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SHORT COMMUNICATION

Analysis of 21 X-chromosomal STRs in an Algerian population sample

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Abstract Twenty-one X-chromosomal short tandem repeat loci, including the six clusters of linked markers DXS10148-DXS10135-DXS8378 (Xp22), DXS7132-DXS10074-DXS10079 (Xq12), DXS6801-DXS6809-DXS6789 (Xq21), DXS7424-DXS101 (Xq22), DXS10103-HPRTB-DXS10101 (Xq26), DXS8377-DXS10146-DXS10134-DXS7423-DXS10011 (Xq28), and the loci DXS6800 and GATA172D05 were typed in a northwestern Algerian population sample (n=210; 104 men and 106 women). Allele and haplotype frequencies were calculated. No evidence of linkage disequilibrium was observed between pairs of loci within clusters of linked markers. At locus DXS10148, sequence analysis of a subset of alleles displaying unusual amplicon length (≥ 36 repeat units) and anomalous electrophoretic mobility showed that this marker has a complex molecular structure with different repeat variants within alleles of identical amplicon size.

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Introduction

X-chromosomal short tandem repeat (STR) loci provide an extremely useful tool in paternity testing, especially in deficiency cases with female offspring. As a consequence, the number of established X-STR markers suitable for forensic usage has risen continually during recent years [1]. Since haplotype analysis of X-STRs is straightforward in hemizygous males and it can be used to detect kinship between alleged relatives in large and incomplete pedigrees, many authors have focused on the development of multiplex polymerase chain reaction (PCR) systems allowing the typing of clusters of closely linked markers [2–4].

The use of X-STR markers in forensic practice requires that the peculiar properties of the X chromosome are taken into account [5]. X chromosomes spend two thirds of their life in females, therefore a lower genetic diversity is expected, due to less frequent mutational events in females than in males [6]. However, since only three X chromosome copies are present in the population for every four autosomes, stronger effects of genetic drift are possible [7]. Because only two thirds of the X chromosomes can recombine in females at each generation, longer linkage disequilibrium (LD) intervals are expected for the X chromosome compared with autosomes [8]. Therefore, for likelihood ratio calculations in relationship testing with X-STRs, especially when clusters of closely linked markers are employed, a precise knowledge of allele and haplotype frequencies, genetic linkage, and LD in the appropriate population is required [9].

With this in mind, we have collected data on the genetic diversity of a large set of X-STR markers in the Algerian population. As a matter of fact, no previous population studies regarding the distribution of X-STRs in North Africa are available at present. The typed markers included those comprised in two multiplex PCR sets which were formerly developed in-house [10], those contained in a commercial typing kit (Argus X-8) [11] and four additional X-STRs (DXS10148, DXS10079, DXS10103, and DXS10146) chosen in order to implement haplotipic data provided with the Argus X-8 kit. In all, 21 X-STRs were analyzed including six clusters of linked markers: DXS10148-DXS10135-DXS8378 (Xp22); DXS7132-DXS10074-DXS10079 (Xq12); DXS6801–DXS6809–DXS6789 (Xq21); DXS7424–DXS101 (Xq22); DXS10103-HPRTB-DXS10101 (Xq26); DXS8377-DXS10146-DXS10134-DXS7423-DXS10011 (Xq28), and loci GATA172D05 and DXS6800. The physical and genetic localizations on the X chromosome of the 21 typed markers are shown in Table S1.

Materials and methods

Blood samples were obtained from unrelated healthy adults residing in northwest Algeria (104 men and 106 women) after informed consent. The sampling was anonymous in order to prevent linkage to the original donor. DNA was extracted using the standard salting-out method [12]. X-STR markers were amplified in four separate multiplex PCR reactions. Loci DXS8378, DXS6809, DXS6789, DXS101, GATA172D05, DXS8377, and DXS7132, DXS6800, DXS6801, DXS7424, HPRTB, DXS10011 were amplified as described by Robino et al. [10], with minor modifications. In particular, a 5-dye (FAM, VIC, PET, NED, LIZ) chemistry was used in order to prevent ambiguous typing of loci DXS6801 and DXS7424, which, in the original 4-dye protocol, were both labeled with JOE and generated amplicons of almost overlapping size (113–137 bp and 147–180 bp). In the new set of primers, DXS6801 is now labeled with VIC and DXS7424 with PET. A slightly modified reverse primer (R: 5′ acaagagcgaaactccaactc) for amplification of locus DXS8378 was used [13]. Loci DXS10135, DXS8378, DXS7132, DXS10074, HPRTB, DXS10101, DXS10134, and DXS7423 were amplified by means of the Mentype Argus X-8 kit (Biotype GE, Dresden Germany) according to the manufacturer's instructions.

