Int J Legal Med (1999) 113 : 38–42

ORIGINAL ARTICLE

S. Füredi · J. Woller · Z. Pádár · M. Angyal

Y-STR haplotyping in two Hungarian populations

Received: 14 July 1998 / Received in revised form: 8 March 1999

Abstract A set of seven Y-chromosomal STR loci (DYS19, DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393) with the addition of the bilocal marker DYS385 was used to generate male-specific haplotype databases for two Hungarian population samples, Caucasians from the Budapest area and Romanies from Baranya county. At the locus DYS385 three types of intermediate sized alleles were detected in six males. The presence of a (GA) dinucleotide, probably due to an (AA) deletion in the second (GAAA) repeat of the polymorphic repeat region leads to an intermediate allelle 17.2. The intermediate alleles 17.-1 and 18.-1 with the consensus repeat structure of $(GAAA)_{17}$ and $(GAAA)_{18}$, respectively, were found to lack a T in the same $(T)_7$ stretch located within the 3′ flanking region of each allele. The forensic efficiency values for the Romany population were significantly lower than those found in the Central Hungarian and other non-isolated Causasian populations, which may imply a possible common paternal ancestry of some haplotypes in the Romany sample. With pairwise comparisons of inter-population molecular variance, the two populations analyzed here and an Italian population sample, could be clearly distinguished using the seven monolocal Y-STRs. A sizing precision of ≤ 0.14 nucleotide standard deviation was obtained with capillary electrophoresis carried out on an ABI Prism 310 Genetic Analyzer. Objective and accurate genotyping is thus possible using an internal size standard with a high density of fragments.

Key words Y-STR haplotype analysis · Capillary electrophoresis · Allele sizing precision · Population studies · Variant alleles

S. Füredi (✉) · J. Woller · Z. Pádár
Department of Biology, Institute for Forensic Sciences,
P.O. Box 314/4, H-1903 Budapest, Hungary

M. Angyal
Department of Forensic Medicine,
Medical University School of Pécs, P.O. Box 99,
H-7643 Pécs, Hungary

Introduction

The haploid and non-recombining nature of most Y-chromosomal short tandem repeats (Y-STRs) and their relative simplicity when used as polymorphic markers, makes them useful tools for male identification purposes in paternity testing and forensic stain analysis [1, 2]. The aim of this study was to generate and evaluate haplotype databases of eight recently established Y-STRs for two Hungarian population samples, Budapest Caucasians and Baranya Romanies, which were analyzed previously with autosomal DNA polymorphisms [3, 4]. A forensically adopted basic set of seven Y-STRs (DYS19 [5], DYS389-I, DYS389-II, DYS390, DYS391, DYS392, DYS393 [6]) with the addition of the bilocal marker DYS385 [6] was used for haplotyping. In addition, to evaluate the precision of allele sizing an experiment was performed for the capillary electrophoresis system used in this study.

Materials and methods

Blood samples were collected from 116 Hungarian Caucasian males living in the Budapest area (Central Hungary) and 78 Romany males residing in Baranya county (south-western Hungary).

Coamplification of the loci was performed in three non-overlapping multiplex PCR systems: triplex I (DYS19, DYS389-I/II), triplex II (DYS390, DYS391, DYS393), and duplex (DYS392, DYS385). PCR amplification of the triplexes was performed according to Kayser et al. [2]. PCR reactions for DYS392 and DYS385 were carried out from 5 ng DNA template using 1U AmpliTaq Gold (Perkin Elmer) in a 25 µl reaction volume with 0.8 and 0.25 µM primers [6], respectively. Cycling conditions for the duplex system (9600 PE thermocycler) were 10 cycles at 95 °C for 11 min, 94 °C for 30 s, 59–55 °C for 30 s (with each cycle touchdown 0.4 °C), 72 °C for 45 s, then 20 cycles with 55 °C annealing temperature and a final extension of 60 °C for 30 min.

