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SHORT COMMUNICATION

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Y-Chromosome STR haplotypes in an Italian population sample

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Abstract Seven Y-chromosome STRs were investigated in a male population sample from the Modena area by means of one quadruplex reaction (systems DYS19, DYS390, DYS391, DYS393), one duplex reaction (systems DYS389-II, DYS392) and two single PCR reactions (DXYS156 and DYS389-I/II). In 100 males, 71 different haplotypes could be observed, 57 of which were seen only once. The haplotype diversity/discrimination index is 0.97. The resulting database could be used for routine forensic application like paternity testing and stain investigation.

Introduction

Until recently only a limited number of polymorphic loci on the non-recombinant portion of the human Y chromosome have been identified and even fewer were amenable to PCR amplification (Mathias et al. 1994). Subsequently, highly polymorphic Y-specific microsatellites showing a high level of Y chromosomal heterogeneity within and between populations were described (Roewer et al. 1996) and tested on different population samples in order to establish locus-specific databases for routine forensic application as well as for human evolutionary studies (Kayser et al. 1997).

The aim of this investigation was the analysis of seven Y-STRs in a male population sample from the Modena area in northern Italy in order to establish a haplotype database.

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Materials and methods

DNA samples

Fresh bloodstains were obtained from a random sample of 100 unrelated donors of the Modena area. DNA was extracted from bloodstains using the Chelex-100 extraction method (Walsh et al. 1991) and quantified by means of the slot blot technique and the human specific probe D17Z1 (Gibco BRL UK) (Waye et al. 1989).

Amplification conditions

PCR was performed in a volume of 25 µl in 50 mM KCl,10 mM Tris-HCl (pH 8.3), 1.5 mM $MgCl_2$, 200 µM dNTPs, 2 U of gold Taq polymerase (Perkin-Elmer) and 10 ng of genomic DNA. For the quadruplex system (DYS19-DYS390-DYS391-DYS393), the following primers were used: 0.5 µM forward primer DYS393, 1 µM forward primer DYS19, 0.3 µM forward primer DYS391, 1 µM forward primer DYS390 and 0.4 µM reverse primer DYS393, 0.8 µM reverse primer DYS19, 4 µM reverse primer DYS391, 1.4 µM reverse primer DYS390. For the duplex system (DYS389-II and DYS392), primer concentrations were as follows: 0.5 µM of both primer for DYS389-II and 1 µM of both primer for DYS392. The forward primers were labeled with the following dyes: DYS19 Fam, DYS390 Hex, DYS391 Hex, DYS393 Fam, DYS 389 Fam, DYS392 Fam. For the amplification of the DXYS156, the primer concentration was 1.7 µM for the forward primer and 1 µM for the reverse primer for the DYS389-I/II locus both primers were 0.5 µM and 1 U of Taq polymerase was used in the singleplex reactions. The other conditions were the same. Primers sequences for DYS19, DYS389-I/II, DYS390, DYS391, DYS392 and DYS393 are described in Kayser et al. (1997), primer sequences for DXYS156 were taken from Chen et al. (1994). The sequence of the forward primer that amplifies only 389-II is TCATCTGTA-TTATCTATGTGTG, the reverse primer is identical to that in Kayser et al. (1997). Cycling conditions for DYS19, DYS390, DYS391, DYS393 and DYS389 and DYS392 were: 94 °C for 10 min, then 30 cycles of 94 °C for 90 s, 55 °C for 30 s, 72 °C for 30 s and for DXYS156: 94 °C for 6 min, then 30 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 1 min (Thermocycler: Biometra Triothermoblock, Germany).

Detection systems for quadruplex and duplex reactions

Aliquots of 1–3 µl of PCR products mixed with 4 µl formamide, 0.5 µl loading buffer and 0.5 µl internal standard GS 2500 (Applied Biosystems) were separated in a 6% polyacrylamide/bisacrylamide (19:1) gel containing 8 M urea, 1 X TBE buffer with a sep-

Table 1 The 71 Y chromosome haplotypes detected in 100 unrelated males from the Modena-area. Nr. = number of the haplotype, *n* = individuals observed for each haplotype

