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CASE REPORT

Chiara Turchi,[1] *B.Sc.; Mauro Pesaresi,*[1] *M.D.; Federica Alessandrini,*[1] *B.Sc.; Valerio Onofri,*[1] *B.Sc.; Alessia Arseni,*[1] *M.D.; and Adriano Tagliabracci,*[1] *M.D.*

Unusual Association of Three Rare Alleles and a Mismatch in a Case of Paternity Testing

ABSTRACT: This study reports a paternity case analyzed by the AmpFℓSTR Identifiler Kit (AB) in which father and daughter shared three rare alleles for D19S433, D18S51 and TH01 microsatellites. The case also showed an apparent exclusion, due to a mutation at the D3S1358 microsatellite. Sequencing analysis was performed to assess the size of the rare alleles and to establish their structure, which revealed some molecular variations in regions flanking the motif repeats.

KEYWORDS: forensic science, paternity testing, rare alleles, mismatch, STRs, DNA

Microsatellites are short tandemly repeated DNA sequences widespread throughout the human genome, which exhibit individual genetic differences exploited extensively in several fields, including genetic mapping, linkage analysis, population studies, personal identification, and paternity testing. One of the most frequently used coamplification kits for paternity testing is the AmpFℓSTR Identifiler Kit (Applied Biosystems, Foster City, CA) which amplifies 15 microsatellites and the Amelogenin locus. This kit, which includes all 13 of the required loci for the combined DNA Index System (CODIS) (1) and two additional loci (D2S1338, D19S4433), has a Probability Identity (Pi) value of 5.01×10^{-18} in Caucasians (2). This paper describes an unusual case of paternity testing performed in our laboratory on a Caucasian family from Central Italy, in which the putative father exhibited three rare alleles, shared by the daughter, and a mismatch for the D3S1358 locus.

Materials and Methods

DNA from buccal swabs was extracted by Chelex (3) and quantitation was carried out using the dot-blot procedure with a primate-specific alpha satellite probe, D17Z1 (Gibco-BRL, Gaithersburg, MD) (4). DNA was amplified with the AmpFℓSTR Identifiler™ Kit on a GenAmp System 9700 thermal cycler (Applied Biosystems) in two different laboratories, following the manufacturer's recommendations. Capillary electrophoresis was carried out on an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The length of the amplified fragments was established from the internal run standard by the Southern Local method, using Genescan Analysis 3.1.2 software (Applied Biosystems). Genotyping was accomplished by comparison with the ladder, which was run twice at the start and end of work sessions. Sequencing of rare alleles was carried out on fragments eluted from gel by the DNA Gel Extraction Kit (Millipore Corporation, Bedford, MA) and transferred to microfuge tubes. Cycle sequencing was performed on both strands using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems). The resulting data were analyzed with AB Software Sequencing Analysis 3.0, and sequences were aligned by Sequence Navigator Software 1.0.1 (Applied Biosystems). Alleles were designated on the basis of number of repeats, according to the recommendations of the International Society for Forensic Genetics (5). All experiments were carried out twice. Negative controls were included in the extraction and PCR stages, and positive controls were introduced at the PCR step (6).

Results and Discussion

Father and daughter shared alleles at all 15 loci except one, locus D3S1358, in which an allele 17, present in the daughter's genotype, was absent in relatives (Table 1). Three rare alleles were present in both father and daughter genotypes, two of them not included in the allele ladder supplied by AB (Applied Biosystems): one allele between alleles 12 and 12.2 at locus D19S433; one intermediate allele between alleles 7 and 9 at locus D18S51; and allele 10 at locus TH01, the frequency of which is only 0.0043 in Caucasians (2). In the ladder, the unknown alleles fell in the positions corresponding to allele 12.1 at locus D19S433 and allele 8 at locus D18S51. Sequencing performed to assess the allele sizes of these two variants and to establish their molecular structure confirmed the genotyping tentatively made by capillary electrophoresis.

Allele 12.1 showed 12 motifs repeat AAGG and an adenine (A) insertion between the 7th and 8th motif repeats (Fig. 1). This variant was described by Binda et al. (7) in a population study on 206 Swiss, occurring at a low frequency of 0.005. Since these authors did not

[1] Istituto di Medicina Legale, Universita di Ancona, Policlinico Torrette, I-60020 Ancona, Italy.

