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Forensic Science International 135 (2003) 158–162

Forensic Science International

www.elsevier.com/locate/forsciint

Results of the GEP-ISFG collaborative study on two Y-STRs tetraplexes: GEPY I (DYS461, GATA C4, DYS437 and DYS438) and GEPY II (DYS460, GATA A10, GATA H4 and DYS439)

Paula Sánchez-Diz[a], Leonor Gusmão[b], Sandra Beleza[a,b], Alfonso Benítez-Páez[c], Azucena Castro[d], Oscar García[e], Lourdes Prieto Solla[f], Helena Geada[g], Pablo Martín[h], Begoña Martínez-Jarreta[i], Maria de Fátima Pinheiro[j], Eduardo Raimondi[k], Sandra María Silva de la Fuente[l], Maria Conceição Vide[m], Martin R. Whittle[n], María Teresa Zarrabeitia[o], Angel Carracedo[a], António Amorim[b,p,*]

GEP-ISFG (The Spanish and Portuguese Working Group of the International Society for Forensic Genetics)[1]

[a]*Institute of Legal Medicine, University of Santiago de Compostela, Compostela, Spain*
[b]*IPATIMUP, Institute of Pathology and Immunology of University of Porto, Porto, Portugal*
[c]*Genetics and Molecular Biology Laboratory, Bogota, Colombia*
[d]*DataGene, Bilbao, Spain*
[e]*Ertzaintza Laboratory, Bilbao, Spain*
[f]*DNA Laboratory, Scientific Police, Madrid, Spain*
[g]*Faculty of Medicine, Forensic Genetics Service, National Institute of Legal Medicine, University of Lisbon, Lisbon, Portugal*
[h]*Biology Section, Institute of Toxicology, Madrid, Spain*
[i]*Departament of Legal Medicine and Forensics, University of Zaragoza, Zaragoza, Spain*
[j]*Porto Department, National Institute of Legal Medicine, Porto, Portugal*
[k]*PRICAI, Favaloro Foundation, Buenos Aires, Argentina*
[l]*Clinical and Molecular Analysis Laboratory, San José, Costa Rica*
[m]*Coimbra Department, Forensic Genetics Service, National Institute of Legal Medicine, Portugal*
[n]*Genomic Engenharia Molecular Ltda., São Paulo, Brazil*
[o]*Unit of Legal Medicine of Santander, University of Cantabria, Cantabria, Spain*
[p]*Faculty of Sciences, University of Porto, Porto, Portugal*

Received 31 January 2003; accepted 6 May 2003

Abstract

A collaborative exercise was carried out by the Spanish and Portuguese ISFG Working Group (GEP-ISFG) in order to evaluate the performance of two Y-chromosome STR PCR tetraplexes, which include the loci DYS461, GATA C4, DYS437 and DYS438 (GEPY I), and DYS460, GATA A10, GATA H4 and DYS439 (GEPY II).

The participating laboratories were asked to type three samples for the eight markers, using a specific amplification protocol. In addition, two control samples, with known haplotypes, were provided.

The results obtained by the 13 different participating laboratories were identical, except for two laboratories that failed to type correctly the same two samples for GATA C4.

* Corresponding author. Present address: IPATIMUP, Institute of Pathology and Immunology, University of Porto, Rua Dr. Roberto Frias s/n, Porto, Portugal. Tel.: +351-22-5570700; fax: +351-22-5570799.
E-mail address: aamorim@ipatimup.pt (A. Amorim).

[1] All authors of this article belong this working group.

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doi:10.1016/S0379-0738(03)00200-7

By sequence analyses, two different GATA C4 allele structures were found. One control sample (allele 21) and two questioned samples (allele 22, correctly typed by all the laboratories, and allele 25) presented the following repeat structure: $(TCTA)_4(TGTA)_2(TCTA)_2(TGTA)_2(TCTA)_n$, but different from the one found for allele 26 in one sample included in this exercise, as well as in the second control sample (allele 23), namely $(TCTA)_4(TGTA)_2(TCTA)_2(TGTA)_2(TCTA)_2(TGTA)_2$-$(TCTA)_n$.

