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CASE REPORT

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Non-amplification of an allele of the D8S1179 locus due to a point mutation

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Abstract During a population study of 128 Korean families (626 persons) with the AmpF*l*STR Profiler Plus PCR amplification system, we found an unusual homozygous genotype at the D8S1179 locus in 4 families. Therefore, a new pair of primers was designed for the D8S1179 locus from GenBank data (GenBank Accession No. G08710) to evaluate the cause. The newly designed primers amplified alleles that were not amplified with the AmpF*l*STR Profiler Plus PCR amplification system. We sequenced alleles of the family members who had non-amplified alleles and we found a G-to-A transition at the position of the 147th base of the GenBank sequence.

Keywords D8S1179 · STR · Mutation · Multiplex · PCR

Introduction

During a population study of Korean families with the AmpF*l*STR Profiler Plus PCR amplification system (Profiler Plus Kit, PE Applied Biosystems, Foster City, Calif.), we found an unusual homozygous genotype at the D8S1179 locus in four families that was hard to explain by Mendel's hereditary principles. The genotyping results suggested the possibility of non-amplified alleles due to deletion of an allele or non-binding of the primer. We therefore designed new primers and performed further studies on these families to evaluate the cause.

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Material and methods

Buccal swab samples or blood samples from 17 family members of 4 genetically unrelated families from the 128 proven Korean families (n = 636) were used for DNA extraction.

Design of new primers

A new set of fluorescence-labelled primers was designed for the D8S1179 locus from GenBank data (GenBank Accession No. G08710) with the web-based Primer 3 program (Whitehead Institute for Biomedical Research/MIT Center for Genome Research). According to the user's manual of the Profiler Plus Kit, the size of allele 12 is 144 bp, but we tried to design primers that can amplify the maximum size of PCR product. Thus, the products that were amplified with the new primers were 100 bp longer than the products of the Profiler Plus Kit, although having the same the number of the repeat motif, The new primers were as follows:

– D8S1179 (forward) 5′HEX-TGG CAA CTT ATA TGT ATT TTT G-3′
– D8S1179 (reverse) 5′-ATT CTT GTT CCC AGT TTC TT-3′

Electrophoresis and sequencing

After PCR, electrophoresis was carried out on an ABI 377 Genetic Analyzer (PE Applied Biosystems). Fragment sizes were determined using Genescan software ver. 2.1 (PE Applied Biosystems) and genotyping was done using Genotyper software ver. 2.1.

For the sequencing, the DNA fragments were eluted from the polyacrylamide gels as described by Möller and Brinkmann (1994). The sequencing reactions for both strands were performed using the BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems) and the sequencing results were analysed using sequencing analysis software ver. 2.1 (PE Applied Biosystems).

Results

All of the alleles that had not been amplified with the Profiler Plus Kit were amplified with the newly designed primers, so we excluded the possibility of deletion of one allele.

In the case of family 1, it was revealed that the father's genotype at the D8S1179 locus was not (12/12) but (12/16)

Table 1 The different genotyping results of the D8S1179 locus between the amplifications with the AmpF*l*STR Profiler Plus PCR amplification system and with the new primers

Family	AmpF*l*STR profiler plus				New primers			
	Father	Mother	Child 1	Child 2	Father	Mother	Child 1	Child 2
Family 1								
First generation	12/12	13/13	13/13[a]	13/13	12/16	13/13	13/16	13/16
Second generation	13/13[a]	16/16	16/16	–	13/16	16/16	16/16	–
Family 2	12/12	14/15	14/14	–	12/15	14/15	14/15	–
Family 3	10/13	15/15	10/10	–	10/13	15/16	10/16	–
Family 4	13/16	13/13	16/16	–	13/16	13/16	16/16	–

[a] The father in the 2nd generation of family 1 is child 1 in the first generation