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TABLE 1—*Genotyping results of PCR analysis of 15 microsatellites and Amelogenin test.*

Locus	Father	Mother	Daughter
Amelogenin	XY	X	X
D3S1358	18	18	17–18
vWA	17	14–18	14–17
FGA	21–23	19–23	21–23
D5S818	10–11	12–13	10–13
D13S317	11–12	10–12	10–11
D7S820	8–10	11	8–11
D8S1179	10–14	13	13–14
D21S11	29–30	29–31	29
D18S51	8–18	15–17	8–15
TH01	6–10	7–9	9–10
TPOX	8	8–10	8
CSF1PO	10–12	10–11	10–12
D16S539	12–13	12–13	12–13
D2S1338	20–23	23	20–23
D19S433	12.1–14	15.2–17	12.1–15.2
SE33	19–21	17–19	17–19

describe the sequence of their variant, we do not know if our variant is the same as that carrying the adenine insertion. Additionally, sequencing of allele 12.1 revealed the presence of an A at base 124 of the 3′-flanking region (Fig. 1), outside primer annealing, in place of the T described in the sequence loaded in Gen Bank (G08036). This variation was confirmed in two other samples sequenced as controls.

Allele 8 of D18S51 locus was sequenced by Griffiths et al. (8) and reported as a variant at very low frequency (1 in 658 subjects) in a Russian sample by Kornienko et al. (9). Sequencing displayed 8 AGAA tetranucleotide repeats (Fig. 2), as reported by Griffiths et al. (8), and showed a T insertion at position 50.1 of the flanking region—unlike the sequence loaded in Gen Bank (X91255). This insertion is quite common, because it was found in the two other samples sequenced as controls.

Study of a few available ancestors revealed the presence of the above-mentioned 12.1 D19S433 and 10 TH01 alleles in the paternal grandmother and allele 8 D18S51 in a paternal uncle. The presence of these alleles in parental lineages gives evidence of genetic

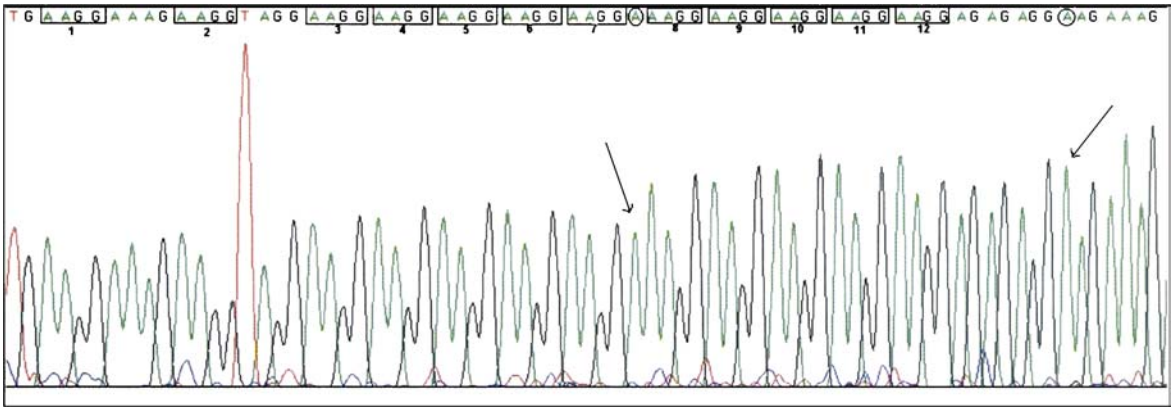

FIG. 1—*Sequencing electropherogram of allele 12.1 at locus D19S433 showing pattern of repeated sequence* $(AAGG)_1AAAG(AAGG)_1TAGG(AAGG)_5A(AAGG)_5$*, adenine insertion after 7th motif repeat* (arrow, left) *and presence of an A in place of T in 3′-flanking region* (arrow, right).

