiler® reverse primer from binding to its target, which resulted in the silent allele. Reverse primers were designed specifically for a wild-type and silent allele. The silent and wild-type alleles were then amplified selectively from sample D using the primer set WILD or SILENT. Each set contained an allele-specific primer, and direct sequencing analysis was performed. The wild-type and silent alleles had 14 and 13 repeats, respectively.

D19S433 typing was performed using the primer sets COM and ASP, separately. The COM set contained a forward and reverse primer common to wild-type and silent alleles. The 3′ end of the reverse primer of COM was located one base downstream from the point mutation (Fig. 2). The ASP set contained the common forward primer and two reverse primers, each specific for a wild-type and silent allele. The 5′ end of the silent allele-specific primer was located three bases downstream from that of the wild-type-specific primer. Hence, the PCR product of the silent allele was three bases longer than that of the wild-type allele, which contained the same number of repeat units. ASP was especially useful in positively detecting the silent allele from an individual who had the wild-type and silent alleles with the same repeat number. Figure 3 shows the D19S433 electropherogram profiles of Identifiler®, COM, and ASP. Typing results were concordant (13, 14.2) among the three primer sets in sample E, which was typed as a heterozygote using the Identifiler® kit. Sample F was typed as a homozygote (14.2, 14.2) using the Identifiler® kit; however, the silent allele with 13 repeats was detected by COM. While the spacing between the two peaks in the COM set electropherogram profile was about six bases, it was reduced to about three bases in the ASP set electropherogram profile. This indicated that the allele with 13 repeats was amplified with the silent allele-specific reverse primer of the ASP set. Sample G was typed as a homozygote (13, 13) using the Identifiler® kit and the COM set. If sample G had contained the silent allele with the 13 repeat units, two peaks at a spacing of about three bases should have been observed in the ASP electropherogram profile. Thus, the possibility of the sample G containing the silent allele was eliminated.

The silent alleles were detected in four individuals out of 176 (0.0114) in Shizuoka and four out of 156 (0.0128) in Okinawa. The eight individuals were heterozygotes for wild-type and silent alleles. It was possible that a mutation other than the G32A mutation was the cause of the silent allele. However, the spacing between the two peaks in the ASP electropherogram profile indicated that the eight silent alleles had the G32A mutation. The Identifiler® reverse primer was highly specific for the wild-type allele, as no nonspecific amplification of the silent allele was observed. Allele frequency data obtained using the Identifiler® kit and the