The remaining loci were amplified in a single multiplex reaction using primers previously described by Hundertmark et al. (DXS10148) [4], Hering et al. (DXS10079) [3], and Edelmann et al. (DXS10146) [14]; for locus DXS10103, the following primers were used (Szibor, personal communication): F: 5′ tcataatcacatatcacatgagc; R: 5′ aaacagaaccagg ggaatgaa. For this last multiplex reaction, primer sequences, concentrations, and dye-labeling are listed in Table S2. PCR was performed in a 12.5-μl volume containing 1–10 ng of template DNA and 1× QIAGEN Multiplex PCR Master Mix (Qiagen, Hilden, Germany). The PCR protocol consisted of a 15-min pre-PCR heat step at 95°C, followed by 32 cycles of denaturation at 94°C for 30 s, annealing at 57°C for 90 s, and extension at 72°C for 1 min, with a final 30-min extension step at 60°C.

Typing was done by capillary electrophoresis on the ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) in comparison to allelic ladders and control DNA from K562 and 9947A cell lines (Promega, Madison, WI) [15] using the GeneScan and Genotyper software version 3.7 (Applied Biosystems).

Sequencing of DXS10148 alleles was performed with the Big Dye Terminator Sequencing kit (Applied Biosystems), using the unlabelled multiplex PCR primers.

Arlequin software [16] was used to perform exact test of differentiation (100,000 Markov steps) between male and female allele frequencies, test of Hardy–Weinberg equilibrium (HWE) in the female subsample, test of LD between pairs of markers within clusters of linked loci in the male subsample, and to calculate locus by locus population pairwise genetic distances (F_{ST}) between northwestern Algerians and other population samples drawn from the literature. In order to summarize the relationship between Algerians and reference populations, principal component analysis (PCA) was performed by using the R-package software v2.0.1 (http://www.r-project.org).

The following statistical parameters of forensic interest were calculated using the online functions provide by the ChrX-STR.org 2.0 database (http://www.chrx-str.org) [15]: expected heterozygosity (h) [17], polymorphism information content (PIC) [18], power of discrimination in females (PD^F) and males (PD^M) [19], mean exclusion chance for autosomal markers in trios (MECI) [20], X-STRs in trios with daughters (MECII) [21], and X-STRs in father/daughter duos (MECIII) [19].

Results and discussion

Since the three markers DXS8378, DXS7132, and HPRTB were amplified both in the multiplex PCR reactions developed in-house and the Argus X-8 system, a partial concordance study between the two assays was performed. Genotype incompatibilities at STR loci between samples typed with different PCR primers are indeed possible, due to primer binding site mutations [22], and were previously described also for X-STRs [13]. In this case, full genotype

Table 1 Allele frequencies of X-STR loci in northwestern Algerian population (316 chromosomes) and *P* values for HWE (standard error ± 0.001)