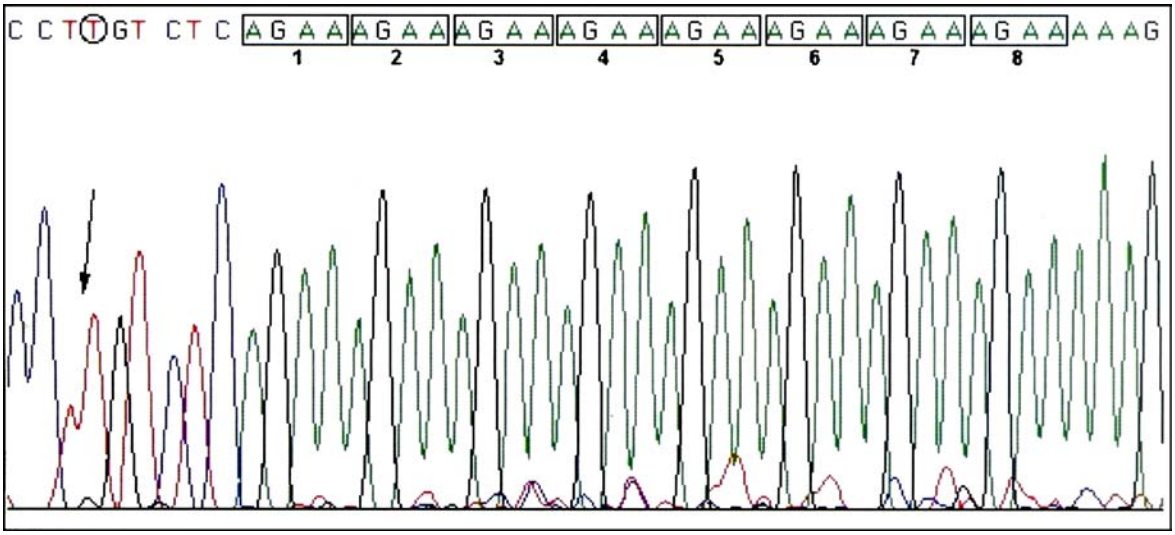

FIG. 2—*Sequencing electropherogram of allele 8 at locus D18S51 showing pattern of repeated sequence* $(AGAA)_8$ *and T insertion* (arrow) *in 5′-flanking region.*

reshuffling responsible for the association of these three rare alleles in the father-child pair, rather than mutational events during gametogenesis, i.e., linked to genetic defects.

Despite the high indication for paternity derived from the putative father-child sharing of three rare alleles, analysis revealed a mismatch for locus D3S1358, since the daughter showed alleles 17–18 and the parents were homozygous for allele 18. Considering the compatibility for all the loci examined except this one, a mutational event was hypothesized. Microsatellites are characterized by a high mutation rate (10^{-3}) which may alter their length by the insertion or deletion of a small number of repeat units. Previous studies have suggested that microsatellite mutations occur more frequently in longer alleles and that interrupted alleles are more stable than uninterrupted ones (10). Two mechanisms may be responsible for mutation: DNA slippage during replication, and recombination between DNA strands, both depending on repeated motif, allele size, chromosome position, GC content in flanking DNA, cell division, sex and genotype, e.g., mutations at mismatch-mutation-repair (MMR) genes (11). DNA MMR genes correct replication errors and inhibit recombination between diverged sequences (12). The mutation rate for D3S1358 has been calculated to be 0.11% and <0.02 % for paternal and maternal meioses, respectively (13). An attempt to identify the paternal origin of this event was made by sequencing both parental alleles in the hope of finding different molecular structure in relatives, since D3S1358 is a locus having compound structure with interpersed irregular units TCTA $(TCTG)_{2-3}(TCTA)_n$. However, parental alleles showed the same structure.

The paternity index was calculated on allele frequencies reported for U.S. Caucasians (2), also taking into account the mismatch at locus D3S1358 by the formula for mutational events suggested by Brenner (14), in which paternity index (PI = X/Y) is even to $\mu/4P(Q)$, where μ represents mutation rate for mutated locus and P(Q) is the frequency of the Q mutated allele in the population.

The results were strongly indicative of kinship (PI = 0.99999999998). Stronger indications for paternity were obtained when the locus in question was excluded from the calculus (PI = 0.99999999999624).

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Additional information and reprint requests:
Adriano Tagliabracci, M.D.
Istituto di Medicina Legale
Policlinico Torrette
I-60020 Ancona (Italy)
E-mail: tagliabr@unian.it