The collaborative exercise results proved that both Y-tetraplexes produce good amplification results, with the advantage of being efficiently typed using different separation and detection methodologies. However, since GATA C4 repeat presents a complex structure, with alleles differing in sequence structure, efficient denaturing conditions should be followed in order to avoid typing errors due to sizing problems.
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Keywords: Y-chromosome STRs; PCR multiplex; GEP-ISFG

1. Introduction

Y-chromosome haplotypes, defined by STR loci, are now in current use in forensics. Extensive databases, using a ''minimal haplotype'', have been constructed and are constantly updated (http://www.ystr.org; http://www.ystr.org/usa; http://www.ystr.org/asia). Although the discriminatory power provided by the ''minimal haplotype'' is, indeed, great, specific forensic needs sometimes require more information and, on the other hand, confusion between identity by state (IBS) and identity by descent (IBD) can be relieved if more markers are used [1].

The aim of this collaborative exercise was to evaluate the performance of two Y-chromosome STR tetraplexes: (a) GEPY I, which includes the loci DYS461, GATA C4, DYS437 and DYS438 and (b) GEPY II, comprising DYS460, GATA A10, GATA H4 and DYS439.

2. Materials and methods

Five bloodstains, from male voluntary donors, were sent out to the participating laboratories. Stains were prepared with 50 µl of blood applied to stain cards (FTA® Classic Cards, Whatman) and dried at room temperature.

The five samples included two controls, with known haplotypes, and three samples to be typed for the Y-STRs under study, using a specific amplification protocol.

2.1. Amplification protocol

The eight most polymorphic loci, out of the 12 described by Ayub et al. [2] and White et al. [3], were selected for the present study (Table 1).

Two PCR tetraplex reactions (GEPYs I and II) were optimised so that the size range of the alleles of each locus would not overlap and the detection of the amplified products could be carried out using either monochromatic (ALF-DNA sequencers, Amersham Pharmacia Biotech) or polychromatic platforms (ABI sequencers, Applied Biosystems), as well as by using silver staining of polyacrylamide gels.

For both multiplexes, PCR amplification was recommended to be carried out using 5–50 ng of genomic DNA in a 25 µl reaction volume containing 2 mM $MgCl_2$, 200 µM of dNTPs, 1× Gold buffer (Applied Biosystems) and 0.5 U Taq Gold Polymerase (Applied Biosystems). Primer sequences and concentrations are given in Table 1. The cycling conditions (optimised in a GeneAmp PCR system 2400 thermocycler, PE) were: pre-incubation for 11 min at 95 °C, followed by 32 cycles of 30 s at 94 °C, 20 s at 60 °C, 30 s at 70 °C, and then a final incubation step of 70 °C for 45 min.

2.2. Amplification detection and allele nomenclature

Since the detection of the amplified products could be carried out using either monochromatic or polychromatic platforms, as well as silver staining, the participant laboratories were asked to use their own detection protocols and label the primers accordingly.

To allow a comparative analysis of the results, laboratories were asked to use the same allele nomenclature (in Table 2) as proposed by Gusmão et al. [4].

3. Results and discussion

The results obtained by the 13 different participating laboratories were identical (Table 3), except for two laboratories that failed to type correctly the same two samples for GATA C4. These two laboratories typed samples Z2058 and Z2060 as 23 and 24, and, 22 and 25, respectively.

These typing errors were found to be due to a different mobility between GATA C4 short and long alleles, when specific separation protocols are used. In one case, the separation was undertaken by vertical electrophoresis (DDH-300-20 C.B.S Scientific Company, Del Mar, CA, USA) and DNA fragments were detected by silver staining. In the other case, typing was carried out on an ALF DNA sequencer (Amersham Pharmacia Biotech, Uppsala, Sweden).

