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Increased forensic efficiency of a STR-based Y-specific haplotype by addition of the highly polymorphic DYS385 locus

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Abstract The polymorphic short tandem repeat (STR) locus DYS385 mapping to the male-specific region of human Y chromosome, was used to reinvestigate 125 unrelated Italian males, from our data archive, who had been previously typed for 7 different Y-specific STRs (DYS19, DYS389 I and II, DYS390, DYS391, DYS392, DYS393), defining a haplotype now widely adopted in the forensic context. The aim of this study was to improve the information value of the original haplotype in view of its application to issues of personal identification and parental analysis. DYS385 proved to be highly polymorphic (94.5% gene diversity) and the overall individualization capacity of the 8-loci haplotype was raised to 93.6%, with 117 unique assets out of 125 tested samples.

Key words Y-chromosome · DYS385 · Y-haplotype · Short tandem repeats

Introduction

In the last few years, information on the genetic diversity of the male-specific portion of the human Y-chromosome has considerably increased (Mathias et al. 1994; Jobling and Tyler-Smith 1995). Once believed to be poor in gene diversity (Malaspina et al. 1990), this region of the human genome contains a widely assorted set of polymorphisms, including restriction sites, point mutations and short tandem repeats (STRs) (Jakubiczka et al. 1989; Jobling et al. 1996), and it has become popular among molecular evolutionists and forensic analysts (Roewer and Epplen 1992; Hammer 1995; Hammer and Horai 1995; Pääbo 1995, Cooper et al. 1996; Roewer et al. 1996; Underhill et al. 1996; Jobling et al. 1997). The haploid state, paternal inheritance and exposure to male-driven genetic drift confer Y-specific polymorphisms the role of the male complement of the mitochondrial heredity. Y-specific STRs are apparently as highly variable and short in length as their autosomal counterpart (Roewer et al. 1992) – a fact that qualifies them as ideal candidates for a vast range of forensic applications. A limited number of tetranucleotide repeat STRs is presently available (Jobling and Tyler-Smith 1995). Of these, seven have recently been selected as first-choice markers for forensic profiling, in the course of a multilaboratory cooperative study addressing population genetics and practical applications of several STRs (Kayser et al. 1997; de Knijff et al. 1997). This set of markers has been already helpful in reconstructing some particular aspects of the recent history of human populations (Zerjal et al. 1997). We recently used a subset of this STR haplotype to characterize the evolutionary relationship between Sardinians and Italians from the mainland (Caglià et al. 1997). For this latter study, an archive of peninsular Italians, typed for the seven Y-specific STRs, has been established in our laboratory, which can be used for reference in male identification. At present, our collection (125 individuals) comprises 87 unique haplotypes and 16 assets occurring more than once. Although showing considerable discrimination, this haplotype can obviously be brought to a higher level of forensic efficiency by integrating further STRs in the typing protocol.

Therefore, we decided to type our collection of male DNA samples with another Y-specific tetranucleotide STR (DYS385 G00-316-257). PCR analysis of this system with a pair of specific oligonucleotide primers coamplifies two products – presumably reflecting a gene duplication – spanning from 360 to 412 bp and defining an interesting polymorphism. We here briefly report on the DYS385 gene frequencies and the properties of the 8-STRs Y specific haplotype which results from inclusion of this polymorphism in our male haplotypic profiles.

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Materials and methods

DNA samples from 125 healthy male volunteers of Central and Southern Italy have been stored in our laboratory since previous studies. Classification of these individuals by other Y-specific markers has been reported in part elsewhere (Kayser et al. 1997).

For this study PCR amplification was carried out from 20 ng DNA template in 50 µl reaction volume with primers (one of which fluorescein labeled) ranging from 0.4 to 1 µM final concentration. Primers were: 5′F* AGC ATG GGT GAC AGA GCT A 3′ and 5′ TGG GAT GCT AGG TAA AGC TG 3′.

For PCR amplification (9600 and 2400 Perkin Elmer thermocyclers) the following conditions were used: pre-denaturation: 94° C-5 min; 28 annealing cycles at 94° C-1 min, 56° C-1 min, 72° C-1 min; final extension: 72° C-5 min.

The amplification products were processed by an ALFexpress DNA sequencer (Pharmacia) in sequence-format denaturing gels with standard electrophoretic conditions. Genotype classification was obtained by side-to-side comparisons with a home-made allelic ladder. A collection of sequenced fragments, supplied by Dr. P. M. Schneider (Institut für Rechtsmedizin, Mainz), was also available to us. The nomenclature adopted here reflects the number of repeat units and complies with the ISFH recommendations (DNA Commission of the ISFH 1994).

The gene diversity was calculated as: $\Sigma (q_i)^2$, where q is the frequency of the ith haplotype. The individualization capacity was assumed as the percentage proportion of unique haplotypes.

