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Y chromosome STR haplotypes: genetic and sequencing data of the Galician population (NW Spain)

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Abstract Recently described Y-STR polymorphisms can be analysed as informative haplotypes which are useful in the forensic field. In order to include these systems in our forensic routine, we have carried out a population study in Galicia (NW Spain) analysing seven Y-STR polymorphisms (DYS19, DYS389-I, DYS389-II, DYS390, DYS393 and DYS385: two loci). The results were compared with other population studies. In addition various alleles for each system (except DYS385) were sequenced and the corresponding allelic ladders constructed.

Key words Y chromosome haplotypes · STRs · Sequencing data · Galician population

Introduction

In recent years a number of Y-chromosome polymorphisms have been described although the Y chromosome appears to be the least polymorphic human chromosome. Several Y polymorphisms have been studied such as some microsatellites (Roewer et al. 1992, 1996; Mathias et al. 1994; Kayser et al. 1997; de Knijff et al. 1997), base substitutions (Hammer and Horai 1995; Whitfield et al. 1995; Underhill et al. 1995) and other polymorphisms (Santos et al. 1995). Some of them such as base substitutions are quite useful to distinguish between main population groups. However, STRs are more informative to study variation within a particular population due to their polymorphism. There are several important applications of these Y-STRs in human evolution, genealogical and forensic studies (Jobling and Tyler-Smith 1995). The non-pseudoautosomal part of the Y chromosome and mitochondrial DNA have some similarities because of the lack of recombination and the mode of inheritance. The Y chromosome is paternally inherited and passed down from father to son unchanged except by the accumulation of mutations.

Tetrameric STR polymorphisms can be used to construct highly discriminative Y haplotypes which are useful in forensic science (Roewer et al. 1996; Cooper et al. 1996; Jobling et al. 1997; Prinz et al. 1997; Kayser et al. 1997; de Knijff et al. 1997). The availability of allelic ladders (Roewer et al. 1996; Heyer et al. 1997; Kayser et al. 1997; de Knijff et al. 1997) and the collaborative studies carried out by Kayser et al. (1997) and de Knijff et al. (1997) have greatly contributed to the introduction of Y-STRs in the forensic field. Here we present sequencing and population data from Galicia (NW Spain) on the Y chromosome STRs DYS19, DYS385, DYS389-I, DYS389-II, DYS390 and DYS393 and a comparison of the results with other population studies.

Material and methods

Samples

Blood samples from 116 random males were obtained from healthy individuals from Galicia (NW Spain) and from routine paternity cases (fathers). Additionally 35 father/son combinations were included for studying mutations. DNA was extracted from EDTA blood using a phenol-chloroform procedure (Valverde et al. 1993) and quantified using the DyNA Quant 200 fluorometer (Hoefer Pharmacia Biotech, San Francisco, Calif.). Oligonucleotides used as primers were obtained from Pharmacia Biotech (Sweden) and selected primers were 5′end labeled with fluorescein.

PCR conditions

Standard PCR reactions were performed in a total of 25 µl for all the systems. DYS393, DYS390: 1–20 ng DNA, 0.2 µM each primer and 0.65 U Taq DNA polymerase. DYS19, DYS385, DYS389I and II: 1–20 ng DNA, 0.5 µM each primer and 1 U Taq DNA polymerase (2 U for DYS19 and DYS385). PCR cycling conditions are shown in Table 1.