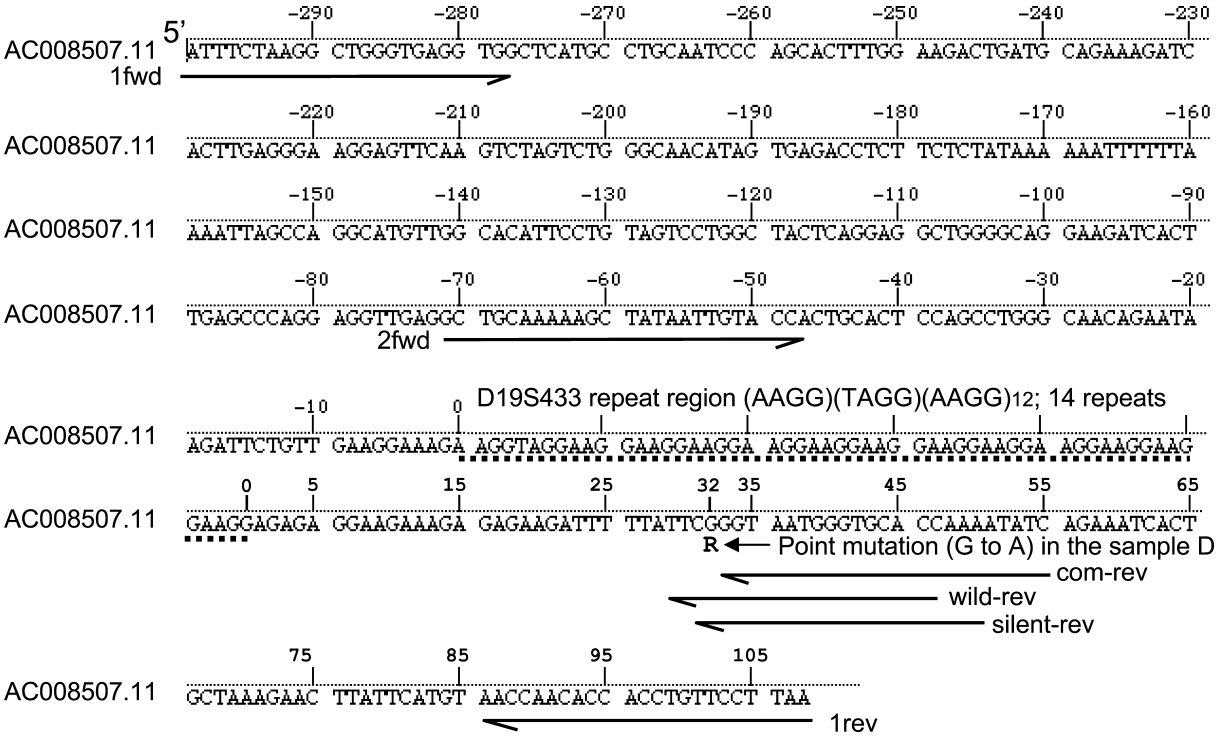

FIG. 2—*A point mutation from guanine to adenine at 32 nucleotides downstream from the D19S433 repeat region and the positions of the designed primers.*

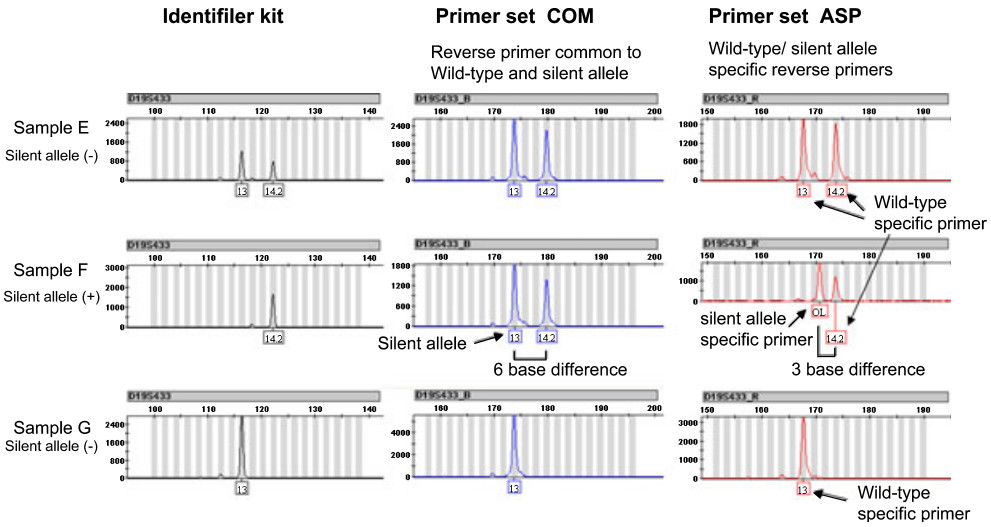

FIG. 3—*Electropherograms of PCR products amplified with three primer sets separately.*

TABLE 2—*D19S433 allele frequency data in Shizuoka and Okinawa obtained using the Identifiler® kit, and the primer sets COM and ASP.*

	Identifiler®			Primer Set COM and ASP		
Allele	Shizuoka	Okinawa	Combined	Shizuoka	Okinawa	Combined
12	0.0341	0.0705	0.0512	0.0341	0.0673	0.0497
12.2	0.0085	0.0000	0.0045	0.0085	0.0000	0.0045
13	0.2727	0.3333	0.3012	0.2727	0.3333	0.3012
13 with the G32A mutation	–	–	–	0.0114	0.0064	0.0090
13.2	0.0171	0.0224	0.0196	0.0171	0.0224	0.0196
14	0.3722	0.2949	0.3358	0.3665	0.2917	0.3313
14 with the G32A mutation	–	–	–	0.0000	0.0064	0.0030
14.2	0.0994	0.0481	0.0753	0.0966	0.0481	0.0738
15	0.0398	0.0737	0.0557	0.0398	0.0705	0.0542
15.2	0.1335	0.1218	0.1280	0.1307	0.1186	0.1250
16	0.0085	0.0064	0.0075	0.0085	0.0064	0.0075
16.2	0.0114	0.0289	0.0196	0.0114	0.0289	0.0196
17.2	0.0028	0.0000	0.0015	0.0028	0.0000	0.0015
n	176	156	332	176	156	332
Observed heterozygosity	0.7614	0.6923	0.7289	0.7841	0.7180	0.7530
Expected heterozygosity	0.7582	0.7755	0.7690	0.7574	0.7709	0.7658
p-value	0.6103	0.0606	0.1606	0.6315	0.0877	0.1453

p-value of the exact test for Hardy–Weinberg equilibrium.

combination of COM and ASP are shown in Table 2. The distributions of genotypes in Shizuoka, Okinawa, and the combined population were consistent with the Hardy–Weinberg equilibrium.