Results and discussion

The phenotype frequency distribution emerging from the DYS385 typing work is shown in Table 1. Since it was impossible to assign coamplified fragment to either locus, frequencies of two fragment assets are reported. Under this assumption, in 125 unrelated italian males we observed 37 different combinations of 12 individual fragments, each occurring at low frequencies (0.8%–6.4%), except for the

Table 1 Phenotype frequencies of the DYS385 polymorphism in 125 Italians

Allele combination	q	Obs.	Allele combination	q	Obs.
10–14	1	0.008	13–19	5	0.04
10–18	1	0.008	13–20	1	0.008
11–11	5	0.04	14–14	2	0.016
11–14	20	0.16	14–15	5	0.04
11–15	5	0.04	14–16	6	0.048
11–16	3	0.024	14–17	3	0.024
11–17	2	0.016	14–18	2	0.016
12–12	2	0.016	15–15	2	0.016
12–13	2	0.016	15–16	3	0.024
12–14	6	0.048	15–18	1	0.008
12–15	3	0.024	16–16	3	0.024
12–16	1	0.008	16–17	2	0.016
12–17	2	0.016	16–18	4	0.032
13–13	2	0.016	16–19	5	0.04
13–14	2	0.016	16–21	1	0.008
13–15	5	0.04	17–17	1	0.008
13–16	5	0.04	17–18	2	0.016
13–17	8	0.064	17–19	1	0.008
13–18	1	0.008			

Table 2 A list of the 121 Y-specific STR haplotype combinations from peninsular Italians. Pairs of still unresolved individuals after DYS385 typing are in bold-italic typeset. For DYS19, DYS389 I and II, DYS390, DYS391, DYS392, DYS393 loci, allele designation was achieved by comparison with allelic ladders kindly provided by Dr. P. de Knijff (Leiden). For DYS389-II the nomenclature of de Knijff et al. 1997 is adopted although a different classification has recently been proposed (Cooper et al. 1996)

DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	N	Surname identity
10	9	27	24	10	11	13	14 18	1	
13	9	25	22	10	16	11	12 16	1	
13	9	26	23	10	11	14	16 19	1	
13	9	27	24	11	11	13	16 16	1	
13	10	27	23	10	12	13	17 17	1	
13	10	27	24	10	11	13	16 18	1	
13	10	27	24	10	11	13	17 18	1	
13	*10*	*27*	*24*	*10*	*11*	*13*	*16 19*	*2*	*no*
13	10	27	24	10	11	14	16 18	1	
13	10	27	24	11	13	12	11 14	1	
13	10	27	24	11	13	13	11 14	1	
13	10	28	24	11	11	13	16 17	1	
13	11	27	23	10	13	13	13 17	1	
13	11	28	24	10	11	13	17 19	1	
13	11	28	25	11	11	13	16 21	1	
13	11	29	24	10	11	13	15 16	1	
14	9	24	24	11	13	13	11 11	1	
14	9	25	22	10	11	12	10 18	1	
14	9	25	22	10	11	13	13 13	1	
14	9	25	23	9	11	12	14 16	1	
14	9	25	23	10	11	13	15 15	1	
14	9	25	23	10	11	13	16 18	1	
14	9	25	24	10	11	13	13 14	1	
14	9	26	23	10	11	13	17 18	1	
14	10	23	24	10	13	13	12 14	1	

Table 2 (continued)

DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	N	Surname identity
14	10	25	23	10	11	12	13 17	1	
14	10	25	24	11	13	14	11 11	1	
14	10	25	24	11	14	12	12 14	1	
14	10	26	22	11	13	13	11 14	1	
14	10	26	23	9	13	13	11 11	1	
14	10	26	23	10	11	12	12 15	1	
14	10	26	23	10	11	12	12 17	1	
14	10	26	23	10	11	12	13 18	1	
14	*10*	*26*	*23*	*10*	*11*	*13*	*13 19*	*2*	*no*
14	10	26	23	10	13	14	15 18	1	
14	10	26	23	10	14	12	13 14	1	
14	10	26	23	11	11	12	14 18	1	
14	10	26	23	11	13	13	11 14	1	
14	10	26	23	11	14	13	11 17	1	
14	10	26	23	11	14	13	14 16	1	
14	10	26	24	10	11	12	13 15	1	
14	10	26	24	10	11	12	13 19	1	
14	10	26	24	10	11	13	13 17	1	
14	10	26	24	10	13	12	11 14	1	
14	10	26	24	10	13	13	11 15	1	
14	*10*	*26*	*24*	*10*	*13*	*13*	*11 14*	*2*	*no*
14	10	26	24	11	11	12	11 16	1	
14	10	26	24	11	13	13	10 14	1	
14	10	26	24	11	13	13	11 15	1	
14	10	26	24	11	13	13	11 17	1	
14	10	26	24	11	13	13	12 13	1	
14	10	26	25	10	13	13	12 14	1	
14	10	26	25	11	13	12	12 14	1	