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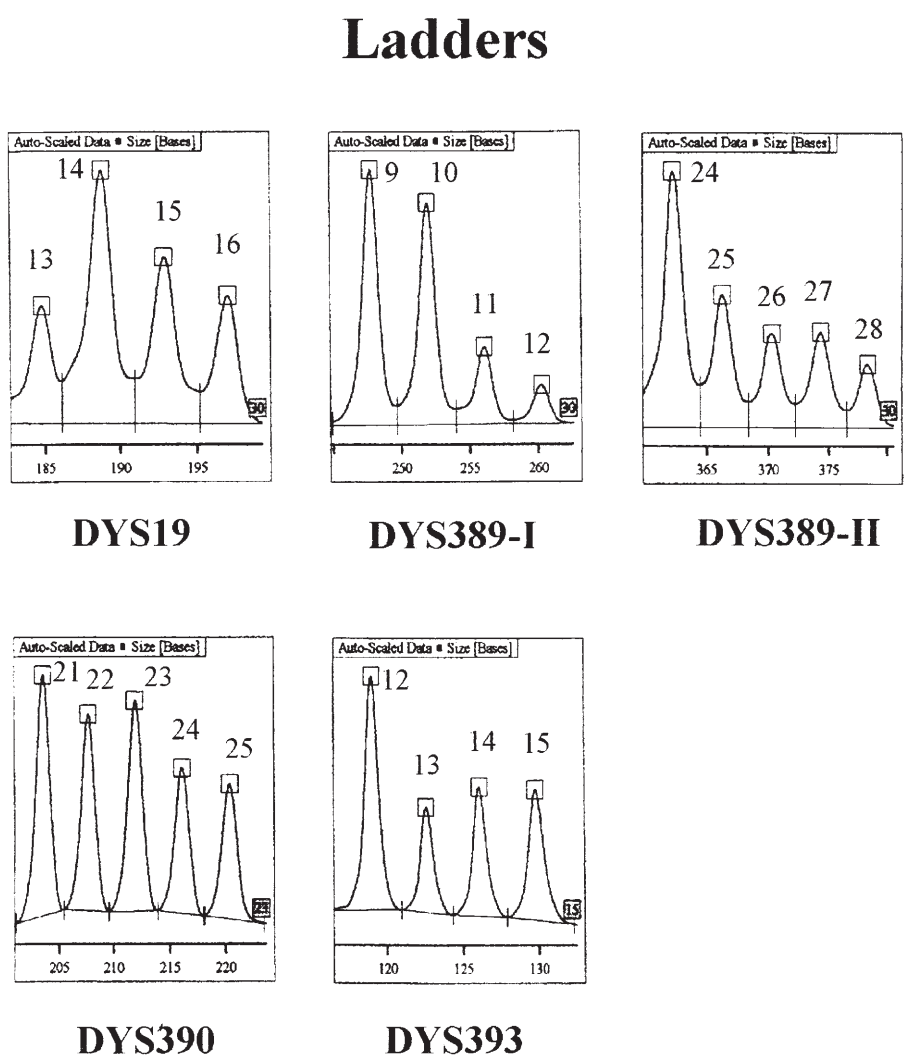
Table 1 PCR cycling conditions of the systems included in this study

	Predenaturation	Denaturation	Annealing	Extension	Cycles	Final extension
DYS393 DYS389-I DYS389-II	94 °C-2 min	94 °C-15 s	58 °C-15 s	72 °C-20 s	30	72 °C-10 min
DYS390	94 °C-2 min	94 °C-15 s	58 °C-15 s	72 °C-20 s	5	
		94 °C-15 s	54 °C-15 s	72 °C-20 s	30	72 °C-10 min
DYS19		94 °C-1 min	58 °C-1 min	72 °C-2 min	30	
DYS385	94 °C-3 min	94 °C-30 s	59 °C-30 s	72 °C-1 min	3	
		94 °C-30 s	57 °C-30 s	72 °C-1 min	2	
		94 °C-30 s	56 °C-30 s	72 °C-1 min	29	

Fig. 1 Sequenced allelic ladders of the STRs studied. Nomenclature according to Kayser et al. (1997) and de Knijff et al. (1997)



Primer sequences

DYS19 (locus GDB:121409):
Primer1: *5′CTACTGAGTTTCTGTTATAGT3′
Primer2: 5′ATGGCATGTAGTGAGGACA3′

DYS385 (locus GDB:316257):
Primer1: *5′AGCATGGGTGACAGAGCTA3′
Primer2: 5′GGGATGCTAGGTAAAGCTG3′

DYS389 I and II (locus GDB:366108):
Primer 1: *5′CCAACTCTCATCTGTATTATCTATG3′
Primer 2: 5′TCTTATCTCCACCCACCAGA3′

DYS390 (locus: GDB:366115):
Primer 1: *5′TATATTTTACACATTTTTGGGCC3′
Primer 2: 5′TGACAGTAAAATGAACACATTGC3′

DYS393 (locus GDB:456649):
Primer 1: *5′GTGGTCTTCTACTTGTGTCAATAC3′
Primer 2: 5′AACTCAAGTCCAAAAAATGAGG3′

* Forward primer was labeled with fluorescein.

