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A three-fragment phenotype in the D16S309 locus

Trzy-fragmentowy fenotyp w lokus D16S309

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An allele with internal restriction site or another kind of mutation has been found in D16S309 locus. Using a probe MS205 in combination with the enzyme Hinf I 3-fragment, a pattern was observed in a woman from the Upper Silesia population – the mother of a child involved in paternity testing. DNA from this woman was additionally digested with Alu I and Mbo I enzymes. All the digested samples showed a 3-fragment pattern with the probe MS205, while other probe/enzyme combinations detected only the expected phenotypes. The disputed child inherited a second, normal allele from its mother.

U kobiety z populacji Górnego Śląska – matki dziecka – uczestniczącej w sprawie spornego ojcostwa spotkano 3-fragmentowy fenotyp w lokus D16S309(MS205) w cięciu z Hinf I, Alu I i Mbo I. Inne kombinacje próba/enzym dały typowe fenotypy w badanych loci SLP. Dziecko odziedziczyło od matki normalny allel w lokus D16S309.

Key words: DNA typing, D16S309(MS205), three-fragment phenotype, the Upper Silesia population
Słowa kluczowe: oznaczanie DNA, D16S309 (MS205), 3-fragmentowy fenotyp, populacja Górnego Śląska

INTRODUCTION

The D16S309 locus has been assigned to the proterminal DNA of the short arm of chromosome 16. Its relatively small alleles (8-87 repeats of 45-54bp unit) are detected with the MS205 probe after Hinf I, Pst I, Alu I and Mbo I restriction. They cannot be detected in Hae III, Rsa I and Hpa II cleavage, because these enzymes cut within the repeat unit [1-5]. Heterozygosity of this locus varies from 89.8% [6] to 99.7% [3] in Europeans,; its sex-averaged germline mutation rate is 0.4% per gamete, but with a stronger male mutation rate [3].

Pseudo-exclusion from paternity due to maternal uniparental disomy 16 was described by Bein et al. [7]. Raczek and Berent [8] presented two cases of pseudo-non-paternity: the first pseudo-exclusion was caused by an existence of a covert allele in a pair: putative father-child, and in the second case, the length mutation in the alleged father was observed. In the practice of the Department of Forensic Medicine in Katowice, 5 cases of opposite homozygotes have been met in a pair: putative father-child, and 2 cases in a pair: mother-child.

This article shows a three-fragment pattern of restriction with Hinf I, Alu I and Mbo I in the D16S309 locus in a woman from the Upper Silesia population (Poland).

To date, no two-part D16S309 allele has been reported [9], while three-fragment phenotypes have been met in D12S11 [10, 11], D1S7 [12], D7S21 [12], D5S43 [13] and D4S139 [14]. Besides, the reason which confirms the necessity of remarking the appearance of 3-fragment phenotype in the locus D16S309 in the era of STR domination is the usefulness of SLPs in clarifying complicated paternity testing (the letter from D. Patzelt dated September 28, 2006, containing the request: …"It is important to collect vials of fresh blood in order

to perform RFLP-test"…) and a very high value of PI for SLP loci.

MATERIALS AND METHODS

DNA was isolated according to Kunkel et al. [15] with small modifications, from individuals – participators of paternity testing, living in the Upper Silesia (Poland). Restriction (Hinf I, Alu I – Invitrogen, Rsa I , Hae III – Life Technologies, Mbo I – EURX), electrophoresis and hybridization (MS205, MS43a, MS31 and MS8 – Tepnel) were carried out according to the manufacturer's recommendations [16].

Fragment sizes (in bp) were calculated on the ground of comparison to NICETM (Life Technologies)/ MW100 (Tepnel) molecular weight marker using the Vilber Lourmat program [17].

Mutation rate was calculated according to Henke and Henke [12].

RESULTS AND DISCUSSION

mong 570 unrelated adults from the Upper Silesia investigated in paternity testing, in case of one woman a three-fragment phenotype at the D16S309 locus has been observed. The MS205 probe in combination with the restriction enzyme Hinf I has detected 3 fragments: 3523, 3417 and 2473bp (Table I). The disputed child has inherited a normal (3523bp) allele from its mother:, she has not transferred the two-part allele to her child (Fig. 1A, 1B). DNA from the investigated woman and her child have been additionally digested with the enzymes Alu I and Mbo I. All the digested samples from the child's mother have shown a 3-fragment pattern with probe MS205 (Table I, Fig. 1B).