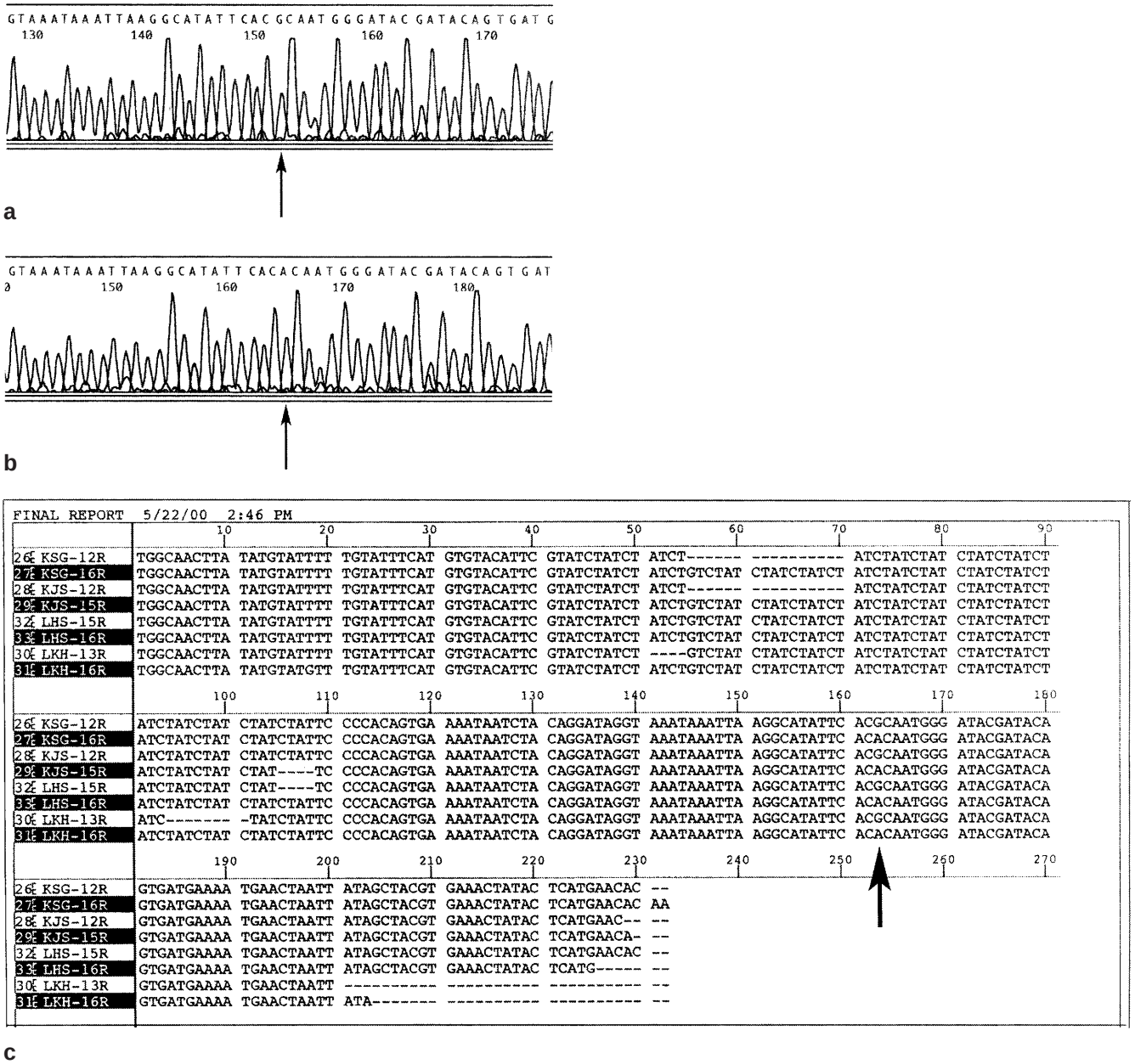

Fig. 1 **a** Electropherogram showing the sequencing results of the D8S1179 allele not amplified with the AmpF*l*STR Profiler Plus PCR amplification system compared to **b** the results of amplification with the newly designed primers. The shadowed alleles are non-amplified alleles of each family member. The sequencing result **c** confirms a guanine to adenine (G → A) transition of the non-amplified allele (*arrows*) (*KSG* grandfather of family 1, *KJS* father of family 2, *LHS* mother of family 3, *LKH* mother of family 4)

and the genotype of both children was (13/16). This result means that the allele 16 of the father was not amplified with the Profiler Plus Kit and both children have inherited this allele 16 from the father. So exclusion of paternity seen in both the 1st generation and 2nd generation children, turned to non-exclusion of paternity in this family when the newly designed primers were used to amplify D8S1179 locus. (Table 1)

In the same way, the father in family 2, the mother in family 3 and the mother in family 4 all had an allele that was amplified only with the new primers. (Table 1) All three families showed no exclusion when the new primers were used to amplify the D8S1179 locus.

The sequencing results show that the alleles that were not amplified with the Profiler Plus Kit, have a G-to-A transition at the 147th base of a 3′ flanking region of the GenBank sequence in common (55th position upstream to the repeat), even though the repeat number is different, while all other alleles exhibited a guanine (G). (Fig. 1)

Discussion

The new set of primers presented in this work allowed an understanding of the basis for the apparent mother/child and paternity exclusions when employing the Profiler Plus kit. From the genotyping and the sequencing results, we considered that an appropriate explanation for non-amplification of these alleles would be a point mutation positioned in the primer annealing sites as also seen in the MBP-STR alleles (Gusmao et al. 1996).

According to the user's manual the size of allele 12 is 144 bp and the position showing the mutation is the 147th base of the GenBank sequence (No. G08710). If we assume that the manufacturer designed the primers using the GenBank sequence of D8S1179 locus, there is a strong possibility that the primer of D8S1179 locus that is included in Profiler Plus Kit is positioned at the sequence showing a mutation. To confirm this possibility, the sequences of both primers are absolutely necessary, but we could not get information about the primer sequences of D8S1179 locus from the manufacturer.

We found 4 of 128 families (3.125%) showing non-amplification of 1 allele. When we tested with the Profiler Plus kit, all four families showed an exclusion but using the new primers it was revealed that the results of the Profiler Plus Kit were wrong. Even though this mutation seems as yet to be limited to Koreans and has not been described for other populations (Hantschel et al. 1999; Neuhuber et al. 1999; Perez-Lezaun et al. 2000), we recommend caution in the interpretation of the results obtained with the Profiler Plus Kit, especially when a mismatch is seen in the D8S1179 locus due to homozygote genotypes.

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