TECHNICAL NOTE

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Swiss Allele Frequencies and Haplotypes of 7 Y-Specific STRs

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ABSTRACT: In view of application to personal identification and paternal analysis, the allele distribution of the loci DYS19, DYS389 I and II, DYS390, DYS391, DYS392, and DYS393 were determined in a sample of 126 unrelated males from the area of Bern (Switzerland). The 7 Y-STR loci were coamplified in a total of two multiplex reactions using fluorescently-labeled primers. PCR products were separated and detected on a capillary electrophoresis ABI Prism 310 instrument. All loci were polymorphic and the allele distributions are similar to other caucasian data.

KEYWORDS: forensic science, Y chromosome, Y-haplotype analysis, short tandem repeat

Y-chromosomal STR polymorphisms are becoming of increasing interest in the forensic field, because of their possible application in stain analyses and in paternity testing particularly in male lineage cases (1–3). Since the majority of sexual offenses have a male perpetrator and a female victim, using Y-chromosome specific primers can improve the chances of being able to detect small amounts of perpetrator DNA in a high background of heterologous female DNA (4). Other possible applications include the determination of the number of male contributors in a mixed sample. In order to evaluate the statistical significance of Y-STR allele results, a database for Swiss males has been established for seven Y-chromosomale STRs (DYS19, DYS389 I/II, DYS390, DYS391, DYS 392, and DYS393) (5).

Materials and Methods

Population Sample

One hundred twenty-six Swiss males from the area of Bern were analyzed at the following tetranucleotide STR loci: DYS19, DYS389I/II, DYS390, DYS391, DYS393 and at the trinucleotide locus DYS392. Primer sequences were those described by Kaiser et al. (6).

Multiplex 1: DYS19: Primer 1-JOE labeled
DYS389 I/II: Primer 1- JOE labeled
DYS390: Primer 1- FAM labeled
Multiplex 2: DYS391: Primer 1-FAM labeled
DYS392: Primer 1-JOE labeled
DYS393: Primer 1-FAM labeled

Amplifications Conditions

Multiplex 1—2 ng template-DNA, 2 U AmpliTaq Gold (Perkin Elmer), 0.25 μM each primer DYS19 and DYS390, 0.125 μM each primer DYS389, 200 μM dNTPs, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris HCl pH 8.3, 0.01% gelatin, 160 μg BSA in a total volume of 20 μL.

Multiplex 2—2 ng template-DNA, 2 U AmpliTaq Gold (Perkin Elmer), 0.3 μM each primer DYS391 and DYS393, 1 μM each primer DYS392, 200 μM dNTPs, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris HCl pH 8.3, 0.01% gelatin, 160 μg BSA in a total volume of 20 μL.

Cycling conditions (Perkin Elmer TC9600)—95°C − 11 min, 94°C − 1 min, 55°C − 1 min, 72C° − 2 min, 30 cycles, 60°C − 30 min.

Electrophoresis—Multiplex 1 and multiplex 2 amplification products were subjected to electrophoresis on an ABI PRISM 310 Genetic Analyzer instrument. After amplification, 1 μL of PCR product and 1 μL of GeneScan™-500 (Rox) Internal Lane Size Standard (GeneScan™-350 (Rox) was used for the multiplex 2 products) were added to 24 μL of deionized formamide, denaturated at 95°C for 3 min and immediately placed on ice. The PCR products were injected for 5 s at 15 kv and electrophoresis was performed in Performance Optimized Polymer sieving medium (POP4™; 1 mL syringe). Data were collected using ABI PRISM 310 Collection software application, with the module GS POP4 A (virtual filter set A).

Allele assignement for the 7 Y-STRs was made possible by comparison with an in-house constructed allelic ladder. Allele nomenclature: according to reference tables (5).

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Results and Discussion

Population Studies

A total of 126 unrelated individuals from the area of Bern (Switzerland) were analyzed, and the allele frequencies of each system are shown in Table 1. The gene diversity values were calculated to be 61.2% (DYS19), 58.2% (DYS389 I), 74.5% (DYS389 II), 71.4% (DYS390), 48.9% (DYS391), 64.5% (DYS392), and 41.9% (DYS393). The estimated values were similar to those published previously (6). One rare intermediate allele 12.2 was observed at the locus DYS393, this allele was not sequenced but migrated to a position 2 bp smaller than the allele 13.