The PCR products, which were fluorescein-labeled by the forward primers, were analyzed by capillary electrophoresis in the denaturing polymer POP-4 on an ABI Prism 310 Genetic Analyzer (PE). Standard electrophoretic conditions using the run module GS STR POP4 F (1 ml) were applied in 47 cm, 50 µm ID uncoated capillaries. For allele sizing of individual samples the fluorescent ladders CXR 60–400 (Promega Corp.) and GeneScan-500 (ROX) (GS500, PE) were used as internal size markers for the triplex and

duplex systems, respectively. Data from the ABI Prism 310 instrument were analyzed with GeneScan 2.1 software using the local Southern sizing and light smoothing algorithms. Automatic allele assignment for the Y-STRs was possible by comparison with a reamplified allelic ladder utilizing Genotyper 2.1 software and setting a ± 0.5 nucleotide (nt) allele size window around the ladder fragments. An allele nomenclature was used according to de Knijff et al. [7]. Allelic designation was done according to Bär et al. [8].

The sizing precision of the 310 instrument was determined in two different modes using population database samples and by running allelic ladders for the 6 monolocal tetramer Y-STR loci in 50 consecutive injections. In the latter case the two different size standards were compared. The allelic ladders used for sizing precision analysis contained the alleles DYS19: 13–17, DYS389-I: 9–11, DYS389-II: 25–29, DYS390: 18–26, DYS391: 9–12 and DYS393: 11–15.

Following standard protocols for the purification of PCR products [9] sequencing analysis of purified DYS385 alleles was carried out on an ABI 310 instrument using Dye Terminator Cycle Sequencing FS AmpliTaq kit (PE). Sequencing was done with forward as well as reverse primers.

Inter-population variance (Φ_{ST}) of analysis of molecular variance (AMOVA) was computed in a pairwise method using the software ARLEQUIN v1.1 [10, 11]. Haplotype diversity was calculated according to Nei [12].

Results and discussion

A measure of the precision of the computerized allele sizing was examined by analyzing individual samples as well as Y-STR allelic ladders on the ABI Prism 310 instrument (Table 1). In each of the precision experiments, the achieved sizing precision of ≤ 0.14 nt standard deviation allows a ± 0.5 nt allele size window to be set for genotyping. The extreme values of both the standard deviations (SD) and the windows of estimated allelic size detected by applying CXR60–400 for the ladders of the six monolocal tetramer Y-STR loci, with the only exception obtained at DYS390, were smaller than those measured using GS500 (Table 1). In the comparison of two different size standards, there was a definite advantage observed using the size standard with a greater density of fragments (i.e. CXR60–400).

Due to the high resolution power of this capillary electrophoresis system three types of intermediate sized alleles could be detected at the locus DYS385. All genotypes carrying variant alleles showed the two-band pattern. These variant alleles were designated as 17.-1, 18.-1 and 17.2 according to their sequence stucture (Fig. 1). Alleles 17.-1 and 18.-1 were found to lack a T in the same block of a $(T)_7$ stretch located within the 3 flanking region of each allele. At the allele 17.2 the presence of a (GA), probably due to an (AA) deletion in the second (GAAA) repeat of the polymorphic repeat region was detected. Interestingly, all five individuals carrying either the 17.-1 or 18.-1 allele have the same five-locus haplotype of the markers DYS389-I, DYS390, DYS391, DYS392, and DYS393. In addition, among three Budapest Caucasians out of these five males there was no allelic difference found for the seven monolocal Y-STR loci. This observation may suggest that the T deletion detected at these variant alleles is likely to have occurred recently. However, this hypothesis should be reinforced by searching for unique polymorphisms, because the relative high recurrent mutation rate of Y-STRs could lead to different Y chromosomes carrying the very similar or same distribution of Y-STR alleles [1, 7, 13]. As recently described, except for isolated point mutations (substitutions), no systematic differences were observed either in the repeat sequence or in the flanking regions between the two DYS385 fragments of a given individual [9]. Thus the mutations reported previously do not seem be suitable to identify allele- or locus-specific differences. The further examina-

