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SHORT COMMUNICATION

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Y chromosome polymorphisms and haplotypes in West Saxony (Germany)*

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Abstract In order to apply a set of useful and high polymorphic Y-STRs in paternity testing, we performed a population genetic study from Saxony. The allele distributions of the systems DYS19, DYS385, DYS389I/II and DYS390 were investigated in a sample of 250 unrelated males from the area of Leipzig. PCR products were detected using native polyacrylamide gel electrophoresis as well as capillary electrophoresis and GenScan Software on the ABI Prism 310 DNA sequencer. Haplotype frequency data of 164 different types were obtained which show that these four systems are very useful for special cases of paternity and forensic stain analysis. In addition several confirmed father-son pairs were examined using the paternity cases of the institute. One mutation was found in the system DYS390 and sequencing data are presented.

Key words Short tandem repeat · Y-chromosome · Y-haplotype analysis

Introduction

Y-linked polymorphisms are haploid therefore most of the chromosome does not recombine. The male specificity makes it useful for forensic studies because the majority of crimes are committed by males [2]. Y-chromosomal STRs have recently been established for routine casework in paternity testing especially in deficiency cases as well as in forensic stain analysis [2, 6, 9, 10]. More than 14 Y-specific STR markers are known [3] and provide simple, reproducible and sensitive markers for male identification. Although these systems provide much information there are limitations especially in cases of non-exclusions [2].

* The results of DYS19 and DYS390 were presented at the 17th International Congress of the ISFH / Oslo 1997

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Materials and methods

DNA was extracted from blood using KCl-ethanol procedures. PCR amplification of DYS19, DYS389I/II and DYS390 were performed according to Roewer et al. [8,9], Kayser et al. [3] and DYS385 to Schneider et al. [10], using the following primer sequences:

DYS19 [8]
Primer I: 5′-CTACTGAGTTTCTGTTATAGT-3′ (6-FAM labeled)
Primer II: 5′-ATGGCATGTAGTGAGGACA-3′

DYS385 [10]
Primer I: 5′-AGCATGGGTGACAGAGCTA-3′ (6-FAM labeled)
Primer II: 5′-GGGATGCTAGGTAAAGCTG-3′

DYS389I/II [3]
Primer I: 5′-CCAACTCTCATCTGTATTATCTAT-3′ (TET labeled)
Primer II: 5′-TCTTATCTCCACCCACCAGA-3′

DYS390 [9]
Primer I: 5′-TATATTTTACACATTTTTGGGCC-3′ (HEX labeled)
Primer II: 5′-TGACAGTAAAATGAACACATTGC-3′

Amplification conditions: 5 ng DNA, 1U AmpliTaq (PE), 200 μM dNTPs, 100 mM Tris-HCl pH 8.3, 500 mM KCl, 15 mM $MgCl_2$, 0.01 w/v gelatine (PE), 0.25 μM each primer.

Cycling conditions (Biometra): DYS19/DYS390: 94 °C – 1 min, 56 °C – 1 min, 72 °C – 1 min, 29 cycles
DYS389I/II: 94 °C – 3 min, 94 °C – 15 s, 58 °C – 20 s, 72 °C – 20 s, 5 cycles, 94 °C – 15 s, 54 °C – 20 s, 72 °C – 20 s, 30 cycles
DYS385: 94 °C – 3 min, touchdown PCR as follows 94 °C – 30 s, 59–57 °C – 30 s (3 × 2 cycles at each temp.), 72 °C – 1 min, then 29 cycles with 56° C annealing temperature and final extension 72 °C – 7 min.

Electrophoresis: PCR fragments of DYS19 were separated in native polyacrylamide gels (6%T, 3% C, 750 μm) and visualized by silver staining. Alleles were designated according to the number of repeats using a commercially available allelic ladder (Invitek). PCR amplification of DYS385, DYS389I/II, DYS390 and also DYS19 was performed using fluorescently labeled primers, followed by capillary electrophoresis in denaturing polymers (POP4, PE) and detection in the ABI 310 sequencer (Applied Biosystems / Perkin Elmer). Allele assignment for DYS385, DYS389I/II and DYS390 was possible by comparison with a self-made allelic ladder.