Nr.	DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DXYS156	n
1	13	10	26	24	11	13	13	7	1
2	13	10	27	23	10	11	14	7	1
3	13	10	27	24	10	11	13	7	2
4	13	10	28	24	10	13	13	7	3
5	13	11	27	24	10	13	13	7	1
6	13	11	28	24	10	11	13	7	1
7	13	12	29	24	10	13	13	7	1
8	14	9	24	24	10	13	13	7	1
9	14	9	25	23	10	11	13	7	1
10	14	9	25	24	10	13	13	7	1
11	14	9	25	24	11	13	13	7	2
12	14	9	26	22	10	11	14	7	1
13	14	9	26	25	10	13	13	7	1
14	14	10	25	22	11	13	13	7	2
15	14	10	25	24	10	14	13	7	1
16	14	10	25	24	11	13	13	7	1
17	14	10	25	25	10	14	12	7	1
18	14	10	26	23	10	11	12	7	1
19	14	10	26	23	11	12	13	7	1
20	14	10	26	23	11	13	13	7	1
21	14	10	26	24	10	13	13	7	1
22	14	10	26	24	10	14	13	7	1
23	14	10	26	24	11	11	14	7	1
24	14	10	26	24	11	13	13	7	13
25	14	10	26	24	11	13	13	8	1
26	14	10	26	24	12	13	12	7	1
27	14	10	26	24	12	13	13	7	1
28	14	10	26	25	11	13	13	7	3
29	14	10	26	25	11	13	14	7	1
30	14	10	27	22	10	12	12	7	1
31	14	10	27	23	10	13	13	7	1
32	14	10	27	23	11	13	12	7	1
33	14	10	27	24	10	13	13	7	1
34	14	10	27	24	11	13	13	7	1
35	14	10	27	24	11	14	12	7	1
36	14	10	27	25	10	13	12	7	1
37	14	10	28	23	10	10	13	7	1
38	14	10	28	23	11	13	13	7	2
39	14	10	28	24	10	11	13	7	1
40	14	11	26	24	11	13	13	7	2
41	14	11	27	23	10	15	14	7	2
42	14	11	27	24	10	13	12	7	1
43	14	11	27	24	10	13	13	7	3
44	14	11	27	24	11	12	13	7	1
45	14	11	27	24	11	13	12	7	1
46	14	11	27	24	11	13	13	7	1
47	14	12	28	24	11	13	13	7	1
48	15	9	25	22	10	10	13	7	1
49	15	9	25	24	11	13	13	7	1
50	15	9	25	25	10	11	13	7	2
51	15	9	27	24	10	13	12	7	1
52	15	10	25	24	11	13	13	?	1
53	15	10	26	23	9	11	12	7	2
54	15	10	26	23	10	12	14	7	1
55	15	10	26	23	10	13	13	7	2
56	15	10	26	23	10	13	14	7	1

Table 1 (continued)

Nr.	DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DXYS156	n
57	15	10	26	23	11	13	13	7	1
58	15	10	26	24	9	12	12	7	2
59	15	10	26	24	10	13	13	7	1
60	15	10	26	24	11	13	13	7	2
61	15	10	26	24	11	13	14	7	1
62	15	10	27	22	11	11	14	7	1
63	15	10	27	25	11	11	13	7	1
64	15	11	27	23	10	11	12	7	1
65	16	9	26	25	10	11	12	7	1
66	16	9	27	21	10	11	15	7	1
67	16	9	27	22	10	12	13	7	1
68	16	10	26	24	9	11	12	7	1
69	16	10	26	24	11	13	13	7	1
70	16	10	27	24	11	13	13	7	1
71	16	10	29	24	11	11	13	7	1

aration distance of 24 cm. The gel solution was filtered and degassed before the addition of 180 µl 10% ammonium peroxodisulfate solution and 24 µl TEMED (N′N′N-′N′-tetramethylethylendiamine). Electrophoresis was carried out on the ABI 373A automated sequencer (Applied Biosystems Division/Perkin Elmer) at 2000 V, 50 mA, 35 W for 3 h and the data were automatically sized using GeneScan 672 software (Applied Biosystems, Foster City, CA).

