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SHORT COMMUNICATION

**María T. Zarrabeitia · José A. Riancho · Leonor Gusmão
María V. Lareu · Carolina Sañudo · António Amorim
Angel Carracedo**

Spanish population data and forensic usefulness of a novel Y-STR set (DYS437, DYS438, DYS439, DYS460, DYS461, GATA A10, GATA C4, GATA H4)

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Abstract DNA typing of 8 recently described STRs on the Y chromosome was carried out by means of 2 multiplex amplification reactions for 134 unrelated males from Cantabria, a region in northern Spain. Multiplex 1 included loci DYS460 (GATA A7.1), GATA A10, GATA H4 and DYS439; multiplex 2 included DYS461 (GATA A7.2), GATA C4, DYS437 and DYS438. Haplotype diversity was found to be 99.36%, similar to that obtained with the standard 9-STR set ("minimal haplotype") of the European Y-user group (99.35%). The 13-locus haplotype resulting from the combination of the standard minimal haplotype and the 4-locus multiplex 1 showed a 99.89% diversity. Further inclusion of the 4 loci in multiplex 2 resulted in a haplotype diversity of 99.93%. The combination of the "minimal haplotype" and the multiplex 1 in the present study may be an efficient way of increasing the power of discrimination in forensic cases.

Keywords Microsatellites · Short tandem repeats · Y-chromosome · Haplotype diversity · Population genetics

M. T. Zarrabeitia (✉) · C. Sañudo
Unidad de Medicina Legal, Facultad de Medicina,
Universidad de Cantabria C/Herrera Oria, s/n,
39011 Santander, Spain
Tel.: +34-942-201984, Fax: +34-942-201903,
e-mail: zarrabet@unican.es

J. A. Riancho
Universidad de Cantabria, Departamento de Medicina Interna,
Santander, Spain

M. V. Lareu · A. Carracedo
Universidad de Santiago, Instituto de Medicina Legal,
Santiago de Compostela, Spain

L. Gusmão · A. Amorim
Universidad do Porto,
Instituto de Patologia e Imunologia Molecular, Porto, Portugal

Introduction

Polymorphisms on the Y chromosome have become a very useful tool in forensic and population genetics. A number of short tandem repeat (STR) polymorphisms have been widely studied in Europe and other areas, and large databases have been made publicly available [1]. Thus, population frequency data of a standard set of STRs combined in a so-called minimal haplotype are now available through the internet database of the Y-user group [1]. Such a haplotype is established by typing 9 loci: DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and DYS385I-II. Other STRs have also been recently suggested as interesting for forensic purposes [2, 3, 4]. However, given the linkage among different loci on the non-recombining part of the Y chromosome, it is unclear if they add useful information to the widely studied minimal haplotype, or result in redundant information.

Therefore, we planned to study allele population distribution and forensic power of a recently described set of eight STRs, and compare them with the minimal haplotype.

Materials and methods

Subjects

Unrelated male individuals (n=134) living in Cantabria, a region in northern Spain with a population about 500,000, were studied. Genomic DNA was extracted from peripheral blood by a commercial method according to the manufacturer's instructions (Qiagen, Germany) and quantified by light absorbance (Genequant, Pharmacia, Sweden). Aliquots containing 10–30 ng DNA were used to amplify the regions of interest by PCR, with the primers reported by González-Neira et al. [4], except for loci DYS460 (GATA A7.1), DYS461 (GATA A7.2) and GATA H4. These loci were amplified with new primers designed to decrease the size range of the alleles, as recently reported in the collaborative exercise by the GEP-ISFG (Spanish and Portuguese Group of the International Society of Forensic Genetics) [5, 6] (Table 1). Two multiplex reactions were carried out. Multiplex 1 included FAM-labelled primers to amplify GATA A7.1 (0.3 µM), GATA A10 (0.2 µM), GATA H4 (0.5 µM), and DYS439 (0.2 µM). Multiplex 2 contained TET-labelled primers

