P. Leyenda,[1] *B.Sc; S. Dios, B.Sc; and B. Caeiro, Ph.D.*[1]

TECHNICAL NOTE

Population Genetics of Two Tetranucleotide Repeats (UGB and D3S1349): a Preliminary Study in Galicia, Spain*

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ABSTRACT: The polymorphism of two tetranucleotide repeats, UGB and D3S1349 in the population of Galicia, Spain was carried out. Up to 7 and 8 alleles respectively were found, which determine values of $H = 0.820$ and $H = 0.808$ for these two STRs. A non-isotopic technique using thin layer polyacrylamide gel electrophoresis followed by silver staining, for the screening of both systems, is presented and discussed. Formal genetic studies from 51 meioses are concordant with an autosomal co-dominant way of inheritance of these systems.

KEYWORDS: forensic science, DNA typing, population genetics, short tandem repeats, tetranucleotide, UGB, D3S1349, Galicia, Spain

We report the genetic analysis of two new tetranucleotide repeats: UGB (Uteroglobin Gene) $(ATTT)_n$ (1) and D3S1349 $(AGAT)_n$ (2) by non-isotopic technique, to assess their suitability in routine analysis for genetic characterization of forensic biological evidence and human genetic studies.

Materials and Methods

Blood samples from unrelated healthy individuals from Galicia (NW Spain) were analyzed (200 for UGB and 250 for D3S1349). DNA was extracted using either the phenol-chloroform method (3) or chelating resins (4). Amplification took place in a Perkin Elmer GeneAmp 2400 PCR thermocycler (Norwalk, Connecticut) in 25 μL reaction mix according to the following conditions: for UGB, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 75 μM dNTPs, 0.8 μM each primer (1), 10–50 ng DNA and 0.5 U Taq DNA Polymerase (Promega, Madison WI). For D3S1349, 10 mM Tris-HCl pH 8.3, 50 mM KCl, 2 mM $MgCl_2$, 150 μM dNTPs, 0.125 μM each primer (2), 50–200 ng DNA and 0.5 U Taq DNA polymerase treated with 1.5 μL of monoclonal antibodies anti-Taq DNA polymerase (Clontech, Palo Alto CA). The cycling conditions were: 94°C 30 s, 52°C 30 s, and 72°C 30 s for 30 cycles and a further elongation at 72°C 5 min, for UGB. For D3S1349: initial denaturation at 93°C for 5 min followed by 35 cycles at 94°C 1 min, 59°C 1 min, 72°C 1 min, and a final extension at 72°C for 10 min.

Amplified DNA was electrophoresed in horizontal polyacrylamide gels (0.4 mm thick) (5% T, 3% C for UGB; 9% T, 5% C for D3S1349) in 19 and 12 cm long gels for UGB and D3S1349, respectively. Tris-HCl (375 mM), pH 8.8 and Tris-Glycine (125 mM), pH 8.8 were used as gel and bridge buffer, according to the conditions described in Luis and Caeiro (5). Electrophoresis was accomplished at 200 V for 3 h at 18°C for UGB and 160 V, 2 h 30 min at 4°C for D3S1349. Finally, the DNA bands were visualized after a silver staining (6). The alleles of UGB and D3S1349 were named according to the nomenclature proposed by Stöhr and Weber (1) and Li et al. (2).

An exact test for analyzing genetic equilibrium was applied (7). The values of PIC and DP were calculated as detailed by Botstein et al. (8). Population comparisons of allele frequencies were carried out using the G statistic (9).

Results and Discussion

Figure 1A and 1B show the phenotypes of UGB and D3S1349. The main problems for UGB phenotyping lie in obtaining a good molecular separation between consecutive bands, as their respective alleles only differ by about 1% in size. In order to overcome this limitation, different technical improvements were employed. Discontinuous electrophoresis at constant pH contributes to improving the resolution (10,6) and, in our experience the use of Tris-HCl-Glycine buffers at pH 8.8 gives satisfactory results. Bearing in mind that the temperature affects the viscosity of the gel and, accordingly the molecular sieving, different temperatures from 4 to 30°C were tested. An improved separation at 18–20°C was observed. Progressively higher temperatures led to undesired distortion of the banding. A further improvement was achieved in the separation of the bands after increasing the length of the gels to 19 cm.