Detection systems for DYS156 reaction

The alleles were separated in a discontinuous horizontal ultrathin-layer polyacrylamide gel (8% T, 3% C) with piperazine diacrylamide as crosslinker, in 8 mM tris/sulfate pH 9. Ammonium peroxodisulfate (10%) and TEMED were added for gel polymerisation. Separation distance 25 cm, 2% agarose plugs in 2X tris/borate (0.5 M tris, 0.28 M borate pH 9). Electrophoresis was carried out at initially 1000 V, 40 mA and 5 W, with ramping every 90 min up to 15 W which was continued until the bromophenol blue marker front had reached the anode. Bands were visualized by silver staining (Budowle et al. 1991).

Alleleic ladders, Nomenclature and statistical calculations

The designation of Y-STR alleles followed the recommendations of the International Society of Forensic Haemogenetics (DNA Commission of the ISFH 1994) based on the number of variable repeats. The nomenclature is described in detail in the reference table appendix of de Knijff et al. 1997 and Kayser et al. 1997. Allelic ladders for each polymorphism were made of sequenced alleles and were run on every gel to ensure correct allele typing. Initially, our ladders were compared to those described in de Knijff et al. 1997 and Kayser et al. 1997 that we recieved as a gift from Peter de Knijff, Leiden. Gene or haplotype diversity was calculated according to the formula $D = 1 - \Sigma x_i^2$ with x_i being the frequency x of the ith haplotype or allele (Nei 1987). In Y-linked systems this value is identical to the discrimination index DI (Sensabaugh 1982) and to the power of exclusion (Chakravarti and Li 1983).

Results and discussion

The analysis of 100 males with 7 Y-STRs markers allowed the distinction of 71 haplotypes (Table 1) and 57 haplotypes were seen only once. The haplotype diversity/discrimination index is 0.97. Considering only the Yh1 haplotype format (Kayser et al. 1997) the discrimination capacity is 70%. This figure is somewhat lower than those described for other Caucasoid populations (Kayser et al. 1997), where these values were between 74 and 90%.

No new alleles outside the allelic ladder or alleles that migrate between ladder alleles have been found at the Y-STRs loci surveyed. In one case it was not possible to type the Y allele for the DXYS156 locus probably due to a sequence change in the primer binding region or a chromosome deletion. The frequencies of the single alleles for each polymorphism are reported in Table 2. By means of seven well defined Y-polymorphisms we constructed highly informative Y chromosome haplotypes (Kaiser et al. 1997). In our population sample collected from a defined geographical area we have obtained a haplotype diversity discrimination index of 97%.

Like mtDNA, the non-recombining region of the Y chromosome is of special interest since this is haploid and does not recombine at meiosis (Wolf et al. 1992). Consequently the chromosome markers in this region are transmitted together as haplotypes from fathers to sons, thus establishing patrilineages. Therefore, these markers are a powerful tool of exclusion, particularly helpful in analysing mixed DNA samples, in identifying the victims of crimes or accidents, as well as in deficiency cases in paternity testing where the alledged father is not avaible but other patrilined relatives are (Jobling et al. 1997). However, in cases of non-exclusion, autosomal marker systems must support the Y evidence because identical Y haplotypes could be found in all relatives in the patrilineage of the suspect of a crime. Nevertheless, the increasing size of available databases and the characterization of further highly informative Y-STRs should increase the power of exclusion of Y chromosome haplotypes and the usefulness of these markers in crime investigations.

Table 2 Allele frequencies and gene diversity obtained for the seven Y-STRs loci studied

Locus	Allele	Frequency	Gene diversity
DYS19	13	0.1	0.56
	14	0.61	
	15	0.22	
	16	0.07	
DYS389-I	9	0.16	0.52
	10	0.65	
	11	0.17	
	12	0.02	
DYS389-II	24	0.01	0.67
	25	0.14	
	26	0.45	
	27	0.32	
	28	0.06	
	29	0.02	
DYS390	21	0.01	0.55
	22	0.06	
	23	0.19	
	24	0.63	
	25	0.11	
DYS391	9	0.03	0.54
	10	0.44	
	11	0.51	
	12	0.02	
DYS392	10	0.02	0.52
	11	0.19	
	12	0.07	
	13	0.66	
	14	0.04	
	15	0.02	
DYS393	12	0.16	0.43
	13	0.73	
	14	0.10	
	15	0.01	
DXYS156	7	0.98	0.04
	8	0.01	
	?	0.01	

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