Table 1 Primer sequences of the Y-chromosome STRs investigated

Locus	Primer sequences
DYS437	GAC TAT GGG CGT GAG TGC AT AGA CCC TGT CAT TCA CAG ATG A
DYS438	TGG GGA ATA GTT GAA CGG TAA GTG GCA GAC GCC TAT AAT CC
DYS439	TCC TGA ATG GTA CTT CCT AGG TTT GCC TGG CTT GGA ATT CTT TT
DYS460	AGC AAG CAC AAG AAT ACC AGA G TCT ATC CTC TGC CTA TCA TTT ATT A
DYS461	AGG CAG AGG ATA GAT GAT ATG GAT TGA TGC TGT GTC ACT ATA TTT CTG
GATA A10	CCT GCC ATC TCT ATT TAT CTT GCA TAT A ATA AAT GGA GAT AGT GGG TGG ATT
GATA C4	AGT GTC TCA CTT CAA GCA CCA AGC AC GCA GCA AAA TTC ACA GTT GGA AAA ATG T
GATA H4	GTT ATG CTG AGG AGA ATT TCC AA CCT CTG ATG GTG AAG TAA TGG AAT TAG A

to amplify GATA A7.2 (0.2 µM), GATA C4 (0.5 µM), DYS437 (0.16 µM), and DYS438 (0.5 µM). Both reaction mixes contained 2 mM $MgCl_2$, 200 µM dNTPs and 0.5 U Taq Gold polymerase (Applied Biosystems, USA) in a 25 µl volume. Cycling conditions for both multiplexes were: pre-incubation at 95° for 7 min, followed by 32 cycles of 30 s at 94°, 20 s at 60° and 30 s at 70°, with a final extension step of 45 min at 70°C.

DYS385 locus was analysed with the protocol proposed by Schneider et al. [7]. Other loci in the minimal haplotype were amplified with the primers described by Gusmão et al. and Kayser et al. [8, 9, 10], as previously reported [11].

Amplicon analysis

Aliquots containing 1.2 µl of PCR product were mixed with 24 µl formamide and 1 µl TAMRA as a internal size standard, heated for 5 min at 95°C, quenched in an ice bath for 10 min and injected into an ABI 310 capillary electrophoresis system (Applied Biosystems). Amplicon size was determined with the local Southern method implemented in Genescan software. Allele designation followed the ISFG and other previously published recommendations [12, 13]. Sequenced alleles were used as controls.

Table 2 Allele frequencies of loci included in multiplex 1 (n=134)

Allele	DYS460		GATA A10		DYS 439		GATA H4	
	Freq.	SE	Freq.	SE	Freq.	SE	Freq.	SE
9	0.022	0.013			0.007	0.007		
10	0.388	0.042			0.112	0.027		
11	0.575	0.043			0.224	0.036		
12	0.015	0.010			0.485	0.043		
13			0.015	0.010	0.149	0.031		
14			0.291	0.039	0.022	0.012		
15			0.575	0.043				
16			0.104	0.026				
17			0.007	0.007				
18			0.007	0.007				
26							0.037	0.016
27							0.388	0.042
28							0.493	0.043
29							0.075	0.023
30							0.007	0.007
Locus diversity	0.522		0.578		0.684		0.604	

Table 3 Allele frequencies of loci included in multiplex 2 (n=134)

Allele	DYS461		DYS437		DYS438		GATA C4	
	Freq.	SE	Freq.	SE	Freq.	SE	Freq.	SE
9	0.007	0.007			0.045	0.018		
10					0.261	0.038		
11	0.201	0.034			0.075	0.023		
12	0.664	0.041			0.612	0.042		
13	0.112	0.027			0.007	0.007		
14	0.015	0.010	0.470	0.043				
15			0.410	0.043				
16			0.112	0.027				
17								
18			0.007	0.007				
20							0.030	0.015
21							0.164	0.032
22							0.075	0.023
23							0.604	0.042
24							0.127	0.029
Locus diversity	0.509		0.602		0.554		0.589	

Table 4 Haplotypes resulting from the analysis of loci included in multiplex 1, multiplex 2 and the minimal haplotype (n=104)