Table 2 Sequencing data of the systems DYS19, DYS389-I and II, DYS390 and DYS393

DYS19
Consensus structure:
P1(21bp)- 8bp $(at)_5$ agtatt $(at)_4$ agtgtt $(at)_5$ agtgttt **$(TAGA)_3$** tagg **$(TAGA)_n$** tata 31bp -P2(19bp)

Allele (bp)	Sequence
13 (186):	P1(21bp)- 8bp $(at)_5$ agtatt $(at)_4$ agtgtt $(at)_5$ agtgttt **$(TAGA)_3$** tagg **$(TAGA)_{10}$** tata 31bp -P2(19bp)
14 (190):	P1(21bp)- . . . . . **$(TAGA)_{11}$** . . . . . -P2(19bp)
15 (194):	P1(21bp)- . . . . . **$(TAGA)_{12}$** . . . . . -P2(19bp)
16 (198):	P1(21bp)- . . . . . **$(TAGA)_{13}$** . . . . . -P2(19bp)
17 (202):	P1(21bp)- . . . . . **$(TAGA)_{14}$** . . . . . -P2(19bp)

DYS389-I
Consensus structure:
P1(25bp) TATC $(TGTC)_3$ **$(TATC)_n$** cctccctcTATCaatcTATCtattTATCtagc 71bp TGTC 43bp-P2(20bp)

Allele (bp)	Sequence
9 (247):	P1(25bp) TATC $(TGTC)_3$ **$(TATC)_9$** cctccctcTATCaatcTATCtattTATCtagc 71bp TGTC 43bp -P2(20bp)
10 (251):	P1(25bp)- . . . . . **$(TATC)_{10}$** . . . . . -P2(20bp)
11 (255):	P1(25bp)- . . . . . **$(TATC)_{11}$** . . . . . -P2(20bp)
12 (259):	P1(25bp)- . . . . . **$(TATC)_{12}$** . . . . . -P2(20bp)

DYS389-II
Consensus structure:
P1(25bp)-6bp **$(TCTG)_n$ $(TCTA)_m$** 48bp $(TCTG)_3$ **$(TCTA)_p$** 152bp-P2 (20 bp)

Allele (bp)	Sequence
24 (359):	P1(25bp)-6bp **$(TCTG)_5$ $(TCTA)_{10}$** 48bp $(TCTG)_3$ **$(TCTA)_9$** 152bp -P2(20bp)
25 (363):	P1(25bp)-6bp **$(TCTG)_4$ $(TCTA)_{12}$** 48bp . . . . . **$(TCTA)_9$** 152pb -P2(20bp)
26 (367):	P1(25bp)-6bp **$(TCTG)_5$ $(TCTA)_{11}$** 48bp . . . . . **$(TCTA)_{10}$** 152bp -P2(20bp)
27 (371):	P1(25bp)-6bp **$(TCTG)_5$ $(TCTA)_{11}$** 48bp . . . . . **$(TCTA)_{11}$** 152bp -P2(20bp)
28 (375):	P1(25bp)-6bp **$(TCTG)_5$ $(TCTA)_{11}$** 48bp . . . . . **$(TCTA)_{12}$** 152bp -P2(20bp)
29 (379):	P1(25bp)-6bp **$(TCTG)_5$ $(TCTA)_{14}$** 48bp . . . . . **$(TCTA)_{10}$** 152bp -P2(20bp)

DYS390
Consensus structure:
P1(23bp)-26bp $(TCTA)_2$ **$(TCTG)_n$ $(TCTA)_m$** TCTG $(TCTA)_4$ TCA $(TCTA)_2$ 29bp-P2(23bp)