Table 2 (continued)

DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	N	Surname identity
14	10	27	22	10	11	12	12 12	1	
14	10	27	22	10	11	12	13 20	1	
14	10	27	22	10	13	13	14 16	1	
14	10	27	23	10	11	12	13 17	1	
14	10	27	23	10	12	14	14 16	1	
14	10	27	23	10	12	14	16 16	1	
14	10	27	23	10	13	13	11 14	1	
14	10	27	23	11	13	13	11 14	1	
14	10	27	24	10	11	13	16 19	1	
14	10	27	24	11	13	14	11 11	1	
14	10	28	23	10	11	12	13 19	1	
14	11	26	23	10	11	12	13 16	1	
14	11	26	23	10	13	13	11 14	1	
14	11	26	23	11	12	14	15 16	1	
14	11	26	23	11	14	12	11 14	1	
14	11	27	23	10	11	12	11 16	1	
14	11	27	23	10	13	13	14 16	1	
14	11	27	23	10	15	12	16 19	1	
14	11	27	23	11	13	13	11 14	1	
14	11	27	24	11	13	13	11 14	1	
14	11	27	24	11	13	13	11 15	1	
14	11	27	25	11	13	13	14 15	1	
14	11	28	22	9	11	12	13 17	1	
14	11	28	22	10	11	12	11 11	1	
14	11	28	23	10	11	12	13 16	1	
14	11	28	23	10	11	12	16 18	1	
14	11	28	24	10	11	12	13 15	1	
14	11	28	24	10	11	13	11 14	1	

Table 2 (continued)

DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	N	Surname identity
14	11	28	24	10	13	13	11 14	1	
14	11	29	24	10	11	12	13 17	1	
15	9	24	24	10	11	12	14 17	1	
15	9	24	24	10	11	14	14 15	1	
15	9	25	22	10	11	12	13 17	1	
15	9	25	22	10	11	13	14 14	1	
15	9	25	23	10	11	11	14 16	1	
15	9	25	24	10	11	12	14 17	1	
15	9	26	21	10	11	15	13 15	1	
15	9	26	21	10	11	15	14 17	1	
15	9	26	22	10	11	13	12 14	1	
15	9	26	22	10	11	13	13 13	1	
15	9	26	22	10	11	14	12 14	1	
15	9	26	24	10	11	12	13 17	1	
15	9	27	23	10	12	13	11 15	1	
15	9	27	23	10	12	14	13 15	1	
15	10	25	23	10	11	13	12 13	1	
15	*10*	*25*	*24*	*11*	*14*	*13*	*11 14*	*2*	*yes*
15	10	26	22	11	11	13	14 14	1	
15	10	26	22	11	11	14	14 15	1	
15	10	26	23	11	13	13	11 15	1	
15	10	26	24	10	11	13	13 19	1	
15	10	27	22	10	11	12	13 16	1	
15	10	27	23	10	11	12	12 17	1	
15	10	27	23	10	11	12	13 16	1	
15	10	27	23	11	13	13	13 15	1	
15	10	27	24	12	11	13	11 14	1	
15	10	27	25	10	11	13	11 16	1	

Table 2 (continued)

DYS19	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS385	N	Surname identity
15	11	28	22	10	11	13	12 15	1	
15	11	29	23	10	12	13	15 15	1	
15	12	28	23	9	11	12	13 16	1	
16	9	27	22	9	12	13	16 17	1	
16	10	27	25	11	11	13	11 14	1	
16	10	27	26	10	12	11	11 14	1	
16	10	28	25	11	11	13	12 15	1	
16	11	28	23	10	13	14	16 16	1	
17	9	25	25	10	11	12	15 16	1	
17	9	26	22	10	11	13	14 15	1	
17	10	29	24	11	11	14	14 15	1	
17	11	26	23	10	11	13	12 12	1	

11–14 asset (16.0%). No imperfectly repeated fragment was found, nor were mutations in the 14 father-child pairs so far studied. The isolate individualization capacity of DYS385 locus was 29.6%, the gene diversity was 94.5%.

A synopsis of the individual assets resulting from the 8-STR haplotype presently available in our laboratory is reported in Table 2, whence it emerges that, prior to introducing DYS385, 16 haplotypes occurred more than once whereas the remaining 87 combinations were unique (98.8% overall gene diversity; 70% individualization capacity). After complete 8-STR genotyping, there remained only four pairs of unresolved individual assets, each with 1.6% frequency (99.1% gene diversity; 93.6% individualization capacity). Interestingly two of the identical individuals shared both the surname and birthplace, implying a possible common paternal ancestry.