Gene/haplotype diversity: calculated according to Nei [5] – $P(Y) = \Sigma (P_i)^2$
Allele nomenclature: according to reference tables [7]

Sequencing: sequencing of several DYS390 alleles was carried out on a ABI 310 sequencer using TaqFS Dye-Deoxy-Terminator

Table 1 Allele frequencies of DYS19

Allele	obs. (*n*)	obs. (%)
13	14	5.6
14	115	46.0
15	56	22.4
16	43	17.2
17	23	8.8

Table 2 Allele frequencies of DYS390

Allele	obs. (*n*)	obs. (%)
21	2	0.8
22	21	8.4
23	64	25.6
24	82	32.8
25	76	30.4
26	5	2.0

Table 3 Haplotype frequencies of DYS389I/II

Haplotype	obs. (*n*)	obs (%)
8/24	2	0.8
9/24	1	0.4
9/25	28	11.2
9/26	14	5.6
9/27	2	0.8
10/24	1	0.4
10/25	11	4.4
10/26	83	33.2
10/27	52	20.8
10/28	21	8.4
10/29	4	1.6
11/26	1	0.4
11/27	16	6.4
11/28	7	2.8
11/29	4	1.6
11/30	3	1.2

Cycle Sequencing Kit (ABI) and 1.5 pmol primer. Sequencing was done with forward as well as reverse primers.

Results and discussion

For DYS19 we found no differences between the results obtained by silver staining and fluorescence detection using the ABI Prism 310. The results are reported in Tables 1–4. For our population study we calculated gene- and haplotype diversities as shown in Table 5.

The results for DYS19, DYS385 and DYS390 were compared to those reported for other Caucasians and especially German populations [3, 4, 7, 10]. The gene- and haplotype frequencies showed no large differences as shown in Tables 6-8 (the gene-/haplotype diversities were calculated by the reported frequencies).

A total of 164 different haplotypes could be identified (Table 9) as a result of combining the 33 alleles of the 4 Y-linked systems containing 6 loci. This shows the enormous power for male identification.

To obtain information about mutation rates 41 confirmed father-son pairs were analysed. All alleles were in-

Table 4 Haplotype frequencies of DYS385

Haplotype	obs. (*n*)	obs. (%)
9/14	1	0.4
10/14	17	6.8
10/15	5	2.0
10/16	2	0.8
11/13	9	3.6
11/14	103	41.2
11/15	17	6.8
11/16	4	1.6
12/13	1	0.4
12/14	13	5.2
12/15	2	0.8
12/16	1	0.4
12/17	1	0.4
13/13	2	0.8
13/14	12	4.8
13/15	9	3.6
13/16	1	0.4
13/17	5	2.0
14/14	6	2.4
14/15	11	4.4
14/16	2	0.8
15/15	2	0.8
15/16	5	2.0
15/17	2	0.8
15/19	1	0.4
16/16	1	0.4
16/17	2	0.8
16/18	7	2.8
16/19	1	0.4
17/18	4	1.6
17/19	1	0.4

Table 5 Calculated gene and haplotype diversities

System	Gene/haplotype diversity
DYS19	0.698
DYS385	0.808
DYS389I/II	0.816
DYS390	0.727
YH	0.987

YH: DYS19+DYS385+DYS389I/II +DYS390

Table 6 Gene diversities of DYS19 in nine European populations

Population	Number of individuals	Gene diversity
Leipzig	250	0.698
Münster [7]	272	0.605
Köln [7]	100	0.547
Jena [7]	143	0.632
Heidelberg [7]	113	0.651
Magdeburg [7]	210	0.682
Berlin* [7]	233	0.722
Leiden [7]	88	0.471
Roma [7]	100	0.642

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Table 7 Gene diversities of DYS390 in six European populations

Population	Number of individuals	Gene diversity
Leipzig	250	0.727
Münster [7]	114	0.740
Heidelberg [7]	104	0.753
Berlin* [7]	70	0.737
Leiden [7]	88	0.708
Roma [7]	100	0.671

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Table 8 Haplotype diversities of DYS385 in four European populations

Population	Number of individuals	Gene diversity
Leipzig	250	0.808
Magdeburg [7]	164	0.834
Mainz |10]	146	0.870
Berlin* [7]	77	0.850

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Table 9 Haplotype frequencies of DYS19/DYS390/DYS389I/II/DYS385

