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 AmpF/STR 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 AmpF/STR 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 AmpF/STR 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
<table>
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FIG. 1—Sequencing electropherogram of allele 12.1 at locus D19S433 showing pattern of repeated sequence (AAGG)<sub>1</sub>AAAG(AAGG)<sub>1</sub>TAGG(AAGG)<sub>5</sub>A(AAGG)<sub>5</sub>, 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)<sub>8</sub> 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. 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. DNA MMR genes correct replication errors and inhibit recombination between diverged sequences. The mutation rate for D3S1358 has been calculated to be 0.11% and <0.02% for paternal and maternal meioses, respectively. The paternity index was calculated on allele frequencies reported for U.S. Caucasians, also taking into account the mismatch at locus D3S1358 by the formula for mutational events suggested by Brenner (14), in which paternity index \(\text{PI} = \frac{X}{Y}\), where \(\mu\) 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 \(\text{PI} = 0.999999999998\). Stronger indications for paternity were obtained when the locus in question was excluded from the calculus \(\text{PI} = 0.99999999999624\).

**References**


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