Discussion

Silent alleles caused by mutations at a primer binding site have been identified at several STR loci included in the Identifiler® kit (9–23). In most studies, the presence of a silent allele in an individual has been recognized by observing the discordant genotyping results of an individual after using the AmpFℓSTR® and PowerPlex 16® kits (Promega). While the D19S433 marker is not included in the PowerPlex 16® kit, it is included in the AmpFℓSTR® Identifiler® and AmpFℓSTR® SGM Plus™ kits. The primer sequences in the Identifiler® kit have not been published, but they are consistent with those in the SGM Plus™ kit (5). Therefore, the D19S433 silent allele is not problematic in the human identification setting, because both kits produce the same typing results, even in individuals with the silent allele. In contrast, the silent allele has a great impact on parentage analysis, as it can cause apparent inconsistency between a child and his/her biological parent, when it is transmitted to the offspring.

The cause of the silent allele at D19S433 has been identified as a point mutation at the binding site of the reverse primer in the Identifiler® kit. If the 5′ end of the reverse primer is assumed to be located at the point mutation site, the 5′ end of the forward primer must be located at 37 nucleotides upstream of the repeat region, taking into account the amplicon size and the length of the repeat region. However, this assumption is unlikely to be correct, because a matched or mismatched base of an allele-specific primer is usually located close to its 3′ end (24,25). Therefore, the 5′ end of the forward primer must be located less than 37 nucleotides upstream of the repeat region. Thus, it can be determined that the forward primers of Identifiler® and the COM set are different (Fig. 2). The COM set has been useful in detecting the silent alleles. Silent allele frequency was determined in 176 and 156 individuals in Shizuoka and Okinawa, respectively. Shizuoka is located on the Pacific, ~200 km southwest of Tokyo. Okinawa is located in the Ryukyu archipelago in the East China Sea. The frequency of the silent allele in Shizuoka and Okinawa was 0.0114 and 0.0128, respectively. There was no significant regional difference in frequency of the silent allele. Frequency of individuals homozygous for the silent allele, from whom no peaks will be observed at D19S433 in the Identifiler® electropherogram profile, was estimated at ~1 in 8000 and 6000 in Shizuoka and Okinawa, respectively.

Besides heterozygotes with a silent allele caused by a mutation at a primer binding site, heterozygotes with an extreme "off-ladder" allele, the size of which falls outside the allele size range defined by the kit manufacturer, may also be falsely typed as homozygotes (26,27). No extreme "off-ladder" allele was found at D19S433 in the 332 Japanese individuals.

We first recognized the possibility that sample D contained the silent allele by comparing peak heights of D19S433 and vWA in the Identifiler® electropherogram profile (Fig. 1). The peak height ratio between D19S433 and vWA was calculated for all 332 individuals. These individuals were divided in three groups: D19S433 heterozygotes, homozygotes without the G32A mutation, and false

TABLE 3—*Peak height ratios between D19S433 and vWA within a DNA sample analyzed using the Identifiler® kit.*

Peak Height Ratio	D19S433		
	Heterozygote	Homozygote Without the G32A Mutation	False Homozygote with the G32A Mutation
n	242	82	8
Max	1.584	1.516	0.780
Min	0.695	0.728	0.461
Mean	1.033	1.047	0.581
SD	0.164	0.181	0.110

Peak height is the sum of two peaks, if a sample is heterozygote. Peak height of D19S433 was divided by that of vWA.

TABLE 4—*Probability of observing an inconsistency at D19S433 in parentage testing using the Identifiler® kit, due to passing the silent allele to the offspring.*

Alleles*		Frequency of Mating	Probability of Inconsistency
Father	Mother		
A_i, A_{silent}	A_j, A_k	$\sum_{i=1}^{n} 2p_i\, p_{silent}\, (1 - p_i - p_{silent})^2$	0.5
A_j, A_k	A_i, A_{silent}	$\sum_{i=1}^{n} 2p_i p_{silent}\, (1 - p_i - p_{silent})^2$	0.5
A_i, A_{silent}	A_i, A_j	$\sum_{i=1}^{n} 4p_i^2\, p_{silent}\, (1 - p_i - p_{silent})$	0.25
A_i, A_j	A_i, A_{silent}	$\sum_{i=1}^{n} 4p_i^2\, p_{silent}\, (1 - p_i - p_{silent})$	0.25
A_i, A_{silent}	A_i, A_i	$\sum_{i=1}^{n} 2p_i^3\, p_{silent}$	0
A_i, A_i	A_i, A_{silent}	$\sum_{i=1}^{n} 2p_i^3\, p_{silent}$	0
A_i, A_{silent}	A_i, A_{silent}	$\sum_{i=1}^{n} 4p_i^2\, p_{silent}^2$	0.25
A_i, A_{silent}	A_j, A_{silent}	$\sum_{i=1}^{n} 4p_i\, p_{silent}^2\, (1 - p_i - p_{silent})$	0.75
A_j, A_k	A_{silent}, A_{silent}	$p_{silent}^2\, (1 - p_{silent})^2$	0
A_{silent}, A_{silent}	A_i, A_j	$p_{silent}^2\, (1 - p_{silent})^2$	0
A_i, A_{silent}	A_{silent}, A_{silent}	$2\, p_{silent}^3\, (1 - p_{silent})$	0.5
A_{silent}, A_{silent}	A_i, A_{silent}	$2\, p_{silent}^3\, (1 - p_{silent})$	0.5
A_{silent}, A_{silent}	A_{silent}, A_{silent}	p_{silent}^4	0