Allele (bp)	Sequence
21 (204):	P1(23bp)-26pb $(TCTA)_2$ **$(TCTG)_8$ $(TCTA)_8$** TCTG $(TCTA)_4$ TCA $(TCTA)_2$ 29bp -P2(23bp)
22 (208):	P1(23bp)-26bp . . . . . **$(TCTG)_8$ $(TCTA)_9$** . . . . . 29bp -P2(23bp)
23 (212):	P1(23bp)-26bp . . . . . **$(TCTG)_8$ $(TCTA)_{10}$** . . . . . 29bp -P2(23bp)
	P1(23bp)-26bp . . . . . **$(TCTG)_9$ $(TCTA)_9$** . . . . . 29bp -P2(23bp)
24 (216):	P1(23bp)-26bp . . . . . **$(TCTG)_8$ $(TCTA)_{11}$** . . . . . 29bp -P2(23bp)
25 (220):	P1(23bp)-26bp . . . . . **$(TCTG)_8$ $(TCTA)_{12}$** . . . . . 29bp -P2(23bp)

DYS393
Consensus structure:
P1(24bp)- **$(AGAT)_n$** $(ATGT)_2$ 17bp-P2(22bp)

Allele (bp)	Sequence
11 (115):	P1(24bp)- **$(AGAT)_{11}$** $(ATGT)_2$ 17bp -P2(22bp)
12 (119):	P1(24bp)- **$(AGAT)_{12}$** . . . . . 17bp -P2(22bp)
13 (123):	P1(24bp)- **$(AGAT)_{13}$** . . . . . 17bp -P2(22bp)
14 (127):	P1(24bp)- **$(AGAT)_{14}$** . . . . . 17bp -P2(22bp)
15 (131):	P1(24bp)- **$(AGAT)_{15}$** . . . . . 17bp -P2(22bp)

Each locus was amplified individually except for DYS389 I/II and DYS385 (two loci each with the same set of primers). All loci contain tetranucleotides as repeat units.

Detection systems

Detection of the amplified products was carried out using the Automatic Laser Fluorescent (ALF) DNA sequencer (Pharmacia) following the conditions of Pestoni et al. (1995). Fragment sizes were determined automatically using the Fragment Manager 1.2 software and typed by comparison with the sequenced allelic ladders.

Sequence reactions

PCR products were purified through MicroSpin S-300 HR columns (Pharmacia Biotech) before sequencing. Alleles from the systems DYS389-I and II were separated in a 3% agarose gel and eluted with the Sephaglass BandPrep Kit (Pharmacia Biotech).

The DNA Sequencing Kit, Dye Terminator Cycle Sequencing Ready Reaction (with AmpliTaq DNA Polymerase, FS) (PE Applied Biosystems), the ABI Prism 377 DNA Sequencer and the DNA sequencing analysis software (Perkin-Elmer) were used for sequencing analysis.

Allelic ladders and allelic designation

Sequenced allelic ladders containing all the common alleles found in our population study were constructed (Fig. 1). For the DYS385 system an allelic ladder with five alleles (11, 13, 15, 17, 19) was kindly supplied by P. Schneider (Institute of Legal Medicine, Mainz, Germany). Allelic designation of the systems was made according to Kayser et al. (1997) and de Knijff et al. (1997).

Results and discussion

Sequencing data

Sequencing data of the alleles of the different systems and their consensus structure are shown in Table 2. According to Urquhart et al. (1994) and Gill et al. (1997) these STRs can be classified as simple or compound STRs. Most of these systems have constant and variable repeat sequences and the nomenclature was made according to Kayser et al. (1997) and de Knijff et al. (1997) which is widely accepted in a large number of forensic laboratories.

The sequence of the system DYS389-I is included in the DYS389-II sequence (Cooper et al. 1996) and one set of primers amplifies both loci together because the forward primer anneals in two different places on the same strand. Unambiguous assignation of each allele to each locus is possible because the alleles of each locus differ by at least 100 bp.

