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Study of eight novel Y-chromosome STRs in a sample from Valencia (East of Spain): analysis of gene and haplotypes frequencies

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Abstract

Because of the great interest in Y-chromosome polymorphisms not only in the forensic field but also in evolutionary studies, the number of described Y-chromosome markers has been increased considerably in the recent literature. During the last few years, new Y-chromosome polymorphisms have been described, including binary polymorphisms, microsatellites and minisatellites. In this study, eight recently described tetranucleotide microsatellites have been analysed: DYS434, DYS437, DYS439, Y-GATA A7.1, Y-GATA A7.2, Y-GATA A.10, Y-GATA C4 and Y-GATA H4. Gene and haplotype frequencies have been estimated in the Valencian population, to determine highly informative haplotypes, using these new Y-STRs combined with the classical ones.

Keywords: Y-chromosome; STRs; Population study

1. Introduction

During the last few years, new Y-chromosome polymorphisms have been described, including binary polymorphisms, microsatellites and minisatellites. Specially, the new Y-STRs described could have importance in forensic genetics.

In this study, eight recently described [1,2] tetranucleotide microsatellites have been analysed: DYS434, DYS437, DYS439, Y-GATA A7.1, Y-GATA A7.2, Y-GATA A10, Y-GATA C4 and Y-GATA H4.

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2. Materials and methods

2.1. PCR reactions

2.1.1. Multiplex amplification

Two multiplexes were used. *Multiplex I* includes four STRs: Y-GATA 7.1, Y-GATA A.10, Y-GATA C4 and Y-GATA H4. *Multiplex II* combines two STRs: DYS437 and DYS439.

Multiplex I and II conditions: the reaction mix contained 200 μM of each dNTPs, 2 mM $MgCl_2$, 2 U Taq Gold polymerase, 1×PCR buffer (Perkin Elmer, Foster City, CA), 4 μg BSA and 5–10 ng DNA in a total volume of 25 μl.

2.1.2. Singleplex amplification

The loci Y-GATA A7.2 was amplified following the conditions described by White et al. [1]. DYS434 was amplified using 5 ng of genomic DNA in a 25-μl reaction volume comprising 200 μM of each dNTPs, 1.5 mM $MgCl_2$, 1 U Taq DNA polymerase, 1×PCR buffer (GibcoBRL, Life Technologies, Gaithersburg, MD). Primer concentrations and cycling conditions for all loci are shown in Table 1.

2.2. Primer sequences

The primer used is as described in Refs. [1,2].

2.3. Detection systems

Fragment size determination of the PCR amplified products was performed using an ALF-Express automatic sequencer and Alfwin 1.1 Analysis software (APB, Uppsala, Sweden). Allele identification was carried out using the Allellocator 1.03 software from APB, with allelic ladders kindly provided by the Institute of Legal Medicine (Santiago de Compostela, Spain) and the IPATIMUP (Porto, Portugal).

Table 1
Primer concentration and cycling conditions

Locus	Primer	Pre-incubation	Denaturation	Annealing	Extension	Post-extension	Cycles
Y-GATA 7.1	0.14 μM		95 °C-1 min	64[a]C-1 min*	68 °C-2 min		12
Y-GATA A10	0.12 μM						
Y-GATA C4	0.08 μM	95 °C-10 min	95 °C-1 min	58 °C-1 min	68 °C-2 min		18
Y-GATA H4	0.22 μM					68 °C-60 min	
Y-GATA 7.2	0.3 μM	95 °C-5 min	94 °C-30 s	59 °C-10 s	72 °C-30 s	72 °C-10 min	30
DYS434	0.3 μM	95 °C-2 min	94 °C-30 s	56 °C-10 s	72 °C-1 min		30
DYS437	0.06 μM	95 °C-5 min	94 °C-30 s	60 °C-30 s	70 °C-45 s		30
DYS439	0.16 μM					60°C-20 min	

* Touchdown PCR protocol: the annealing temperature was decreased by 0.5 °C in each cycle.

3. Results

Table 2 shows gene frequencies (GF) and gene diversity (GD). The nomenclature proposed by González-Neira et al. [3] was used. A total of 76 individuals have shown 67 different haplotypes. Out of the 67 different haplotypes, 63 were present once (1.32% each), 3 were found two times (2.63% each), and 1 was found seven times (9.21%). The

Table 2
Gene frequencies and diversity of the eight systems

LOCUS	ALLELES	GF (S.D.)	GD
DYS434	8	0.053 (0.026)	0.1483
	9	0.921 (0.031)	
	10	0.026 (0.018)	
DYS437	16	0.408 (0.057)	0.5751
	17	0.500 (0.058)	
	18	0.092 (0.033)	
DYS439	19	0.026 (0.018)	0.6282
	20	0.276 (0.052)	
	21	0.526 (0.058)	
	22	0.132 (0.039)	
	23	0.026 (0.018)	
	24	0.013 (0.013)	
Y-GATA 7.1	9	0.079 (0.031)	0.6243
	10	0.355 (0.055)	
	11	0.487 (0.058)	
	12	0.079 (0.031)	
Y-GATA 7.2	12	0.013 (0.013)	0.6504
	13	0.066 (0.029)	
	14	0.184 (0.045)	
	15	0.526 (0.058)	
	16	0.184 (0.045)	
	17	0.026 (0.018)	
Y-GATA A.10	20	0.263 (0.051)	0.5084
	21	0.645 (0.055)	
	22	0.079 (0.031)	
	23	0.013 (0.013)	
Y-GATA C4	21	0.013 (0.013)	0.6081
	22	0.053 (0.026)	
	23	0.145 (0.041)	
	24	0.105 (0.035)	
	25	0.592 (0.057)	
	26	0.079 (0.031)	
	27	0.013 (0.013)	
	28	0.013 (0.013)	
Y-GATA H4	25	0.013 (0.013)	0.4122
	26	0.013 (0.013)	
	27	0.039 (0.022)	
	28	0.342 (0.055)	
	29	0.539 (0.058)	
	30	0.053 (0.026)	

results are similar to other populations in the Iberian peninsula (Ref. [4] and González-Neira, personal communication).

Some of these new STRs are very interesting for forensic analysis, most of them are very robust and complement existing Y STRs.

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