TABLE 1—*Allele frequencies of 7 Y-STRs and gene diversity (1- Σp_i^2, p_i allele frequencies).*

DYS19	
Allele	Frequency (%)
13	12.6
14	55.9
15	23.6
16	6.3
17	1.6
Gene diversity = 61.16%	

DYS389 I	
Allele	Frequency (%)
8	1.6
9	24.4
10	57.5
11	16.5
Gene diversity = 58.24%	

DYS389 II	
Allele	Frequency (%)
23	0.8
24	4.7
25	16.5
26	40.2
27	22.8
28	10.2
29	3.9
31	0.8
Gene diversity = 74.49%	

DYS390	
Allele	Frequency (%)
21	3.1
22	15.7
23	24.4
24	42.5
25	14.2
Gene diversity = 71.37%	

DYS391	
Allele	Frequency (%)
9	0.8
10	61.9
11	35.7
12	1.6
Gene diversity = 48.49%	

DYS392	
Allele	Frequency (%)
11	43.7
12	9.5
13	38.9
14	6.3
15	1.6
Gene diversity = 64.49%	

DYS393	
Allele	Frequency (%)
11	0.8
12	7.1
12.2	0.8
13	74.6
14	13.5
15	3.2
Gene diversity = 41.91%	

Sensitivity

The sensitivity of both multiplex PCR systems was approx. 200 pg of template DNA (data not shown).

Haplotype Analysis

For the 126 individuals, 7 loci-haplotypes were constructed (Table 2), 18 haplotypes occurred more than once whereas the remaining 72 combinations were observed once (98.236% overall gene diversity; 71.4% discrimination capacity). The most common haplotype (14/10/26/24/11/23/13) determined in this study had a frequency of 5.6%.

Forensic Case Application

As an example of the application of Y-STRs, a case study is provided. After a sexual assault only very few sperm cells could be recovered from the vaginal swab. A differential lysis was performed and the male fraction of the vaginal swab and the victim's reference sample were amplified with the AmpFISTR Profiler™ kit (Fig. 1). The small area of the Y peak at the amelogenin locus compared to the area of the X peak indicates that very small amounts of male DNA are present in this male fraction. In such a case, typing Y-STR loci may be more effective, because of less competition for reagents with the predominant female DNA. In addition, it is possible that male epithelial cells and leukocytes may be typed in the female fraction. Therefore the male fraction DNA was amplified with the multiplex 1 and 2 system. A DNA profile of 7 Y-STRs could be obtained from the sperm cells (Fig. 2). The Y-haplotype of the sperm cells had not yet been observed in our database of 126 samples. Currently, no suspect was available for comparison.

In conclusion, the multiplex analysis of these 7 Y-STRs can be done in addition to the typing of autosomal markers. Thus, analyses of biological samples with female/male admixture and paternity tests with male children can be more effectively analyzed when autosomal loci typing is uniformative. This swiss haplotype data can be used to provide an estimate of the rarity of the profile.

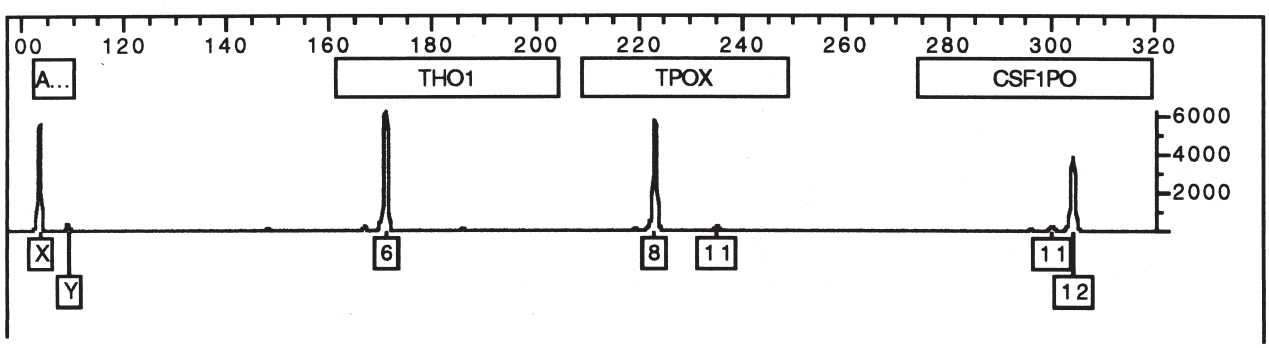

FIG. 1—*Electropherograms of the male fraction of the vaginal swab at the amelogenin, THO1, TPOX, and CSF1PO loci.*