Population data

Allele frequencies of the systems and gene diversity values are shown in Tables 3 and 4. The DYS385 system showed two alleles from two loci that cannot be unambiguously assigned to each loci because of overlapping sizes, so they were analysed as a haplotype. A comparative study was carried out between allele frequencies of the Galician sample and two Spanish population samples (Catalans and Basques) (Pérez-Lezaun et al. 1997) by means of χ^2 contingency tests and no significant differences were found in any of the systems ($p > 0.01$). Y-haplotypes were constructed with the seven loci together (Table 5) and the haplotype diversity value is shown in Table 6. The typing of base substitutions may also be useful to differentiate between identical haplotypes of Y-STR polymorphisms and will provide important additional information for anthropological purposes.

Forensic usefulness

The gene and haplotype diversity values have the same value as the power of discrimination (PD) and chance of exclusion (CE) i.e.1-(matching probability) = $1\text{-}\Sigma p_i^2$; (p_i: allele or haplotype frequencies). The seven STRs described in this study result in informative Y-haplotypes with CE and PD values of 0.9794 (Table 6). A total of 35 male offspring were analysed where paternity had been positively confirmed in order to increase the number of

Table 3 Allele frequencies of the systems

Locus	Allele (bp)	Frequency	Gene diversity value
DYS19 (n = 93)	13 (186)	0.1613	0.5613
	14 (190)	0.6236	
	15 (194)	0.1398	
	16 (198)	0.0645	
	17 (202)	0.0107	
DYS389-I (n = 116)	9 (247)	0.1638	0.5553
	10 (251)	0.6121	
	11 (255)	0.2069	
	12 (259)	0.0172	
DYS389-II (n = 116)	24 (359)	0.0172	0.6539
	25 (363)	0.1380	
	26 (367)	0.5259	
	27 (371)	0.1983	
	28 (375)	0.1034	
	29 (379)	0.0086	
	30 (383)	0.0086	
DYS390 (n = 116)	21 (204)	0.0259	0.6221
	22 (208)	0.0345	
	23 (212)	0.2672	
	24 (216)	0.5345	
	25 (220)	0.1379	
DYS393 (n = 116)	11 (115)	0.0086	0.4331
	12 (119)	0.1380	
	13 (123)	0.7327	
	14 (127)	0.1035	
	15 (131)	0.0172	

Gene diversity value computed as $1\text{-}\Sigma p_i^2$;
p_i: allele frequencies

Table 4 DYS385 phenotype frequencies (two loci amplified together) (n:93)

Phenotype	Frequency	Phenotype	Frequency
9–13	0.0108	13–14	0.0322
10–14	0.0108	13–15	0.0860
11–12	0.0108	13–16	0.0215
11–13	0.0322	13–17	0.0108
11–14	0.3226	14–14	0.0322
11–15	0.0215	14–15	0.0215
11–16	0.0430	14–16	0.0430
12–12	0.0430	14–17	0.0215
12–13	0.0215	14–18	0.0108
12–14	0.0645	15–15	0.0322
12–15	0.0645	16–16	0.0108
13–13	0.0215	16–18	0.0108

Haplotype diversity value: 0.8669

Table 5 Y-STR haplotypes of the Galician population sample (n = 93). The systems conforming the haplotypes are shown in increasing order of the PCR fragment sizes (from left to right) in order to facilitate the analysis of the data