Table 1 Range of standard deviations (SD) and windows of estimated allelic size for the six monolocal tetramer Y-STR loci obtained by analyzing of population database samples (PDS, 194 males) and allelic ladders on an ABI Prism 310 instrument. Two different size standards (CXR60–400 and GS500) were applied to the allelic ladders

nt = nucleotide

Locus	PDS (CXR60–400)		Y-ladder (CXR60–400)		Y-ladder (GS500)	
	SD (nt)	Window (nt)	SD (nt)	Window (nt)	SD (nt)	Window (nt)
DYS19	0.06–0.07	0.22–0.39	0.05–0.06	0.18–0.30	0.08–0.10	0.26–0.37
DYS389-I	0.06–0.08	0.17–0.44	0.05–0.06	0.19–0.26	0.09–0.10	0.33–0.39
DYS389-II	0.09–0.14	0.23–0.55	0.05–0.07	0.26–0.27	0.09–0.13	0.37–0.46
DYS390	0.05–0.10	0.10–0.47	0.07–0.09	0.27–0.43	0.08–0.11	0.24–0.38
DYS391	0.02–0.08	0.04–0.45	0.05–0.05	0.20–0.23	0.07–0.09	0.34–0.40
DYS393	0.03–0.08	0.07–0.45	0.05–0.06	0.22–0.30	0.08–0.09	0.33–0.36

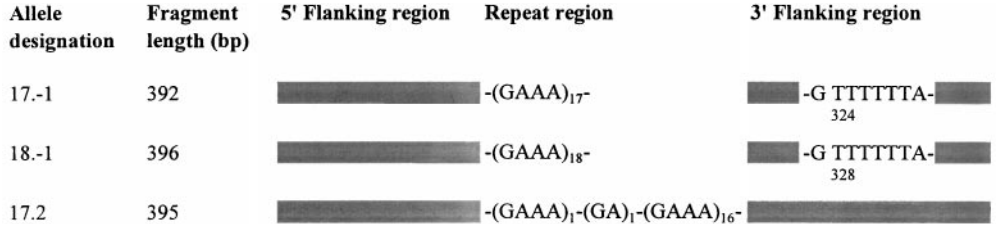

Fig. 1 Sequence structure and fragment length of the three types of intermediate sized DYS385 alleles observed in the population survey. Sequencing was performed on all the six persons carrying variant alleles. In the case of alleles 17.-1 and 18.-1 the starting position of the $(T)_6$ block lacking a T as compared to the reference sequence (EMBL database accession no. Z93950) [9] is indicated

Table 2 A list of 133 Y-STR haplotype combinations for DYS19/DYS389-I/DYS389-II/DYS390/DYS391/DYS392/DYS393/DYS385 observed in 116 Budapest Caucasians and in 78 Baranya Romanies (in parentheses)