All other laboratories, that have typed this marker using either ABI310 or ABI377 sequencers (AB Applied

Table 1
Primer sequences and concentrations for GEPYs I and II multiplexes

Primers	Primer sequence (5′-3′)	Reference	Concentration (μM)
GEPY I			
DYS461-F	AGG CAG AGG ATA GAT GAT ATG GAT	[3]	0.12
DYS461-R	TGA TGC TGT GTC ACT ATA TTT CTG	New*	0.12
DYS437-F	GAC TAT GGG CGT GAG TGC AT	[2]	0.08
DYS437-R	AGA CCC TGT CAT TCA CAG ATG A	[2]	0.08
DYS438-F	TGG GGA ATA GTT GAA CGG TAA	[2]	0.48
DYS438-R	GTG GCA GAC GCC TAT AAT CC	[2]	0.48
GATA C4-F	AGT GTC TCA CTT CAA GCA CCA AGC AC	[3]	0.24
GATA C4-R	GCA GCA AAA TTC ACA GTT GGA AAA ATG T	[3]	0.24
GEPY II			
DYS460-F	AGC AAG CAC AAG AAT ACC AGA G	New*	0.16
DYS460-R	TCT ATC CTC TGC CTA TCA TTT ATT A	[3]	0.16
GATA A10-F	CCT GCC ATC TCT ATT TAT CTT GCA TAT A	[3]	0.2
GATA A10-R	ATA AAT GGA GAT AGT GGG TGG ATT	[3]	0.2
DYS439-F	TCC TGA ATG GTA CTT CCT AGG TTT	[2]	0.2
DYS439-R	GCC TGG CTT GGA ATT CTT TT	[2]	0.2
GATA H4-F	GTT ATG CTG AGG AGA ATT TCC AA	New*	0.24
GATA H4-R	CCT CTG ATG GTG AAG TAA TGG AAT TAG A	[3]	0.24

* Newly designed primer in order to decrease the size range of the alleles [6].

Table 2
Characteristics of the Y-chromosome STRs included in this study

Locus	Repeat structure*	Allele units**	Number of alleles	Size range (bp)
DYS437	$(TCTA)_m(TCTG)_n(TCTA)_4$	13–17	5	180–196
DYS438	$(TTTTC)_1(TTTTA)_{0,1}(TTTTC)_n$	6–14	9	201–241
DYS439	$(GATA)_n$	8–14	7	232–256
DYS460	$(ATAG)_n$	7–13	7	109–133[a]
DYS461	$(TAGA)_n(CAGA)_1$	8/10–15	7	144–172[a]
GATA A10	$(TCCA)_2(TATC)_n$	11–18	8	150–178
GATA C4	$(TCTA)_2[(TCTA)_2(TGTA)_2]_{2,3}$ $(TCTA)_n$	17/19–26	9	238–274
GATA H4	$(AGAT)_4CTAT(AGAT)_2(AGGT)_3(AGAT)_nN_{24}(ATAG)_4(ATAC)_1(ATAG)_2$	25–30	6	272–292[a]

[a] Size range obtained when using the newly designed primers included in this study (see Table 1).
* Nomenclature accordingly to Gusmão et al. [4].
** Alleles described in [2,3,5–13].

Biosystems, Foster City, CA), did not report any error or sizing problems, including one laboratory that used an ALF Express II (Amersham Pharmacia Biotech, Uppsala, Sweden).

In Table 4, The GATA C4 allele sizing results obtained by the different laboratories and typing protocols are shown. With one exception, it is possible to see that, although the allele size can vary (as expected for different apparatus and

Table 3
Typing results on samples included in this collaborative exercise

	GATA A10	GATA C4	GATA H4	DYS437	DYS438	DYS439	DYS460	DYS461
Control 1	15	21	27	14	10	13	11	11
Control 2	14	23	27	14	13	12	11	12
Z2058	14	25	26	16	10	11	10	11
Z2059	16	22	27	15	9	11	11	11
Z2060	15	26	28	14	12	11	10	12

With the exception of two laboratories (samples Z2058 and Z2060 for GATA C4), all the results were concordant.