Haplotype	*n*	DYS393	DYS19	DYS390	DYS389-I	DYS389-II	DYS385
1	1	11	13	22	9	25	11–14
2	1	12	13	24	10	26	11–14
3	1	12	13	24	10	26	16–18
4	1	12	14	22	10	26	11–14
5	1	12	14	23	10	26	14–17
6	1	12	14	23	10	27	13–15
7	1	12	14	23	10	27	13–16
8	1	12	14	23	10	29	14–14
9	1	12	14	23	11	28	12–15
10	1	12	14	24	10	25	11–14
11	1	12	14	24	10	27	12–15
12	1	12	15	23	10	26	13–15
13	1	12	15	24	10	26	12–14
14	1	12	15	24	10	28	13–15
15	1	13	13	23	10	27	11–14
16	1	13	13	24	10	28	16–16
17	1	13	13	24	11	27	13–13
18	2	13	13	24	11	27	13–14
19	1	13	13	24	11	27	13–15
20	1	13	13	24	11	27	14–14
21	1	13	13	24	12	28	11–16
22	1	13	13	25	10	26	12–14
23	1	13	13	25	11	28	13–15
24	1	13	13	25	12	28	11–16
25	1	13	14	22	9	26	14–15
26	1	13	14	23	9	25	13–15
27	2	13	14	23	10	26	11–14
28	1	13	14	23	10	26	12–12
29	1	13	14	23	10	26	12–14
30	1	13	14	23	10	26	13–17
31	1	13	14	23	11	27	10–14
32	1	13	14	23	11	27	12–13
33	1	13	14	23	11	27	12–14
34	1	13	14	24	9	25	11–14
35	1	13	14	24	9	26	11–14
36	1	13	14	24	10	24	11–14
37	1	13	14	24	10	26	11–12
38	2	13	14	24	10	26	11–13
39	9	13	14	24	10	26	11–14
40	1	13	14	24	10	26	11–16
41	1	13	14	24	10	26	12–13
42	1	13	14	24	10	26	12–14
43	1	13	14	24	10	26	13–15
44	1	13	14	24	10	26	14–15
45	1	13	14	24	10	26	14–16
46	1	13	14	24	10	27	11–14
47	1	13	14	24	11	26	11–14
48	2	13	14	24	11	27	11–14
49	1	13	14	24	11	27	11–15
50	1	13	14	24	11	28	11–14
51	2	13	14	25	9	25	11–14
52	1	13	14	25	9	25	12–14
53	1	13	14	25	10	26	9–13
54	1	13	14	25	10	26	11–14
55	1	13	14	25	10	26	11–16
56	1	13	14	25	10	26	12–15
57	1	13	14	25	10	26	13–15
58	1	13	15	22	10	25	12–12

Table 5 (continued)

Haplotype	*n*	DYS393	DYS19	DYS390	DYS389-I	DYS389-II	DYS385
59	1	13	15	23	9	26	13–13
60	1	13	15	23	9	26	14–18
61	1	13	15	23	10	26	13–16
62	1	13	15	24	10	26	11–13
63	1	13	15	24	10	26	11–14
64	1	13	15	24	11	26	11–15
65	1	13	16	21	9	26	14–16
66	1	13	16	23	10	25	12–12
67	1	13	16	25	9	25	14–16
68	1	13	16	25	10	28	11–14
69	1	13	17	24	10	25	12–12
70	1	14	13	24	10	28	13–14
71	1	14	14	23	9	26	14–14
72	1	14	14	24	10	26	11–14
73	1	14	14	24	10	27	12–15
74	1	14	15	23	10	26	14–16
75	1	14	15	23	11	28	15–15
76	1	14	15	23	11	30	15–15
77	1	14	16	21	9	26	14–17
78	1	14	16	23	10	26	15–15
79	2	15	14	24	11	27	12–15
Total:	93						

Table 6 Haplotype diversity values

	Haplotype 1 (DYS389-I, II, DYS390, DYS393)	Haplotype 1+DYS385, DYS19
Number of individuals	116	93
Different haplotypes	50	79
Haplotype diversity value	0.9214	0.9794

samples studied for calculating the mutation rate of these systems. No mutations were found. In addition DNA from 20 females was amplified in order to demonstrate that they are exclusively male polymorphic systems and no amplification products were obtained.

The study was carried out by amplifying all the systems individually. Multiplexing of the systems has also been performed with excellent results. The analysis of these polymorphisms could be useful in certain forensic cases (Prinz et al. 1997; Jobling et al. 1997; Kayser et al. 1997).

In our experience we have also obtained excellent results in some critical forensic cases. We have obtained such results especially in degraded male-female mixtures in rape cases and in paternity testing of male offspring using amniotic fluid taken in the early stages of a pregnancy which has been the result of a rape.

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