Haplotypes	obs. *n*	obs. %	Haplotypes	obs. *n*	obs. %
13/24/10/26/12/14	1	0.4	14/23/10/27/11/14	5	2.0
13/24/10/26/15/16	1	0.4	14/23/10/28/14/16	1	0.4
13/24/10/27/15/17	1	0.4	14/23/10/28/16/18	1	0.4
13/24/10/27/15/19	1	0.4	14/23/11/27/11/13	1	0.4
13/24/10/27/16/18	1	0.4	14/23/11/27/11/14	3	1.2
13/24/10/27/16/19	1	0.4	14/23/11/28/11/14	1	0.4
13/24/10/27/17/18	1	0.4	14/24/08/24/11/15	1	0.4
13/24/10/28/16/16	1	0.4	14/24/09/25/11/16	1	0.4
13/24/10/28/16/18	2	0.8	14/24/09/25/13/15	1	0.4
13/25/10/27/10/15	1	0.4	14/24/09/26/17/19	1	0.4
13/25/10/28/16/18	1	0.4	14/24/10/25/10/14	1	0.4
13/25/10/28/17/18	1	0.4	14/24/10/26/10/14	1	0.4
13/25/10/29/17/18	1	0.4	14/24/10/26/11/14	16	6.4
13/25/11/29/15/17	1	0.4	14/24/10/26/12/14	4	1.6
14/22/09/25/11/14	1	0.4	14/24/10/26/14/14	1	0.4
14/22/09/25/13/14	3	1.2	14/24/10/27/10/14	1	0.4
14/22/09/25/13/15	2	0.8	14/24/10/27/11/14	6	2.4
14/22/09/26/13/13	1	0.4	14/24/10/27/12/14	2	0.8
14/22/09/26/13/14	1	0.4	14/24/10/27/16/17	1	0.4
14/22/09/27/13/14	1	0.4	14/24/10/28/14/15	1	0.4
14/22/10/26/11/13	1	0.4	14/24/11/26/11/14	1	0.4
14/22/10/26/11/15	1	0.4	14/24/11/27/11/14	1	0.4
14/22/10/26/14/14	1	0.4	14/24/11/28/11/14	1	0.4
14/22/10/27/12/14	1	0.4	14/25/10/25/11/15	1	0.4
14/22/10/27/13/15	1	0.4	14/25/10/26/10/14	1	0.4
14/22/11/27/13/15	1	0.4	14/25/10/26/11/14	5	2.0
14/23/08/24/14/14	1	0.4	14/25/10/26/11/15	2	0.8
14/23/09/24/14/14	1	0.4	14/25/10/26/11/16	1	0.4
14/23/09/25/11/15	1	0.4	14/25/10/27/10/14	1	0.4
14/23/09/25/13/14	3	1.2	14/25/10/27/11/13	1	0.4
14/23/09/25/14/15	1	0.4	14/25/10/27/11/14	1	0.4
14/23/09/26/17/18	1	0.4	14/25/10/27/11/16	1	0.4