Haplo-type	N	DYS19	DYS385	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS460	DYS461	A10	C4	H4
H1	1	13	11–13	13	30	24	10	13	13	15	12	12	11	11	15	23	28
H2	1	13	11–14	13	29	24	11	13	13	15	12	12	11	11	15	23	28
H3	1	13	11–14	13	29	25	12	13	13	15	12	13	11	12	16	23	28
H4	1	13	12–14	14	30	24	9	11	13	14	10	10	11	13	14	21	28
H5	1	13	12–17	12	29	24	10	11	12	14	10	12	10	11	14	21	26
H6	1	13	13–14	13	29	24	9	11	13	14	10	10	11	13	15	21	27
H7	2	13	13–14	14	30	24	9	11	13	14	10	10	11	12	14	21	28
H8	1	13	13–14	14	30	24	9	11	13	14	10	10	11	13	14	22	27
H9	1	13	13–14	14	30	24	9	11	13	14	10	10	12	13	14	21	28
H10	1	13	13–15	14	30	24	9	11	13	14	10	10	11	13	14	23	28
H11	1	13	13–15	14	30	24	9	11	13	14	10	11	11	13	14	21	27
H12	1	13	13–16	13	30	22	9	11	13	14	10	12	10	13	14	22	28
H13	1	13	14–15	14	30	24	9	11	13	14	10	10	11	14	14	21	27
H14	1	13	14–16	14	30	24	9	11	13	14	10	10	10	14	14	21	28
H15	1	13	16–17	13	30	24	10	11	13	14	10	12	10	12	15	24	28
H16	1	13	16–18	13	30	24	10	11	13	14	10	13	10	11	15	21	27
H17	1	13	17–18	12	29	25	10	11	12	14	10	11	10	13	15	22	27
H18	1	14	10–11	13	29	23	11	13	14	16	13	11	11	13	15	23	29
H19	1	14	10–14	13	28	25	11	13	13	14	12	13	10	12	14	23	28
H20	1	14	10–14	14	30	25	11	13	13	15	12	12	11	12	15	23	28
H21	1	14	11–11	12	28	23	10	13	13	15	12	13	11	12	15	23	28
H22	1	14	11–11	12	28	24	10	13	13	15	12	13	11	12	15	23	28
H23	1	14	11–13	13	29	25	11	13	13	14	12	12	11	12	16	24	27
H24	1	14	11–14	10	26	23	10	13	13	15	12	12	11	12	15	23	28
H25	1	14	11–14	12	28	24	10	11	13	15	12	11	11	12	15	24	30
H26	1	14	11–14	12	28	24	11	13	13	14	12	12	10	12	15	23	27
H27	1	14	11–14	13	28	24	11	13	13	14	12	12	11	12	15	23	28
H28	1	14	11–14	13	29	24	10	13	13	14	12	12	10	12	15	23	27
H29	1	14	11–14	13	29	24	10	13	13	15	12	11	10	12	14	23	28
H30	1	14	11–14	13	29	24	10	13	13	15	12	11	11	12	14	23	28
H31	1	14	11–14	13	29	24	10	13	13	15	12	11	11	12	15	23	28
H32	1	14	11–14	13	29	24	10	13	13	16	12	12	10	12	15	23	29
H33	1	14	11–14	13	29	24	11	13	13	14	12	12	10	12	15	23	27
H34	1	14	11–14	13	29	24	11	13	13	15	11	11	11	12	16	23	27
H35	1	14	11–14	13	29	24	11	13	13	15	12	12	11	11	15	23	29
H36	1	14	11–14	13	29	24	11	13	13	15	12	12	11	12	15	23	28
H37	1	14	11–14	13	29	24	11	13	13	15	12	14	11	12	16	23	28
H38	1	14	11–14	13	29	24	11	13	14	15	12	13	11	12	15	23	27
H39	1	14	11–14	13	29	24	11	13	14	15	12	14	10	12	15	23	28
H40	2	14	11–14	13	29	25	10	13	13	15	12	12	10	12	15	23	27
H41	1	14	11–14	13	29	25	11	13	13	14	12	12	10	12	15	23	28

Table 4 (continued)