Haplotype	Obs.	*n*	Haplotype	Obs.	*n*
12/10/27/24/10/11/13/17/17	1	(–)	15/9/26/22/10/11/13/13/15	1	(–)
13/9/25/22/10/11/14/14/14	1	(–)	15/9/26/22/10/11/14/15/15	–	(1)
13/9/25/24/11/13/13/12/14	1	(–)	15/9/26/23/10/11/13/12/14	1	(–)
13/9/27/24/10/11/13/16/17	1	(–)	15/9/26/23/10/11/13/13/14	2	(–)
13/10/26/22/10/15/13/14/16	1	(–)	15/10/26/22/10/11/12/14/17	–	(3)
13/10/27/24/10/11/13/15/20	1	(–)	15/10/26/22/10/11/12/15/17	1	(–)
13/10/27/24/11/11/13/17/17	2	(–)	15/10/26/24/11/13/13/12/14	1	(–)
13/11/26/25/10/16/13/13/17	1	(–)	15/10/26/25/10/11/13/11/15	1	(–)
13/11/27/22/10/13/13/14/18	1	(–)	15/10/26/25/10/13/13/11/14	–	(1)
13/11/27/24/9/11/13/13/13	2	(–)	15/10/27/24/11/11/13/14/15	1	(–)
13/11/28/22/8/11/13/12/15	1	(–)	15/10/27/25/10/11/13/11/14	1	(2)
13/11/28/24/10/11/13/16/19	–	(1)	15/10/27/25/10/11/13/12/14	1	(–)
13/11/28/24/10/11/13/18/18	–	(1)	15/10/27/25/11/11/13/10/14	1	(–)
14/9/25/22/10/11/13/13/14	2	(1)	15/10/27/25/11/11/13/12/14	1	(–)
14/9/25/22/10/11/13/14/14	–	(3)	15/10/28/22/10/11/12/13/15	–	(1)
14/9/25/22/10/11/13/14/15	–	(1)	15/10/28/24/11/11/13/11/14	–	(1)
14/9/25/22/10/11/14/14/14	2	(–)	15/10/28/25/10/11/13/11/14	1	(4)
14/9/25/23/10/11/12/15/16	1	(–)	15/10/28/25/11/11/13/11/14	–	(18)
14/9/25/23/10/11/13/13/14	2	(–)	15/10/28/25/12/11/13/11/14	–	(1)
14/9/25/23/10/11/13/13/15	1	(–)	15/10/29/24/12/11/13/14/15	1	(–)
14/9/25/24/10/11/12/14/17.-1	1	(–)	15/10/29/25/9/11/13/11/15	1	(–)
14/9/25/24/10/13/13/11/14	1	(–)	15/11/26/24/10/13/13/12/14	1	(–)
14/9/25/25/10/14/13/11/14	1	(–)	15/11/27/22/10/11/12/15/16	–	(5)
14/9/26/22/10/11/13/13/14	1	(–)	15/11/27/22/10/11/12/15/17	1	(9)
14/9/26/23/10/11/12/13/19	1	(–)	15/11/28/21/11/11/12/14/16	1	(–)
14/9/26/24/11/13/12/11/14	1	(–)	15/11/28/23/10/12/14/13/17	1	(–)
14/9/27/22/10/11/13/13/14	1	(–)	15/12/28/22/10/11/12/15/15	–	(1)
14/10/25/23/10/11/12/12/16	1	(–)	15/12/28/22/10/11/12/15/17	–	(5)
14/10/25/24/10/14/12/11/15	1	(–)	16/9/25/24/11/11/14/13/17.2	1	(–)
14/10/26/22/11/13/13/11/14	1	(–)	16/9/26/24/10/11/12/13/18	1	(–)
14/10/26/23/10/11/13/12/13	1	(–)	16/10/26/24/10/11/13/11/15	1	(–)
14/10/26/23/10/13/13/11/14	–	(1)	16/10/26/24/11/11/13/11/14	–	(1)
14/10/26/23/11/11/12/13/16	–	(1)	16/10/26/24/11/11/13/14/15	1	(–)
14/10/26/23/11/13/13/11/14	2	(–)	16/10/26/25/10/11/13/11/14	1	(–)
14/10/26/24/10/13/13/11/14	1	(–)	16/10/27/24/11/11/13/15/19	–	(1)
14/10/26/24/10/13/13/11/15	–	(1)	16/10/27/25/10/11/13/11/14	1	(–)
14/10/26/24/10/14/12/11/14	1	(–)	16/10/27/25/11/11/13/11/14	2	(–)
14/10/26/24/11/13/12/11/15	1	(–)	16/10/28/22/10/11/12/13/15	1	(–)
14/10/26/24/11/13/13/11/11	1	(–)	16/10/28/24/10/11/13/14/15	1	(–)
14/10/26/24/11/13/13/11/13	1	(–)	16/10/28/24/11/11/13/14/15	1	(–)
14/10/26/24/11/13/13/11/14	1	(1)	16/10/28/24/11/11/13/14/16	1	(–)
14/10/26/25/11/13/13/11/14	1	(–)	16/10/28/25/10/11/13/11/15	1	(–)
14/10/26/25/11/13/13/14/14	1	(–)	16/10/29/24/11/11/13/14/15	2	(–)
14/10/27/22/10/11/12/13/16	1	(–)	16/10/29/24/11/11/13/15/15	1	(–)
14/10/27/23/10/11/12/13/15	1	(–)	16/10/29/25/11/11/13/14/14	–	(1)
14/10/27/23/10/11/12/14/17	1	(–)	16/11/26/23/10/13/13/12/14	1	(–)
14/10/27/24/11/13/12/11/16	1	(–)	16/11/27/24/10/11/13/13/15	1	(–)
14/10/27/25/11/13/13/10/14	1	(–)	16/11/28/24/10/11/13/14/15	–	(1)
14/10/28/23/10/11/12/13/16	1	(–)	16/11/28/24/11/11/13/11/14	1	(–)
14/10/28/23/11/13/12/11/15	1	(–)	16/11/28/25/11/11/13/11/16	1	(–)
14/11/26/23/10/12/12/13/17	1	(–)	16/11/28/26/11/11/13/11/14	1	(–)
14/11/27/23/11/14/14/13/13	1	(–)	17/9/25/26/10/11/14/13/18	1	(–)
14/11/27/24/11/13/13/11/15	1	(–)	17/10/26/25/10/11/13/11/14	1	(–)
14/11/27/25/10/11/13/15/16	–	(1)	17/10/26/25/11/11/13/11/14	1	(–)
14/11/28/22/10/11/12/13/15	1	(–)	17/10/27/23/10/12/15/16/18	1	(–)
14/11/28/23/10/11/12/13/16	1	(2)	17/10/27/25/10/11/13/10/14	1	(–)
14/11/28/24/10/11/12/17/18	–	(4)	17/10/27/26/10/11/13/11/13	1	(–)
14/11/28/24/10/11/12/18/18	–	(1)	17/10/28/24/11/11/13/13/16	1	(–)