TABLE 2—*Haplotype frequencies of the loci DYS19/DYS389I/DYS389II/DYS390/DYS391/DYS392/DYS393.*

Haplotypes	Obs. N	Obs. %	Haplotypes	Obs. N	Obs. %
13/9/26/23/10/11/13	1	0.8	14/11/27/23/10/13/13	1	0.8
13/9/26/24/10/15/13	1	0.8	14/11/27/23/11/13/13	1	0.8
13/9/27/24/11/11/13	1	0.8	14/11/27/24/10/13/13	2	1.6
13/10/23/24/11/13/12	1	0.8	14/11/28/23/11/14/14	1	0.8
13/10/26/24/10/13/13	1	0.8	14/11/28/24/10/11/12	1	0.8
13/10/27/23/10/11/12	1	0.8	14/11/29/22/10/11/14	1	0.8
13/10/27/24/10/11/13	6	4.8	14/11/31/22/10/12/15	1	0.8
13/10/27/25/10/11/13	1	0.8	15/9/24/21/10/11/13	1	0.8
13/10/28/24/10/11/13	2	1.6	15/9/24/23/10/11/13	1	0.8
13/10/28/25/10/11/13	1	0.8	15/9/24/24/10/11/12	1	0.8
14/8/24/22/10/11/13	2	1.6	15/9/25/22/10/11/13	1	0.8
14/9/25/21/10/11/13	1	0.8	15/9/25/23/11/13/13	1	0.8
14/9/25/22/10/11/13	4	3.2	15/9/25/24/11/14/13	1	0.8
14/9/25/22/11/11/13	3	2.4	15/9/26/21/10/11/13	1	0.8
14/9/25/23/10/12/13	1	0.8	15/9/26/21/10/11/15	1	0.8
14/9/25/24/10/14/13	1	0.8	15/9/26/22/10/11/13	1	0.8
14/9/25/24/11/13/13	1	0.8	15/9/26/22/10/11/14	1	0.8
14/9/26/23/11/13/13	1	0.8	15/9/26/25/11/11/14	1	0.8
14/10/25/22/10/13/13	1	0.8	15/9/27/23/11/11/13	1	0.8
14/10/25/24/10/13/13	2	1.6	15/10/26/23/9/11/12	1	0.8
14/10/25/24/10/15/12	1	0.8	15/10/26/23/11/13/13	1	0.8
14/10/25/25/11/12/13	1	0.8	15/10/26/24/11/13/13	1	0.8
14/10/26/22/10/13/13	1	0.8	15/10/26/24/11/14/13	1	0.8
14/10/26/23/10/13/13	3	2.4	15/10/26/25/10/13/12	1	0.8
14/10/26/23/10/13/14	1	0.8	15/10/27/22/11/11/14	1	0.8
14/10/26/23/11/13/13	2	1.6	15/10/27/23/10/12/14	1	0.8
14/10/26/23/11/13/14	1	0.8	15/10/27/25/10/11/13	1	0.8
14/10/26/23/11/14/13	1	0.8	15/10/27/25/11/11/13	1	0.8
14/10/26/23/12/13/13	1	0.8	15/10/28/23/10/11/13	1	0.8
14/10/26/24/10/13/13	6	4.8	15/11/27/23/10/12/14	1	0.8
14/10/26/24/10/14/13	2	1.6	15/11/27/24/10/13/13	2	1.6
14/10/26/24/11/12/13	2	1.6	15/11/28/23/10/12/14	1	0.8
14/10/26/24/11/13/13	7	5.6	15/11/28/25/10/11/13	1	0.8
14/10/26/24/11/13/14	1	0.8	15/11/29/23/10/12/14	1	0.8
14/10/26/24/11/14/13	1	0.8	15/11/29/23/10/12/15	2	1.6
14/10/26/24/12/13/12.2	1	0.8	16/9/24/25/10/11/13	1	0.8
14/10/26/25/11/13/13	2	1.6	16/9/25/23/10/11/13	1	0.8
14/10/27/24/10/11/11	1	0.8	16/9/25/24/11/11/13	1	0.8
14/10/27/24/10/11/12	1	0.8	16/9/26/24/10/11/12	1	0.8
14/10/27/24/11/13/13	3	2.4	16/10/27/25/10/11/13	1	0.8
14/10/27/25/11/13/13	1	0.8	16/10/28/25/11/11/14	1	0.8
14/10/28/22/10/11/14	2	1.6	16/11/28/25/10/11/13	1	0.8
14/11/26/23/11/13/13	1	0.8	16/11/29/23/10/12/14	1	0.8
14/11/26/24/11/13/13	1	0.8	17/10/26/25/10/11/13	1	0.8
14/11/27/22/10/11/14	1	0.8	17/10/28/25/11/11/13	1	0.8

