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A cluster of six closely linked STR-markers: Recombination analysis in a 3.6-Mb region at Xq12-13.1

S. Hering [a,*], C. Augstin [b], J. Edelmann [c], M. Heidel [a], J. Dreßler [a], R. Szibor [d]

[a] *Institute of Legal Medicine, Technical University of Dresden, Germany*
[b] *Institute of Legal Medicine, University of Hamburg, Germany*
[c] *Institute of Legal Medicine, University of Leipzig, Germany*
[d] *Institute of Legal Medicine, University of Magdeburg, Germany*

Abstract. Three tetranucleotide tandem repeats (DXS10079, DXS10074 and DXS10075) which were unutilised so far were evaluated for forensic use. Our investigation was amended to establish a cluster of closely linked markers within a 280-kb region at Xq12. Haplotype stability in 152 meioses was demonstrated in a recombination analysis of 96 three-generation families. Three further known markers, DXS7132, HumARA and DXS981 spanning a region of 3.6 Mb, were also included. No recombination events were detected. Hence, we conclude that this cluster segregates with stable haplotypes, providing a powerful tool in kinship testing. © 2005 Elsevier B.V. All rights reserved.

Keywords: STR cluster; Xq12; Haplotyping; Kinship testing

1. Introduction

Forensic use of X-chromosomal markers requires knowledge about their localisation and linkage [1,2]. Closely linked markers are inherited in haplotypes, providing a high potential in kinship analysis [2]. Searching for suitable tetranucleotide tandem repeats, we detected a cluster of three polymorphic repeats located in the human X contig NT_011669 (components AL049564 and AL049564) within a 280-kb segment. To check the stability of haplotypes within this region of Xq12, we performed a recombination analysis. To obtain more informative constellations, the three known STR markers DXS7132 [3], HumARA [4] and DXS981 (STRX1) [5] were also included.

* Corresponding author. Tel.: +49 351 4582972; fax: +49 351 4582972.
E-mail address: Sandra.Hering@tu-dresden.de (S. Hering).

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2. Materials and methods

Buccal swabs were collected from 96 males with daughters and grandsons (anonymous samples). DNA extraction was carried out using the Chelex method or QIAamp DNA Blood Kit (Qiagen, Hilden/Germany). Primers were designed according to GenBank information using the Primer3 software. The amplification was carried out in a 25-μl PCR reaction volume containing approximately 0.1–1 ng DNA, 200 μM each dNTP, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 1 U Taq polymerase (Applied Biosystems, Foster City, CA) and 1 × PCR buffer in a PTC-200 cycler (MJ Research Inc, Watertown, MA, USA). The cycle conditions were: 95 °C–3 min soak, 94 °C–30 s, 60 °C–1 min (primer pair 1, 2 and 3) or 58 °C (primer pair 4, 5 and 6), 72 °C–1 min, 30 cycles, 72 °C–10 min final extension.

Triplex I	DXS10079 (1)	Forw.: 5′-TET-agattgtgccaatgctctcc-3′
		Rev.: 5′-gtttgcctgtgttgtaacatcctt-3′
	DXS10074 (2)	Forw.:5′-HEX-acttcctactgccccacctt-3′
		Rev.: 5′-gtttcccctcagagagctgacaca-′
	DXS10075 (3)	Forw.: 5′-FAM-aggaggggcctagacaagtg-3′
		Rev.: 5′-cagattatgcttgggcctgt-3′
Triplex II	DXS7132 (4)	Forw.: 5′-TET-tgtggaacttcttagcctcct t-3′
		Rev.: 5′-cactcctggtgccaaactct-3′
	DXS981 (5)	Forw.: 5′-HEX-tggtctgccttcttctctcc-3′
		Rev.: 5′-ttgggtggggacacagag-3′
	HumARA (6)	Forw.: 5′-FAM-accgaggagctttccaga at-3′
		Rev.: 5′-ctcatccaggaccaggtagc-3′

The resulting PCR products were resolved and detected by capillary electrophoresis on the ABI Prism® 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

3. Results and discussion

Amplification of the six markers was performed in two sensitive triplex PCR assays. In a German population study, each locus of the three newly established STR markers exhibited 13 (DXS10079), 14 (DXS10074) and 14 (DXS10075) alleles by length, respectively. Observed heterozygosity was 0.77 (DXS10079), 0.85 (DXS10074) and 0.67 (DXS10075). Fig. 1 demonstrates the electrophe-rogram for one family with two sons: one of the grandsons has inherited the grandpaternal haplotype, while the other grandson shows the alternative haplotype which is derived from the grandmother.

Our family study revealed that the markers DXS10079, DXS10074 and DXS10075 represent a stable cluster in which no crossing over occurred. Transmission of haplotypes free of recombination was observed in all of the 152 cases even when the loci DXS7132, HumARA and DXS981 were also considered.

Segregation analysis of the six markers for all families detected the grandpaternal or grandmaternal origin of the haplotype. In 76 of the 152 cases the mothers transferred the grandpaternal haplotype to their sons. This result meets exactly the statistical expectation.

4. Conclusions

Our study on recombination in the STR cluster was carried out in families including females with two or more sons and their maternal grandfather. No information was obtainable regarding mothers who were homozygous in two or in all three loci. Therefore, we included three further STR markers into our study: HumARA is located near

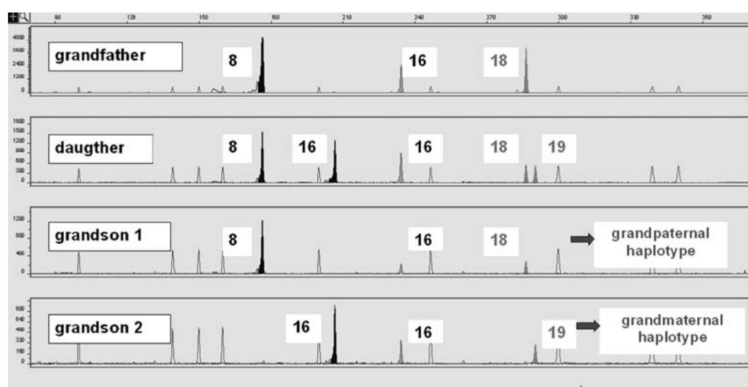

Fig. 1. Electropherogram of triplex I (DXS10074, DXS10075 and DXS10079).

DXS10079, while DXS7132 and DXS981 are outside the cluster spanning a region of 3.6 Mb. On the basis of those results, we conclude that the cluster DXS10079, DXS10074 and DXS10075 segregates with stable haplotypes, providing a powerful tool in kinship testing. Note that HumARA is linked to several disease risks. The use of HumARA is justified only for scientific purposes with anonymised samples [6].

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