Table 2 (continued)

Haplotype	Obs.	*n*	Haplotype	Obs.	*n*
14/11/28/24/11/13/12/12/15	1	(–)	17/10/28/24/11/12/13/14/15	1	(–)
15/9/24/24/10/11/12/13/18.-1	–	(1)	17/10/28/25/10/11/13/10/14	1	(–)
15/9/25/22/10/11/13/14/14	1	(–)	17/10/29/24/11/11/13/15/15	1	(–)
15/9/25/23/10/12/12/15/18	1	(–)	17/11/28/23/10/12/15/15/16	–	(2)
15/9/25/24/10/11/12/12/17.-1	1	(–)	17/11/28/25/10/11/13/10/14	1	(–)
15/9/25/24/10/11/12/13/17.-1	1	(–)	17/11/28/25/11/11/13/11/14	1	(–)
15/9/25/24/10/11/12/14/17.-1	1	(–)	18/10/26/24/11/11/13/14/15	1	(–)
15/9/26/21/10/11/15/13/13	1	(–)	18/10/27/25/10/11/13/11/14	1	(–)
15/9/26/22/10/11/13/13/14	1	(–)			

Table 3 Results of AMOVA analysis in pairwise comparisons of three populations. BuCa = Hungarian Caucasians residing in the Budapest area, BaRo = Hungarian Romanies residing in Baranya county, Italian = peninsular Italian Caucasians [14]

	BuCa	BaRo (significance of Φ_{ST})	Italian
BuCa	–	0.001	$< 10^{-3}$
BaRo	0.0418	–	$< 10^{-4}$
Italian	0.0462	0.1083 (Φ_{ST})	–

tion of the Y chromosomes harboring the variant alleles presented here may provide a possibility for the discrimination between the two loci of the DYS385 marker in special situations.