Haplo-type	N	DYS19	DYS385	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS460	DYS461	A10	C4	H4
H42	1	14	11–14	13	29	25	11	13	13	15	12	12	11	11	14	23	26
H43	1	14	11–14	13	30	23	11	13	13	15	12	13	11	11	15	23	27
H44	2	14	11–14	13	30	24	11	13	13	15	12	13	11	12	15	24	28
H45	1	14	11–14	14	30	23	11	13	14	14	12	12	11	12	15	23	27
H46	1	14	11–14	14	30	24	10	13	13	15	12	12	10	12	16	23	28
H47	1	14	11–14	14	30	24	11	14	13	15	12	11	11	12	15	23	27
H48	1	14	11–14	14	30	25	11	13	12	14	12	11	10	12	15	23	27
H49	1	14	11–14	14	30	25	11	13	13	14	12	12	10	12	15	23	27
H50	1	14	11–14	14	31	24	10	13	13	14	12	14	10	12	15	24	27
H51	1	14	11–14	14	31	24	11	13	13	14	12	12	10	12	15	23	28
H52	1	14	11–14	16	32	24	11	13	12	15	12	11	11	12	15	23	27
H53	1	14	11–15	13	28	24	11	13	13	15	12	12	11	12	16	23	28
H54	1	14	11–15	13	29	22	10	13	12	15	12	13	10	11	18	24	29
H55	1	14	11–15	13	29	24	10	13	13	15	11	11	11	12	15	23	28
H56	2	14	11–15	13	29	24	11	13	13	15	12	12	11	12	14	23	28
H57	1	14	11–15	13	30	24	10	13	13	15	12	11	10	12	15	23	28
H58	1	14	11–15	13	30	24	12	13	13	15	12	11	10	12	15	23	28
H59	1	14	11–15	14	30	24	10	13	13	14	12	12	10	12	15	24	27
H60	1	14	11–15	14	30	24	12	13	13	14	12	12	11	12	15	23	27
H61	1	14	11–15	14	31	24	11	13	13	14	12	11	10	12	14	23	27
H62	1	14	11–15	14	31	24	11	13	13	15	12	12	11	11	15	23	28
H63	1	14	11–16	14	30	24	12	12	13	14	12	12	11	12	15	23	27
H64	1	14	12–14	13	30	25	11	13	13	15	12	13	11	12	15	23	28
H65	1	14	12–14	14	31	24	11	13	13	14	12	12	10	12	15	24	27
H66	2	14	12–14	15	31	24	11	13	13	14	12	12	10	12	14	23	27
H67	1	14	12–15	12	28	24	11	13	13	15	12	13	11	12	15	23	26
H68	1	14	12–15	13	29	24	10	13	13	15	12	11	11	12	15	23	28
H69	1	14	12–15	13	29	24	11	13	13	14	12	13	10	12	15	23	28
H70	1	14	12–16	14	30	23	12	13	13	15	12	12	11	12	15	22	28
H71	1	14	13–14	12	28	24	10	11	12	16	10	12	9	12	15	22	27
H72	1	14	13–14	12	29	22	10	11	13	16	10	11	10	13	15	21	27
H73	1	14	13–15	12	28	22	10	11	13	16	10	11	10	11	16	22	27
H74	1	14	13–15	14	32	23	10	12	12	15	9	10	10	12	14	24	27
H75	1	14	14–16	13	29	23	10	11	12	16	9	11	11	13	15	21	26
H76	1	14	14–19	14	31	23	10	11	12	14	10	11	11	12	16	20	27
H77	1	14	17–18	13	30	24	10	13	12	14	10	11	11	9	15	23	26
H78	1	15	11–14	13	29	24	10	13	13	15	12	12	11	12	14	23	28
H79	1	15	11–14	13	29	24	11	13	13	15	12	12	11	12	16	24	27
H80	1	15	11–14	13	29	24	11	13	13	15	12	12	11	13	14	23	28
H81	1	15	11–14	13	32	25	11	11	13	14	11	10	11	11	15	24	28
H82	1	15	11–14	14	30	25	11	13	13	15	12	12	11	12	15	23	28

Table 4 (continued)

Haplo-type	N	DYS19	DYS385	DYS389-I	DYS389-II	DYS390	DYS391	DYS392	DYS393	DYS437	DYS438	DYS439	DYS460	DYS461	A10	C4	H4
H83	1	15	12–12	13	29	22	10	12	12	14	10	12	10	11	13	23	27
H84	1	15	13–14	12	28	22	10	11	14	16	10	11	11	12	14	21	28
H85	1	15	13–15	12	28	23	10	10	12	15	9	11	11	13	14	24	27
H86	1	15	13–16	13	29	23	10	13	14	14	10	12	11	12	14	21	27
H87	1	15	14–14	13	29	24	10	11	13	14	12	12	11	12	14	23	28
H88	1	15	14–14	13	31	24	11	13	13	16	11	12	12	11	16	24	28
H89	1	15	14–15	12	29	22	10	11	14	16	10	12	11	11	15	21	27
H90	1	15	14–16	12	29	23	10	11	13	16	11	12	10	11	14	21	28
H91	1	15	17–17	13	28	24	10	11	13	15	10	12	11	11	16	20	29
H92	1	15	19–23	13	29	24	10	13	13	14	12	12	10	12	14	23	29
H93	1	16	11–14	12	28	23	10	13	13	15	12	11	10	12	15	23	29
H94	1	16	11–14	12	29	24	12	13	13	15	12	13	11	12	14	23	27
H95	1	16	11–14	14	30	25	11	11	13	14	11	10	11	11	15	23	28
H96	1	16	11–15	13	32	25	11	11	13	14	11	10	11	11	15	23	29
H97	1	16	13–14	12	28	22	10	11	14	16	10	12	9	11	13	21	27
H98	1	17	11–14	12	29	24	10	13	13	15	12	13	11	12	15	23	27
H99	1	17	12–12	13	28	23	10	11	13	15	10	11	10	11	15	22	28