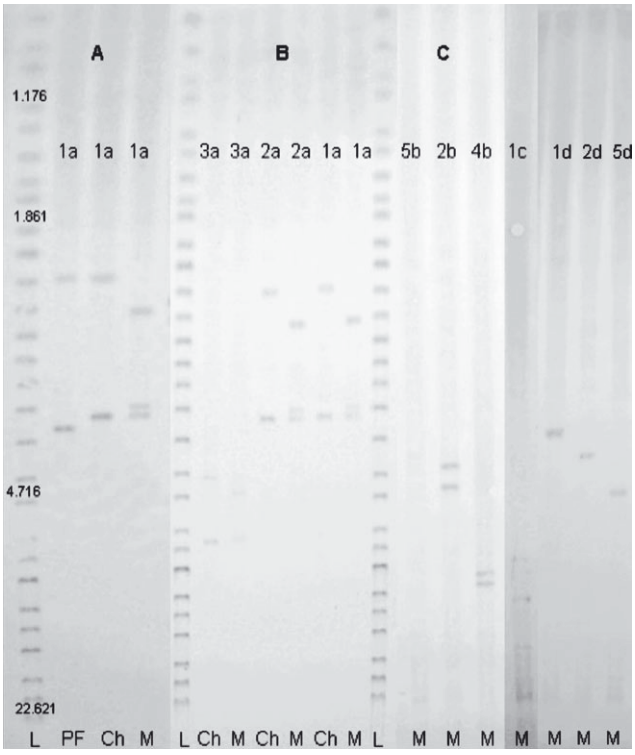
Table I. D16S309 fragment sizes (in bp) derived from various restriction enzymes.

Enzyme Person	Hinf I	Alu I	Mbo I
Mother	3523, 3417, 2473	3544, 3459, 2514	5693, 5570, 4688
Child	3523, 2213	3544, 2226	5693, 4385

Other probe (MS 43a, MS 31, MS 8)/enzymes (Hinf I, Alu I, Mbo I, Rsa I and Hae III) combinations have detected in the woman only 1 or 2 fragments (Fig. 1C), except of the combination MS43a/Hae III, where no fragments could be seen [12].

It is known from other VNTR-markers that occasionally there is the internal restriction site for the commonly employed enzymes [10, 12, 13]. This internal cleavage in locus(probe)/enzyme

Fig. 1A, B, C. Fragment pattern of DNA restriction with Hinf I (1), Alu I (2), Mbo I (3), Rsa I (4) and Hae III (5) enzymes in combination with MS205 (a), MS43a (b), MS31(c) and MS8 (d) probes. M – mother, Ch – child, PF – putative father, L – ladder NICE/MW100.



combination reveals two fragments, which, in the normal digestion, should be one. This additional restriction site might be interpreted as a mutation case [10, 12,13]. In the Upper Silesia population, there were found three-fragment phenotypes at the D5S43 locus [13]; in European populations, the two-part alleles were described in D12S11 [10, 11], D1S7 [12], D7S21 [12] and D4S139 [14]. When the three-fragment phenotype is in more than one locus(probe)/enzyme combination, the interpretation is not that simple. However, Henke and Henke [12] do not provide a firm explanation of this 3-fragment phenotype found by them in the D1S7 locus in cut with different enzymes, only speculate about its genetic background, while Olaisen et al. [11] explain a similar case in the D12S11 locus in terms of a somatic mutation.

In the Upper Silesia population, in the D16S309 locus, there has been also observed a covert allele; it has been revealed after the amplification with 205 A and B primers [8]. Such very short alleles (780bp), according to Armour (private correspondence) …"are occasionally found among Africans and appear to be rare in Europeans. Our database of 686 MVR-mapped alleles only contains 7 examples of

alleles with fewer then 10 repeat; all of there are of African origin".

The observed heterozygosity calculated for the Upper Silesia population at the D16S309 locus is 94.91%;, a mutation rate is 0.35% per gamete for male and 0.17% for female.

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