Table 9 Continued

Haplotypes	obs. *n*	obs. %	Haplotypes	obs. *n*	obs. %
14/23/10/24/12/14	1	0.4	14/25/10/28/10/14	1	0.4
14/23/10/25/11/13	1	0.4	14/25/10/29/11/14	1	0.4
14/23/10/25/11/14	4	1.6	14/25/11/27/11/14	2	0.8
14/23/10/26/10/16	1	0.4	14/25/11/30/16/18	2	0.8
14/23/10/26/11/13	1	0.4	15/21/09/25/13/15	1	0.4
14/23/10/26/11/14	10	4.0	15/21/09/26/12/16	1	0.4
14/23/10/26/11/15	1	0.4	15/22/09/25/13/14	1	0.4
14/23/10/26/11/16	1	0.4	15/22/09/25/13/15	1	0.4
14/23/10/27/11/13	1	0.4	15/22/09/26/12/14	1	0.4
15/22/09/26/13/13	1	0.4	16/24/09/25/10/14	1	0.4
15/22/09/26/14/14	1	0.4	16/24/09/25/10/15	1	0.4
15/22/09/26/14/15	1	0.4	16/24/09/25/13/17	1	0.4
15/22/10/26/14/16	1	0.4	16/24/09/27/14/15	1	0.4
15/22/10/27/14/15	1	0.4	16/24/10/26/11/14	1	0.4
15/23/09/25/13/14	3	1.2	16/24/10/26/14/15	1	0.4
15/23/09/26/14/14	1	0.4	16/24/10/26/15/15	1	0.4
15/23/10/25/11/14	1	0.4	16/24/10/28/14/15	2	0.8
15/23/10/25/15/15	1	0.4	16/24/10/29/13/15	1	0.4
15/23/10/26/11/14	4	1.6	16/24/10/29/14/15	1	0.4
15/23/10/26/11/15	1	0.4	16/25/09/26/12/17	1	0.4
15/23/10/26/16/17	1	0.4	16/25/10/26/10/14	1	0.4
15/23/10/27/11/14	1	0.4	16/25/10/26/11/14	5	2.0
15/23/11/27/11/13	1	0.4	16/25/10/26/11/15	2	0.8
15/23/11/27/13/15	1	0.4	16/25/10/27/10/14	2	0.8
15/23/11/28/12/14	1	0.4	16/25/10/27/11/14	5	2.0
15/23/11/28/15/16	1	0.4	16/25/10/27/11/15	1	0.4
15/23/11/30/15/16	1	0.4	16/25/10/27/12/14	1	0.4
15/24/09/25/11/15	1	0.4	16/25/10/28/11/14	1	0.4
15/24/09/25/13/17	2	0.8	16/25/11/27/11/14	3	1.2
15/24/10/25/11/14	1	0.4	16/25/11/27/11/15	1	0.4
15/24/10/25/11/15	1	0.4	16/25/11/28/11/14	1	0.4
15/24/10/26/10/14	1	0.4	16/26/10/26/09/14	1	0.4
15/24/10/26/11/14	4	1.6	16/26/10/26/11/14	1	0.4
15/24/10/26/15/16	1	0.4	17/24/10/26/11/14	1	0.4
15/24/10/27/11/13	1	0.4	17/24/10/26/11/15	2	0.8
15/24/10/27/11/14	1	0.4	17/24/10/27/10/14	1	0.4
15/24/10/28/14/15	1	0.4	17/24/10/27/11/14	1	0.4
15/24/11/29/15/16	1	0.4	17/24/11/28/11/13	1	0.4
15/25/09/25/13/17	1	0.4	17/25/09/25/13/16	1	0.4
15/25/10/26/11/14	1	0.4	17/25/09/25/13/17	1	0.4
15/25/10/27/10/14	1	0.4	17/25/09/26/10/15	2	0.8
15/25/10/27/11/14	3	1.2	17/25/09/26/11/14	1	0.4
15/25/10/27/12/14	1	0.4	17/25/10/27/10/14	2	0.8
15/25/10/28/11/14	1	0.4	17/25/10/27/10/15	1	0.4
15/25/10/28/14/15	1	0.4	17/25/10/28/10/14	2	0.8
15/25/11/29/11/14	1	0.4	17/25/10/28/10/16	1	0.4
15/25/11/29/12/13	1	0.4	17/25/10/28/11/14	3	1.2
15/26/10/26/11/14	1	0.4	17/25/11/28/11/14	1	0.4
16/23/10/26/11/14	1	0.4	17/26/10/26/11/14	1	0.4
16/23/10/27/11/15	1	0.4			
16/23/11/27/12/15	2	0.8			

herited in a regular way with the exception of one case where a mutation in DYS390 was observed. The father was found to have the allele 24 while the son showed allele 23 which was a single exclusion after investigation of

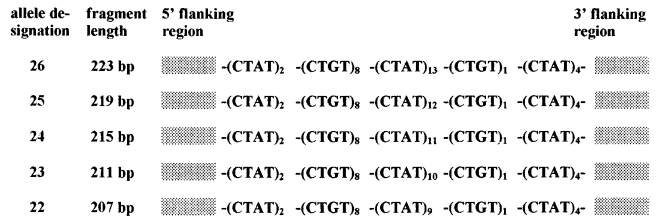

Fig. 1 Sequence structure and fragment length of different DYS390 alleles including the alleles 24 and 23 of a confirmed father-son pair. (Sequencing was performed on three different persons for every allele, always with both primers)

12 classical blood group systems, 4 single locus systems and 8 autosomal PCR-VNTRs. The probability of paternity for this case using all systems investigated except the Y-linked systems was calculated as W= 99.99999999% and PI = $8.43*10^{10}$. Sequencing data showed that an additional [CTAT] repeat unit was present in the father (Fig. 1). The number of the variable [CTAT] repeats is responsible for differences in allele length and we detected 11 repeats for the father and only 10 for the son. Mutations for Y-STRs have been described by Kayser et al. [3] and Heyer et al. [1] who reported that they seem to lie in the range of those for autosomal systems.

In conclusion the Y-chromosomal STRs investigated are useful in forensic casework especially in paternity analysis for deficiency and rape cases. Regional haplotype frequency data should be used.

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