For D3S1349, the main problem for phenotyping lies in the low signal of the amplified products. Different conditions and parameters were tested. Increasing the temperature of annealing was of crucial importance in order to solve this limitation. The best results were obtained with 59°C (Fig. 2*A*). Hot start PCR, including pro-

[1] Section of Anthropology. Faculty of Biology. University of Santiago de Compostela. 15706 Santiago de Compostela, Galicia, Spain.

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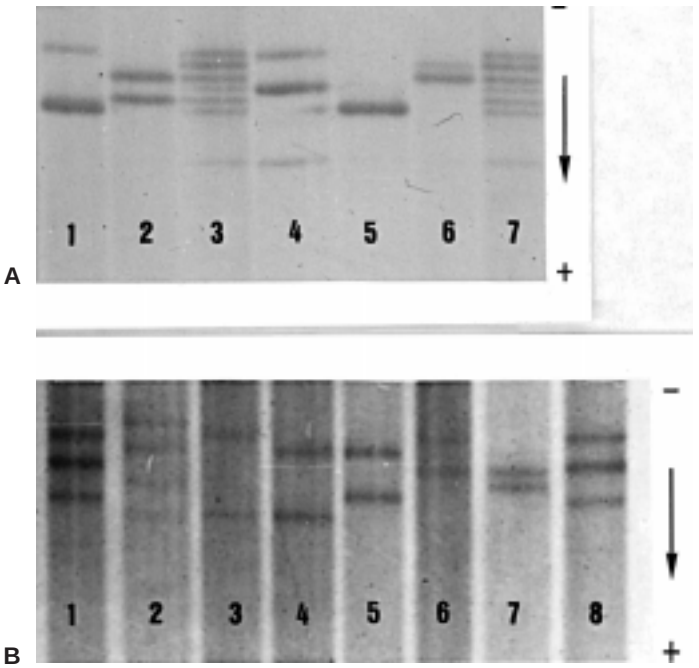

FIG. 1—*(A) Molecular phenotypes of UGB. 1: A3A8, 2: A5A7, 3: Ladder A3A4A5A6A7A8, 4: A3A6, 5: A8, 6: A4A5, 7: A3A4A5A6A7A8.* B *Molecular phenotypes of D3S1349. 1: Ladder A2A4A6, 2: Ladder A1A3A5A7, 3: A2A7, 4: A3A7, 5: A3A6, 6: A2A4, 7: A4A5, 8: Ladder A2A4A6.*

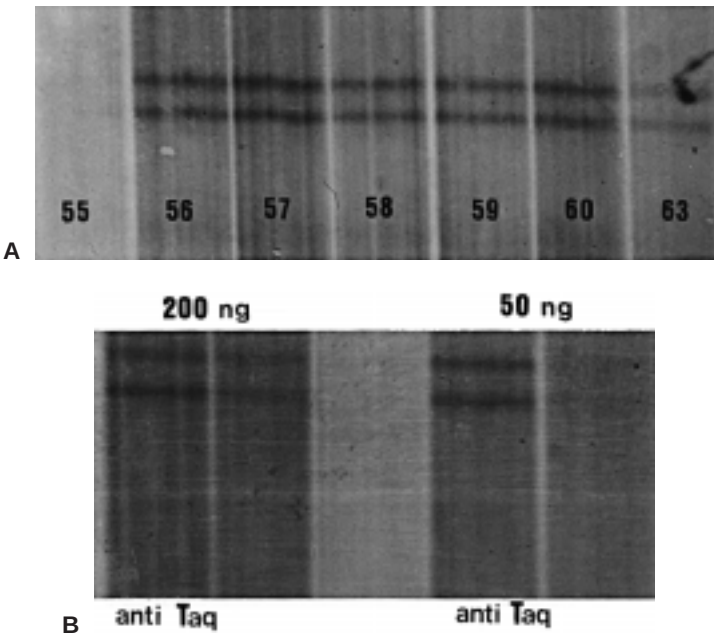

FIG. 2—*(A) Effectiveness of amplification of D3S1349 at different temperatures of annealing. (B) Effect of the treatment of two DNA samples with monoclonal antibodies anti Taq DNA polymerase for D3S1349 amplification.*