Table 4
GATA C4 allele sizes (bp) obtained by different groups

Sample	Size (bp)	Fragment sizes (bp) obtained by different groups															
		Lab 7	Lab 2		Lab 12	Lab 8	Lab 10	Lab 4		Lab 5	Lab 9	Lab 10	Lab 6	Lab 11		Lab 3*	
		ABI310 TET	ABI310 HEX	ABI310 6-FAM	ABI310 6-FAM	ABI310 6-FAM	ABI310 6-FAM	ABI310 6-FAM	ABI377 6-FAM	ABI377 HEX	ABI377 6-FAM	ABI377 6-FAM	ABI377 6-FAM	ABI377 6-FAM	ALF Express II	ALF	ALF*
Control 1	254	254.2	254.70	253.77	252.51	254.76	253.55	253.17	256.01	254.94	255.96	256.80	256.57	255.91	254.5	250	–
Control 2	262	262.4	262.38	262.19	260.77	262.73	261.74	261.33	264.03	263.16	264.04	264.82	264.61	263.84	262.3	–	258
Z2058	270	270.6	271.51	270.68	269.09	271.03	269.85	269.56	271.89	270.92	272.14	272.38	272.15	271.57	270.9	263	264
Z2059	258	258.4	258.96	258.38	257.01	258.82	257.62	257.24	260.08	258.79	259.74	260.81	260.30	259.88	258.4	253	254
Z2060	274	274.6	275.11	274.85	273.50	274.70	274.00	273.59	275.89	275.06	276.03	276.37	276.18	275.39	275.2	267	268

[*] Results obtained by the same laboratory in two different runs. This laboratory typed the sample Z2058 as 24 and Z2060 as 25 (one repeat less than the correct value). The remaining laboratories correctly typed all samples by comparison with control samples.

Table 5
Sequence structure of GATA C4 alleles

Sample	Allele	Repeat structure
Control 1	21	$(TCTA)_4(TGTA)_2(TCTA)_2(TGTA)_2$ $(TCTA)_{11}$
Control 2	23	$(TCTA)_4(TGTA)_2(TCTA)_2(TGTA)_2(TCTA)_2(TGTA)_2$ $(TCTA)_9$
Z2058	25	$(TCTA)_4(TGTA)_2(TCTA)_2(TGTA)_2$ $(TCTA)_{15}$
Z2059	22	$(TCTA)_4(TGTA)_2(TCTA)_2(TGTA)_2$ $(TCTA)_{12}$
Z2060	26	$(TCTA)_4(TGTA)_2(TCTA)_2(TGTA)_2(TCTA)_2(TGTA)_2$ $(TCTA)_{12}$

primer labelling), there are no significant differences in the results obtained by two laboratories using the same detection method, and allele sizes vary in multiples of four base pairs, according to their repeat number. Lab 3 was one of the two laboratories that failed to type correctly the GATA C4, and, apart from a 1 bp difference obtained in two different runs they also detected non-multiple four base pair differences between samples. These results were interpreted as being caused by the presence of different allele sequence structures, according to the previously reported by González-Neira et al. [5], where, apart from a complex repeat structure (TCTA/TGTA), two different sequence subtypes were found in GATA C4 alleles (types A and B).

Indeed, by sequence analyses (Table 5), it was found that the control sample (allele 21) and samples Z2059 and Z2058 (allele 22, correctly typed by all the laboratories, and allele 25) present the same repeat structure, type A, but different from the one found in control 2 (allele 23) and in sample Z2060 (allele 26), type B.

Sequence structure of GATA C4 allele 25 was found to be type A, different from the one already described by González-Neira et al. [5] as type B. Allele 26 has not been previously reported, and it was found in a Northern Portuguese male.

Concerning the performance of both tetraplexes, all the labs obtained good amplification results. For loci DYS460, DYS461, GATA A10, GATA H4, DYS437, DYS438 and DYS439 no typing errors were reported by all 13 participating laboratories, even when using different typing methodologies, including different automatic platforms and different primer labels, as well as manual separation protocols and detection by silver staining.

In conclusion, these two tetraplexes can be used to amplify efficiently these eight loci, however, careful attention should be paid to GATA C4 typing, namely by avoiding the methodologies responsible for an inaccurate sizing of the alleles.

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