Allele	DXS10148	DXS10135	DXS8378	DXS7132	DXS10074	DXS10079	DXS6800	DXS6801	DXS6809	DXS6789	DXS7424	DXS101	GATA172D05	DXS10103	HPRTB	DXS10101	DXS8377	DXS1046	DXS10134	DXS7423	DXS10011
5													.006								
6													.184								
7					.041								.006								
8					.193								.193								
9			.009		.032			.013					.101		.013						
10			.335		.016			.054			.006		.253		.013						
11			.345	.032	.006			.519			.016		.184		.117						
12[a]															.003						
12			.275	.111	.009			.250			.032		.073		.373						
12.1		.006																			
13			.028	.301	.022			.136			.101				.269					.038	
14	.022		.006	.380	.038	.003		.028		.009	.146	.003		.003	.146					.332	
15		.006		.139	.079	.038	.003			.111	.285	.009		.009	.066					.421	
16		.003		.038	.149	.016	.313			.032	.291			.111						.193	
16.1					.003																
16.2					.003																
17	.038	.016			.209	.063	.044			.025	.101	.006		.089						.016	
17.1		.022																			
17.3					.003																
18	.149	.063			.130	.180	.165				.019	.098		.190		.003					
18.1		.006																			
18.2														.006							
18.3						.006	.367														
19	.025	.092			.057	.294				.041		.067		.415							
19.1		.025																			
19.3						.019															
20	.019	.047			.009	.231	.032			.329	.003	.022		.146							
20.1		.028																			
21	.003	.089				.098	.073			.168		.054		.025							.003
21.1	.006	.019																			
22	.006	.054				.044				.184		.057		.006							
22.1	.019	.022																			
23	.041	.054				.006	.003			.095		.060						.003			
23.1	.028	.038																			
24	.003	.089							.003	.006		.161						.009			
24.1	.108	.006																			
24.2																.016					
25	.003	.054										.168				.003		.044			
25.1	.123	.009																			
25.2																.003					
26		.038										.139				.006		.111			.003
26.1	.165																				
27	.006	.038							.006			.079				.006		.146			.013
27.1	.085																				
27.2																.028					
28	.003	.070							.006			.063				.057		.101			.028
28.1	.051																				

Table 1 (continued)

Allele	DXS10148	DXS10135	DXS8378	DXS7132	DXS10074	DXS10079	DXS6800	DXS6801	DXS6809	DXS6789	DXS7424	DXS101	GATA172D05	DXS10103	HPRTB	DXS10101	DXS8377	DXS1046	DXS10134	DXS7423	DXS10011
28.2																.051		.006			.006
29	.006	.019							.044			.006				.054		.130			.025
29.1	.019																				
29.2																.133		.028			.025
30		.025							.104			.003				.076		.073	.006		.025
30.1	.006	.003																			
30.2																.139					.044
31		.009							.139							.079		.051	.019		.038
31.1	.009																				
31.2																.158					.047
31.3																.003					
32		.006							.130			.003				.085		.022	.016		.025
32.1	.003																				
32.2																.025					.041
33		.019							.253							.044		.016	.025		.047
33.2																.009					.025
34		.009							.180							.019		.013	.111		.060
34.1		.003																			
34.2									.003									.009			.006
35		.006							.092										.237		.044
35.2																		.019			.013
36									.029										.206		.073
36.1	.006																				
36.2																		.003			.003
36.3																			.003		
37		.003							.009								.003		.161		.079
37.1	.009																				
37.2																		.009			.003
37.3																			.006		
38																	.006		.082		.070
38.1	.013																				
38.2																		.006			
38.3																			.006		
39																	.013		.032		.054
39.1	.006																				.003
39.2																		.013			
39.3																			.009		
40																	.009		.003		.063
40.1	.003																				
40.2		.003																.044			
40.3																			.032		
41																	.016		.003		.035
41.1	.009																				
41.2																		.016			
41.3																		.003	.019		
42																	.019				.028
42.1	.003																		.003		

Table 1 (continued)

Allele	DXS10148	DXS10135	DXS8378	DXS7132	DXS10074	DXS10079	DXS6800	DXS6801	DXS6809	DXS6789	DXS7424	DXS101	GATA172D05	DXS10103	HPRTB	DXS10101	DXS8377	DXS1046	DXS10134	DXS7423	DXS10011
42.2																		.016			
42.3																			.019		
43																	.054				.032
43.2																		.057			
44																	.013				.013
44.2																		.022			
45																	.057				.013
45.2																		.016			
46																	.070				.003
46.2																		.009			
47																	.117				.006
47.2																		.003			
48																	.130				
49																	.117				
50																	.079				
51																	.117				
52																	.060				
53																	.054				
54																	.028				
55																	.019				
56																	.016				
57																	.003				
P	.307	.187	.257	.163	.223	.141	.562	.963	.016	.757	.354	.111	.041	.036	.916	.823	.706	.035	.830	.921	.034

[a] Apparent intermediate allele with an equivalent size to 11.2 repeats due to an AG deletion at bases 48 and 49 downstream from the repeat unit (D48AGdel)

concordance was observed for overlapping loci amplified with different PCR systems.