TABLE 1—*Allele and genotype frequencies for UGB and D3S1349.*

UGB						D3S1349					
	Genotypes			Alleles			Genotypes			Alleles	
	Number	Proportion		Number	Proportion		Number	Proportion		Number	Proportion
A2A3	1	0.005	A2	2	0.005	A1A2	2	0.008	A1	14	0.028
A2A6	1	0.005	A3	87	0.218	A1A3	5	0.020	A2	65	0.130
A3A3	9	0.045	A4	54	0.135	A1A4	2	0.008	A3	148	0.296
A3A4	13	0.065	A5	103	0.258	A1A5	3	0.012	A4	98	0.196
A3A5	26	0.130	A6	45	0.113	A1A6	1	0.004	A5	96	0.192
A3A6	11	0.055	A7	54	0.135	A1A7	1	0.004	A6	52	0.104
A3A7	11	0.055	A8	55	0.138	A2A2	2	0.008	A7	26	0.052
A3A8	7	0.035				A2A3	24	0.096	A8	1	0.002
A4A4	5	0.025				A2A4	17	0.068			
A4A5	12	0.060				A2A5	9	0.036			
A4A6	5	0.025				A2A6	6	0.024			
A4A7	7	0.035				A2A7	3	0.012			
A4A8	7	0.035				A2A8	0	0.000			
A5A5	15	0.075				A3A3	23	0.092			
A5A6	8	0.040				A3A4	23	0.092			
A5A7	13	0.065				A3A5	26	0.104			
A5A8	14	0.070				A3A6	16	0.064			
A6A6	4	0.020				A3A7	8	0.032			
A6A7	7	0.035				A4A4	7	0.028			
A6A8	5	0.025				A4A5	22	0.088			
A7A7	3	0.015				A4A6	14	0.056			
A7A8	10	0.050				A4A7	6	0.024			
A8A8	6	0.030				A5A5	12	0.048			
Total	200	1				A5A6	8	0.032			
						A5A7	4	0.016			
						A6A6	1	0.004			
						A6A7	4	0.016			
						A6A8	1	0.004			
						Total	250	1			

TABLE 2—*Hardy-Weinberg equilibrium (exact test), heterozygosity, polymorphic information content (PIC), and power of discrimination (PD) values for UGB and D3S1349 in Galicia.*

Locus	Exact Test	$H_e \pm$ s.e.	PIC	PD
UGB	$P = 0.8212$	0.820 ± 0.027	0.793	0.9400
D3S1349	$P = 0.8227$	0.808 ± 0.025	0.779	0.9344

tection of the Taq DNA polymerase with monoclonal antibodies in the initial cycle (11), also contributes to obtain better amplification yield. The efficiency of this treatment is presented in Fig. 2B where this improvement shows a lower efficiency as the amount of template increases.

The distribution of allele frequencies for UGB and D3S1349 are depicted in Table 1. Eight alleles ranging from 114 to 142 bp for D3S1349, and seven alleles from 387 to 411 bp for UGB were found. As the molecular size of the UGB alleles is relatively high, this means that sufficiently lengthy electrophoretic runs have to be carried out in order to offer adequate molecular separation of similarly sized alleles. The distribution of genotypes fit with those expected according to Hardy-Weinberg hypothesis in both STRs: *p* values for the exact test were 0.8212 and 0.8227 for UGB and D3S1349, respectively (Table 2). No significant differences after comparison of Galicia data with those the samples reported for UGB (1) and D3S1349 (2) have been observed ($G = 10.460$, $0.1 < p < 0.2$ and $G = 6.453$, $0.4 < p < 0.5$, respectively). Formal genetic analyses from 10 family groups including 51 meioses are in agreement with an autosomal co-dominant manner of inheritance with no exception.

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Additional information and reprint requests:
Dr. B. Caeiro, Professor
Section of Anthropology
Faculty of Biology
University of Santiago de Compostela.
15706 Santiago de Compostela
Galicia, Spain
Phone: +34-81-563100 Ext. 13333
Fax: +34-81-596904
E-mail:bajlbc95@uscmail.usc.es