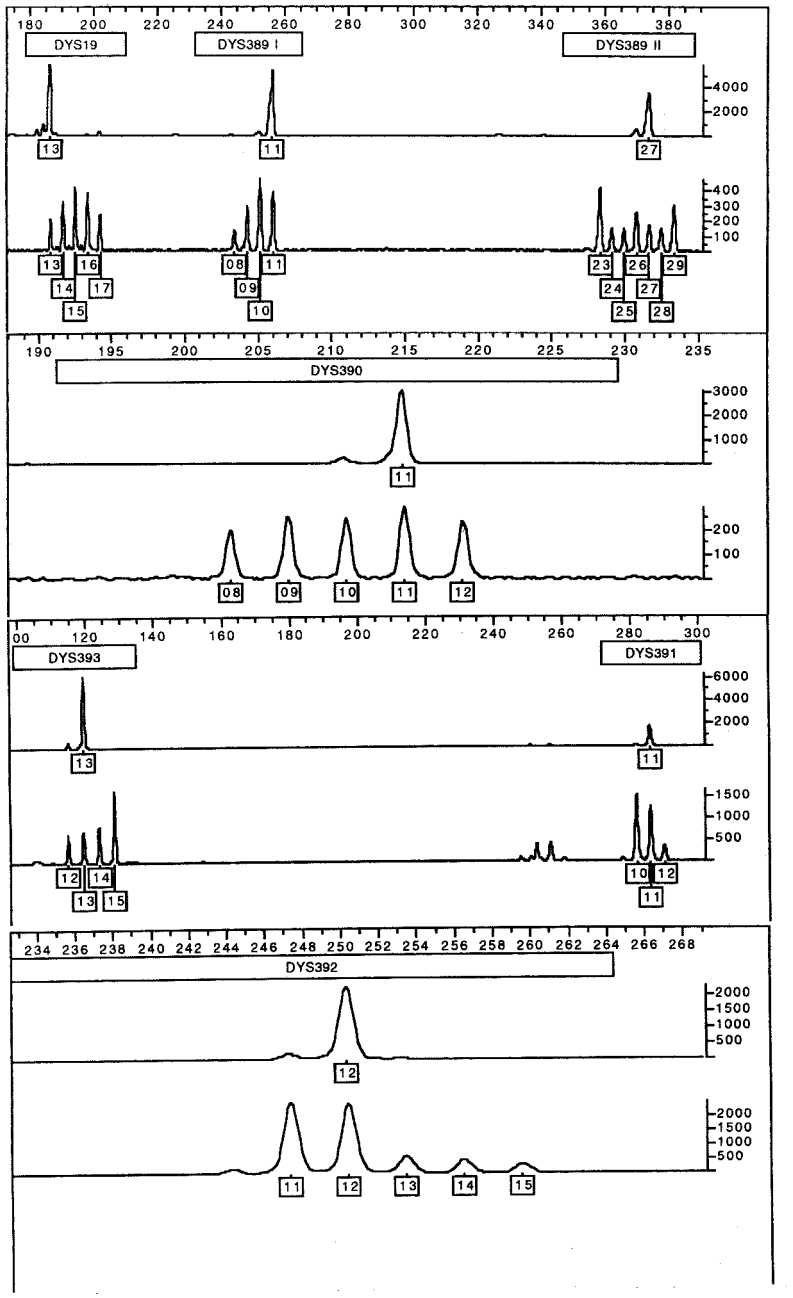

FIG. 2—*Electropherograms of the male fraction of the vaginal swab at the loci DYS19, DYS389I, DYS389II, DYS390, DYS393, DYS391, and DYS392 and allelic ladders for those 7 loci.*

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