No significant deviation in the allelic distribution of X-STRs between the male and female subsamples was seen by exact test, after Bonferroni correction for multiple testing. Allele frequencies for the 21 X-STRs, combined over both sexes, are therefore reported in Table 1. Based on the observed and expected distribution of genotypes in the female subsample, all the markers were found to be in HWE, after Bonferroni correction for multiple testing. As it can be seen in Table 1, at locus DXS10148, about 5% of the analyzed chromosomes showed the presence of alleles characterized by long repeat motifs (> 36), previously undetected in the large German sample described by Hundertmark et al. [4]. All the > 36 alleles found in males were sequenced. Among them, some showed a repeat structure that was slightly different from the one reported by Hundertmark et al. [4]; as it can be seen in Table 2, one out of three of the sequenced alleles 38.1 shows an additional $[AGGA]_3$ repeat block in between of the $[AAGA]_x$ repeat units; moreover, both of the sequenced 41.1 alleles showed an A > T substitution in a single repeat unit of the $[AAGA]_x$ block. This observation prompted us to completely resequence at least one sample for each allelic variant observed in the hemizygous male subsample. As shown in Table 2, variability both in the position of the adenine insertion giving rise to intermediate alleles and in the repeat unit structure could be seen. Moreover, alleles having the same size but different repeat arrays were also found. The complex structure found at locus DXS10148 accounts for the rather large deviations in size (> ± 0.5 bp) observed for some of the typed alleles versus the expected

Table 2 Repeat structure of DXS10148 in a subset of alleles sequenced in the Algerian population sample compared with reference structure as described by Hundertmark et al. [4]

Allele	Repeat structure	Number of sequenced alleles
14	P_F-[GGAA]$_4$-[AAAG]$_6$-N_8-[AAGG]$_2$-[*AAAG*]-[*AAGG*]-N_{104}-P_R	1
17	P_F-[GGAA]$_4$-[AAGA]$_7$-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	1
18	P_F-[GGAA]$_4$-[AAGA]$_8$-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	2
19	P_F-[GGAA]$_4$-[AAGA]$_9$-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	1
20.1	P_F-[GGAA]$_4$-[AAGA]$_{11}$-A^a-[AAAG]$_3$-N_8-[AAGG]$_2$–N_{104}-P_R	1
21	P_F-[GGAA]$_4$-[AAGA]$_{11}$-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	1
22.1	P_F-[GGAA]$_4$-[AAGA]$_{13}$-A^a-[AAAG]$_3$-N_8-[AAGG]$_2$–N_{104}-P_R	2
23	P_F-[GGAA]$_4$-[AAGA]$_{11}$-[AAAG]$_4$-N_8-[AAGG]$_2$-[*AAAG*]-[*AAGG*]–N_{104}-P_R	1
23.1	P_F-[GGAA]$_4$-[AAGA]$_{12}$-*A*^{a}-[AAGA]-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	1
24.1	P_F-[GGAA]$_4$-[AAGA]$_{15}$-A^a-[AAAG]$_3$-N_8-[AAGG]$_2$–N_{104}-P_R	1
24.1	P_F-[GGAA]$_4$-[AAGA]$_{13}$-*A*^{a}-[AAGA]-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	1
25.1	P_F-[GGAA]$_4$-[AAGA]$_{16}$-A^a-[AAAG]$_3$-N_8-[AAGG]$_2$–N_{104}-P_R	2
26.1	P_F-[GGAA]$_4$-[AAGA]$_{17}$-A^a-[AAAG]$_3$-N_8-[AAGG]$_2$–N_{104}-P_R	3
26.1	P_F-[GGAA]$_4$-[AAGA]$_{15}$-*A*^{a}-[AAGA]-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	1
27.1	P_F-[GGAA]$_4$-[AAGA]$_{18}$-A^a-[AAAG]$_3$-N_8-[AAGG]$_2$–N_{104}-P_R	1
28	P_F-[GGAA]$_4$-[AAGA]$_{16}$-[AAAG]$_4$-N_8-[AAGG]$_2$-[*AAAG*]-[*AAGG*]-N_{104}-P_R	1
28.1	P_F-[GGAA]$_4$-[AAGA]$_{17}$-*A*^{a}–[AAGA]-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	2
28.1	P_F-[GGAA]$_4$-[AAGA]$_{19}$-A^a-[AAAG]$_3$-N_8-[AAGG]$_2$–N_{104}-P_R	1
29	P_F-[GGAA]$_4$-[AAGA]$_{17}$-[AAAG]$_4$-N_8-[AAGG]$_2$-[*AAAG*]-[*AAGG*]-N_{104}-P_R	1
29.1	P_F-[GGAA]$_4$-[AAGA]$_{18}$-*A*^{a} -[AAGA]-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	2
31.1	P_F-[GGAA]$_4$-[AAGA]$_{20}$-*A*^{a} -[AAGA]-[AAAG]$_4$-N_8–[AAGG]$_2$–N_{104}-P_R	1
31.1	P_F-[GGAA]$_4$-[*GGAG*]$_3$-*A*^{a}-[*AGGA*]$_{16}$-[*AGAG*]$_3$–[AAAG]-N_8-[AAGG]$_4$–N_{104}-P_R	1
36.1	P_F-[GGAA]$_4$-[AAGA]$_{14}$-*A*^{a}-[AAGA]$_{12}$-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	1
37.1	P_F-[GGAA]$_4$-[AAGA]$_{13}$-*A*^{a}-[AAGA]$_{14}$-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	1
38.1	P_F-[GGAA]$_4$-[AAGA]$_{13}$-*A*^{a}-[AAGA]$_{15}$-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	2
38.1	P_F-[GGAA]$_4$-[AAGA]$_{12}$-*A*^{a}-[*AGGA*]$_3$-[AAGA]$_{13}$-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	1
41.1	P_F-[GGAA]$_4$-[AAGA]$_{17}$-*A*^{a}-[AAGA]$_9$-[*TAGA*]-[AAGA]$_4$-[AAAG]$_4$-N_8-[AAGG]$_2$–N_{104}-P_R	2
Reference structure		
	P_F-[GGAA]$_4$-[AAGA]$_X$-A^a-[AAAG]$_Y$-N_8-[AAGG]$_2$–N_{104}-P_R	