A total of 133 different Y-STR haplotypes could be identified in 194 males from the surveyed population samples (Table 2) and 107 and 32 haplotypes were observed in 116 Budapest Caucasians and 78 Baranya Romanies, respectively. The haplotype diversity and discriminatory capacity values for the Romany population sample (0.912 and 41%) were significantly smaller than those found in the Central Hungarian (0.99 and 92.2%) and other Causasian populations [2, 14, 15]. This observation may imply a possible common paternal ancestry of some haplotypes in such genetically closed population groups as Hungarian Romanies. The two Hungarian populations analyzed here, with the addition of another Caucasian population sample surveyed in Italy [14], can be significantly distinguished with the seven monolocal Y-STR loci by AMOVA analysis (Table 3).

In conclusion, two Hungarian population databases have been established for eight Y-STR loci using a capillary electrophoresis system bearing a sufficient precision and resolution power for reliable genotyping. A significant difference was found between the two Hungarian population samples by performing Y-STR haplotype analysis using AMOVA and by making a comparison between the forensic efficiency values for these populations. Therefore, these results suggest that the forensic interpretation of a Y-STR haplotype as DNA evidence in cases of non-exclusions requires special attention to population substructuring due to prevalent male lineages.

Acknowledgements The authors wish to thank B. Brinkmann, M. Schürenkamp (University of Münster, Germany) M. Kayser (Humboldt University, Berlin, Germany), P.M. Schneider (Johannes Gutenberg University, Mainz, Germany), and P. de Knijff (Leiden University, The Netherlands) for providing allelic ladders and reference DNA samples.

References

1. Jobling MA, Pandya A, Tyler-Smith C (1997) The Y chromosome in forensic analysis and paternity testing. Int J Legal Med 110:118–124
2. 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, Knijff P de, Roewer L (1997) Evaluation of Y-chromosomal STRs: a multicenter study. Int J Legal Med 110:125–133
3. Füredi S, Budowle B, Woller J, Pádár Z (1996) Hungarian population data on six STR loci – HumVWFA31, HumTH01, HumCSF1PO, HumFES/FPS, HumTPOX, and Hum HPRTB – derived using multiplex PCR amplification and manual typing. Int J Legal Med 109:100–101
4. Füredi S, Angyal M, Kozma Z, Sétáló J, Woller J, Pádár Z (1997) Semi-automatic DNA profiling in a Hungarian Romany population using the STR loci HumVWFA31, HumTH01, HumTPOX, and HumCSF1PO. Int J Legal Med 110:184–187
5. 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
6. Jobling MA, Tyler-Smith C (1995) Fathers and sons: the Y chromosome and human evolution. Trends Genet 11:449–456
7. Knijff P de, 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
8. Bär W, Brinkmann B, Budowle B, Carracedo A, Gill P, Lincoln P, Mayr W, Olaisen B (1997) DNA recommendations. Further report of the DNA commisson of the ISFH regarding the use of short tandem repeat systems. Int J Legal Med 110:175–176
9. Schneider PM, Meuser S, Waiyawuth W, Seo Y, Rittner C (1998) Tandem repeat structure of the duplicated Y-chromosomal STR locus DYS385 and frequency studies in the German and three Asian populations. Forensic Sci Int 97:61–70

10. Excoffier L, Smouse P, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131 : 479–491
11. Michalakis Y, Excoffier L (1996) A generic estimation of population subdivision using distances between alleles with special reference for microsatellite loci. Genetics 142 : 1061–1064
12. Nei M (1973) Analysis of gene diversity in subdivided populations. Proc Natl Acad Sci USA 70 : 3321–3323
13. Santos FR, Tyler-Smith C (1996) Reading the human Y chromosome: the emerging DNA markers and human genetic history. Braz J Genet 19 : 665–670
14. Caglià A, Dobosz M, Boschi I, d'Aloja E, Pascali VL (1998) Increased forensic efficiency of a STR-based Y-specific haplotype by addition of the highly polymorphic DYS385 locus. Int J Legal Med 111 : 142–146
15. Kayser M, Knijff P de, Dieltjes P, Krawczak M, Nagy M, Zerjal T, Pandya A, Tyler-Smith C, Roewer L (1997) Applications of microsatellite-based Y chromosome haplotyping. Electrophoresis 18 : 1602–1607