Data analysis

Allele and haplotype frequencies were estimated with Arlequin software (Schneider et al, University of Geneva; available at http://anthro.unige.ch/arlequin). Locus and haplotype diversities were calculated as $(1-\Sigma Pi^2)(n/n-1)$, where Pi is the allele or haplotype frequency [14]. The haplotype matching probability was estimated as 1–haplotype diversity.

Results and discussion

Allele frequencies of the loci included in multiplex 1 are shown in Table 2; and those of loci included in multiplex 2 in Table 3. If only the 4 loci included in multiplex 1 were considered, 59 different haplotypes were found among the 134 subjects studied, whereas multiplex 2 determined 41 different haplotypes. Thus, loci included in multiplex 1 were more useful for discrimination purposes. The combined analysis of both multiplexes resulted in 98 different haplotypes: 2 haplotypes were found in 6 individuals, 1 haplotype in 5, 6 in 3, 10 in 2, and 79 in a single individual. Haplotype diversity was 0.973 for multiplex 1, 0.891 for multiplex 2, and 0.992 for the combined 8-locus haplotype.

Allele frequency distributions in this population group were similar to the distribution in other Iberian populations [6, 12], but different from the Oriental ones [15].

A population subset (n=104) was also genotyped for the standard minimal haplotype, in order to compare haplotype diversity of the different allele combinations (population data for some of those loci have been previously published [11]). The results obtained are shown in Tables 4 and 5. A similar haplotype diversity was obtained with the combined two newer multiplexes reported here, and with the standard minimal haplotype. Adding allele information derived from multiplex 1 to the standard minimal haplotype increased discrimination ability, but further addition of multiplex 2 resulted in only a small gain (Table 5).

The lack of recombination and the subsequent linkage among different loci on the Y chromosome means that marginal utility (i.e., the gain of discrimination power attained by including new loci) decreases as more STRs are included into the analysis. The ideal number of loci to study has not been firmly established, and is probably different depending on the purposes of the study and, in forensic cases, on the availability of other genetic data. Certainly, there is some degree of compromise between the power of

Table 5 Haplotype diversity for different Y-STR sets (n=104 individuals)

Haplotype	Number of loci included	Haplotype diversity
Multiplex 1+multiplex 2	8	0.9936
Minimal haplotype	9	0.9935
Minimal haplotype+multiplex 1	13	0.9989
Minimal haplotype+multiplex 1 +multiplex 2	17	0.9993

discrimination attained and the cost and effort of allele typing.

In the present study, the power of discrimination was similar to that found in a recent European study by Bosch et al. who reported a minimal haplotype diversity of 0.9896, which increased to 0.9988 after including data from 10 additional loci [16]. Somewhat larger diversity values were found in Asian populations. Tsai et al. reported a 0.9999 diversity for the minimal haplotype plus the DYS388 locus in Taiwan [17]. Shin et al. found that a combined haplotype including the minimal loci plus DXYS156 and DYS388 displayed a 0.9995 diversity in Korea [18].

Our results suggest that the 9-locus standard minimal haplotype, as defined by the Y-user group [1] and the 8-locus haplotype determined by using multiplexes 1 and 2 reported here have a similar power of discrimination. Both are likely to be discriminative enough in many routine cases. Combining the minimal haplotype with the multiplex 1 (DYS460, GATA A10, GATA H4, DYS 439) into a 13-locus Y-STR set further improves discrimination power, up to a figure similar to that recently reported for the combined analysis of 19 loci [16]. However, little is gained by the further inclusion of loci in multiplex 2.

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