The increased genetic heterogeneity reflected in the STR Y-specific collection of markers now available to us makes the scenario of its forensic applications significantly more appealing.

References

Caglià A, Novelletto A, Dobosz M, Malaspina P, Ciminelli BM, Pascali VL (1997) Y-chromosome STR loci in Sardinia and Continental Italy reveal islander-specific haplotypes. Eur J Hum Genet 5:288–292

Cooper G, Amos W, Hoffman D, Rubinsztein DC (1996) Network analysis of human Y microsatellite haplotypes. Hum Mol Genet 5:1759–1766

De Knijff P, Kayser M, Caglià A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herrmann S, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Weichhold G, Roewer L (1997) Chromosome Y microsatellites: population genetic and evolutionary aspects. Int J Legal Med 110:134–140

DNA Commission of the ISFH (1994) Report concerning further recommendations of the DNA Commission of the ISFH regarding PCR-based polymorphisms in STR (short tandem repeats). Int J Legal Med 107:169–160

Hammer MF (1995) A recent common ancestry for human Y chromosomes. Nature 378:376–378

Hammer MF, Horai S (1995) Y chromosomal DNA variation and the peopling of Japan. Am J Hum Genet 56:951–962

Jakubiczka S, Arnemann J, Cooke HJ, Krawczak M, Schmidtke J (1989). A search for restriction fragment length polymorphisms on the human Y chromosome. Hum Genet 84:86–88

Jobling MA, Tyler-Smith C (1995) Fathers and sons: the Y chromosome and human evolution. Trends Genet 11:449–456

Jobling MA, Samara V, Pandya A, Fretwell N, Bernasconi B, Mitchell RJ, Gerelsaikhan T, Dashnyam B, Sajantila A, Salo PJ, Nakahori Y, Disteche CM, Thangaraj K, Singh L, Crawford MH, Tyler-Smith C (1996) Recurrent duplication and deletion polymorphisms on the long arm of the Y chromosome in normal males. Hum Mol Genet 5:1767–1775

Jobling MA, Pandya A, Tyler-Smith C (1997) The Y chromosome in forensic analysis and paternity testing. Int J Legal Med 110:118–124

Kayser M, Caglià A, Corach D, Fretwell N, Gehrig C, Graziosi G, Heidorn F, Herrmann S, Herzog B, Hidding M, Honda K, Jobling M, Krawczak M, Leim K, Meuser S, Meyer E, Oesterreich W, Pandya A, Parson W, Penacino G, Perez-Lezaun A, Piccinini A, Prinz M, Schmitt C, Schneider PM, Szibor R, Teifel-Greding J, Weichhold G, de Knijff P, Roewer L (1997) Evaluation of Y chromosomal STRs: a multicenter study. Int J Leg Med 110:125–133

Malaspina P, Persichetti F, Novelletto A, Jodice C, Terrenato L, Wolfe J, Ferraro M, Prantera G (1990) The human Y chromosome shows a reduced level of DNA polymorphism. Ann Hum Genet 54:297–305

Mathias N, Bayés M, Tyler-Smith C (1994) Highly informative compound haplotypes for the human Y chromosome. Hum Mol Genet 3:115–123

Pääbo S (1995) The Y chromosome and the origin of all of us (Men). Science 268:1141–1142

Roewer L, Epplen JT (1992) Rapid and sensitive typing of forensic stains by PCR amplification of polymorphic simple repeat sequences in casework. Forensic Sci Int 53:163–171

Roewer L, Arnemann J, Spurr NK, Grzeschik KH, Epplen JT (1992) Simple repeat sequences on the human Y chromosome are equally polymorphic as their autosomal counterparts. Hum Genet 89:389–394

Roewer L, Kayser M, Dieltjes P, Nagy M, Bakker E, Krawczak M, de Knijff P (1996) Analysis of molecular variance (AMOVA) of Y-chromosome-specific microsatellites in two closely related human populations. Hum Mol Genet 5:1029–1033

Underhill PA, Jin L, Zemans R, Oefner PJ, Cavalli-Sforza LL (1996) A pre-Columbian Y chromosome-specific transition and its implications for human evolutionary history. Proc Natl Acad Sci USA 93:196–200

Zerjal T, Dashnyam B, Pandya A, Kayser M, Roewer L, Santos FR, Schiefenhövel W, Fretwell N, Jobling MA, Harihara S, Shimizu K, Semjidmaa D, Sajantila A, Salo P, Crawford MH, Ginter EK, Evgrafov OV, Tyler-Smith C (1997) Genetic relationships of Asians and Northern Europeans revealed by Y-chromosomal DNA analysis. Am J Hum Genet 60:1174–1183