*A_i, A_j, A_k are not silent alleles, A_j, $A_k \neq A_i$.

homozygotes with the G32A mutation (Table 3). The maximum peak height ratio in false homozygotes exceeded the minimum ratios in heterozygotes and homozygotes without the G32A mutation. Determining the presence of the silent allele only by comparing peaks between D19S433 and vWA appeared to be difficult, even for DNA samples extracted from blood drawn from a vein. Distinguishing homozygotes and false homozygotes based on peak height comparison between D19S433 and vWA is difficult for casework, because some samples contain degraded DNA and exogenous substances that affect PCR efficiency of different loci differently (5,28).

The total probability of observing an apparent genetic inconsistency at D19S433, as a result of passing the silent allele to the offspring, in testing with the Identifiler® kit was calculated as the sum of the mating frequencies multiplied by the probability of inconsistency (Table 4). Using the allele frequency data in Table 2, the probability was calculated to be 0.017, 0.019, and 0.018 for Shizuoka, Okinawa, and the combined population, respectively.

Conclusion

The cause of the D19S433 silent allele was identified as a G to A point mutation at the reverse primer binding site. The frequency of the silent allele in Shizuoka and Okinawa was ~1%. It is anticipated that an "apparent opposite homozygosity" inconsistency at D19S433 between a child and his/her biological parents will be observed about once every 50–60 parentage tests. The primer set COM can be used effectively to resolve the discrepancy. The D19S433 silent allele is not problematic in the human identification setting, as the silent allele is reproducibly not amplified using the Identifiler® kit.

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References

1. Leclair B, Fregeau CJ, Bowen KL, Fourney RM. Enhanced kinship analysis and STR-based DNA typing for human identification in mass fatality incidents: the Swissair flight 111 disaster. J Forensic Sci 2004;49:939–53.
2. Leclair B, Shaler R, Carmody GR, Eliason K, Hendrickson BC, Judkins T, et al. Bioinformatics and human identification in mass fatality incidents: the world trade center disaster. J Forensic Sci 2007;52:806–19.
3. Prinz M, Carracedo A, Mayr WR, Morling N, Parsons TJ, Sajantila A, et al. DNA Commission of the International Society for Forensic Genetics (ISFG): recommendations regarding the role of forensic genetics for disaster victim identification (DVI). Forensic Sci Int Gene 2007;1:3–12.
4. Yoshida K, Mizuno N, Fujii K, Senju H, Sekiguchi K, Kasai K, et al. Japanese population database for nine STR loci of the AmpFℓSTR Profiler kit. Forensic Sci Int 2003;132:166–7.
5. Collins PJ, Hennessy LK, Leibelt CS, Roby RK, Reeder DJ, Foxall PA. Developmental validation of a single-tube amplification of the 13 CODIS STR loci, D2S1338, D19S433, and amelogenin: the AmpFℓSTR Identifiler PCR Amplification Kit. J Forensic Sci 2004;49:1265–77.
6. Yoshida K, Takahashi K, Kasai K. Allele frequencies of 15 loci using AmpFℓSTR Identifiler Kit in Japanese population. J Forensic Sci 2005;50:718–9.
7. Brownstein MJ, Carpten JD, Smith JR. Modulation of non-templated nucleotide addition by Taq DNA polymerase: primer modifications that facilitate genotyping. Biotechniques 1996;20:1004–6, 1008–10.
8. Excoffier L, Laval G, Schneider S. Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinform Online 2005;1:47–50.
9. Takayama T, Takada N, Suzuki R, Nagaoka S, Watanabe Y. Identification of a rare mutation in a TH01 primer binding site. Leg Med (Tokyo) 2007;9:289–92.
10. Vanderheyden N, Mai A, Gilissen A, Cassiman JJ, Decorte R. Identification and sequence analysis of discordant phenotypes between AmpFℓSTR SGM Plustrade mark and PowerPlex® 16. Int J Legal Med 2007;121:297–301.
11. Ricci U, Melean G, Robino C, Genuardi M. A single mutation in the FGA locus responsible for false homozygosities and discrepancies between commercial kits in an unusual paternity test case. J Forensic Sci 2007;52:393–6.
12. Clayton TM, Hill SM, Denton LA, Watson SK, Urquhart AJ. Primer binding site mutations affecting the typing of STR loci contained within the AMPFℓSTR SGM Plus kit. Forensic Sci Int 2004;139:255–9.
13. Hendrickson BC, Leclair B, Forrest S, Ryan J, Ward BE, Petersen D, et al. Accurate STR allele designations at the FGA and vWA loci despite primer site polymorphisms. J Forensic Sci 2004;49:250–4.
14. Leibelt C, Budowle B, Collins P, Daoudi Y, Moretti T, Nunn G, et al. Identification of a D8S1179 primer binding site mutation and the validation of a primer designed to recover null alleles. Forensic Sci Int 2003;133:220–7.