Differences in the position of the adenine insertion and in the repeat unit structure are shown in italics

[a] Insertion giving rise to intermediate alleles

amplicon length, based on sequenced control cell lines, as shown in Table S3. In order to better understand the degree of variability in the molecular structure of DXS10148, possibly clarify its evolutionary meaning, and define a consensus nomenclature [23], further collection of frequency distribution and sequencing data in other populations (e.g., sub-Saharan Africans, Asians) are needed.

Statistical parameters of forensic interest calculated for the 21 X-STR loci are shown in Table S4.

In the male subsample, no significant LD was observed between pairs of markers located within the six clusters of linked loci. The p values of pairwise test of LD, total number of observed haplotypes, and the allelic combination of haplotypes observed in each cluster with frequency $n>1$ are given in Table S5.

Locus by locus population pairwise genetic distances (F_{ST}) between northwestern Algerians and relevant population samples (Angola, Mozambique, Uganda, Ghana, Italy, Portugal, Spain, Kurdistan, and Pakistan) [10, 24–29] are shown in Table S6. As it can be seen, most of the significant F_{ST} values were found in comparison with sub-Saharan African populations. The PCA plot synthesizing the relationship between populations, based on a subset of four unlinked loci (DXS8378, DXS7132, DXS101, and DXS8377) whose allele frequencies were available for each population sample, is shown in Fig. S1. In the plot, Algerians cluster with other Eurasian populations and they are clearly separated from sub-Saharan Africans. These observations are in agreement with a previous study of X-chromosomal single-nucleotide polymorphisms suggesting a high overall genetic homogeneity for the X chromosome in the Mediterranean populations [30].

The obtained results provide the forensic genetics community with a detailed database of X-STR allele and haplotype diversity in northern Africa, to be used in deficiency paternity cases and complex kinship testing. However, since precise information regarding linkage and linkage disequilibrium status of X-linked markers must be taken in account in likelihood calculations, further studies both at family and population level are still required [9].

In the future, the collected data may also prove useful in the population genetics field. The simultaneous analysis of many independent X-STR haplotypes can indeed complement phylogenetic data from mitochondrial DNA (mtDNA) and Y chromosome studies [31]. Moreover, due to a larger effective population size compared with mtDNA and the Y chromosome, analysis of X-chromosomal variability potentially allows to investigate events in human genetic history much older than those described by haploid markers [32].

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