15. Nelson MS, Levedakou EN, Matthews JR, Early BE, Freeman DA, Kuhn CA, et al. Detection of a primer-binding site polymorphism for the STR locus D16S539 using the Powerplex 1.1 system and validation of a degenerate primer to correct for the polymorphism. J Forensic Sci 2002;47:345–9.
16. Moretti TR, Baumstark AL, Defenbaugh DA, Keys KM, Smerick JB, Budowle B. Validation of short tandem repeats (STRs) for forensic usage: performance testing of fluorescent multiplex STR systems and analysis of authentic and simulated forensic samples. J Forensic Sci 2001;46:647–60.
17. Lazaruk K, Wallin J, Holt C, Nguyen T, Walsh PS. Sequence variation in humans and other primates at six short tandem repeat loci used in forensic identity testing. Forensic Sci Int 2001;119:1–10.
18. Cotton EA, Allsop RF, Guest JL, Frazier RR, Koumi P, Callow IP, et al. Validation of the AMPFℓSTR SGM Plus system for use in forensic casework. Forensic Sci Int 2000;112:151–61.
19. Thangaraj K, Reddy AG, Singh L. Mutation in the STR locus D21S1 1 of father causing allele mismatch in the child. J Forensic Sci 2004;49:99–103.
20. Hill CR, Kline MC, Mulero JJ, Lagace RE, Chang CW, Hennessy LK, et al. Concordance study between the AmpFℓSTR MiniFiler PCR amplification kit and conventional STR typing kits. J Forensic Sci 2007;52:870–3.
21. Budowle B, Masibay A, Anderson SJ, Barna C, Biega L, Brenneke S, et al. STR primer concordance study. Forensic Sci Int 2001;124:47–54.
22. Budowle B, Sprecher CJ. Concordance study on population database samples using the PowerPlex 16 kit and AmpFℓSTR Profiler Plus kit and AmpFℓSTR COfiler kit. J Forensic Sci 2001;46:637–41.
23. Delamoye M, Duverneuil C, Riva K, Leterreux M, Taieb S, De Mazancourt P. False homozygosities at various loci revealed by discrepancies between commercial kits: implications for genetic databases. Forensic Sci Int 2004;143:47–52.
24. Bottema CD, Sommer SS. PCR amplification of specific alleles: rapid detection of known mutations and polymorphisms. Mutat Res 1993;288:93–102.
25. Mizuno N, Ohmori T, Sekiguchi K, Kato T, Fujii T, Fujii K, et al. Alleles responsible for ABO phenotype-genotype discrepancy and alleles in individuals with a weak expression of A or B antigens. J Forensic Sci 2004;49:21–8.
26. Heinrich M, Felske-Zech H, Brinkmann B, Hohoff C. Characterisation of variant alleles in the STR systems D2S1338, D3S1358 and D19S433. Int J Legal Med 2005;119:310–3.
27. Huel RL, Basic L, Madacki-Todorovic K, Smajlovic L, Eminovic I, Berbic I, et al. Variant alleles, triallelic patterns, and point mutations observed in nuclear short tandem repeat typing of populations in Bosnia and Serbia. Croat Med J 2007;48:494–502.
28. Mizuno N, Senju H, Sekiguchi K, Yoshida K, Kasai K, Sato H. A standard of AmpliType PM typing from aged evidentiary samples. J Forensic Sci 2001;46:677–80.

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