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SHORT COMMUNICATION

Allele frequencies of 11 X-chromosomal loci of two population samples from Africa

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Abstract Eleven X-chromosomal STRs from two multiplex PCR approaches (DXS6807, DXS8378, DXS7132, DXS6800, DXS9898, DXS7424, DXS101, DXS7133, HPRTB, DXS8377, and DXS7423), located in four different X-chromosomal linkage groups, were typed in two population samples from Africa, Morocco, and Madagascar. Forensic efficiency parameters such as polymorphism information content and mean exclusion chance were calculated. A deviation from the Hardy–Weinberg equilibrium could not be found. The investigation of four father–daughter and five mother–son meioses (from Morocco) revealed no mutations in any STR analyzed. Our data were compared with European, African-American, and Asian populations from the literature.

Keywords X chromosome · STR · Multiplex PCR · Population data · Morocco · Madagascar

Introduction

The analysis of X-chromosomal STRs is especially useful in complex kinship testing, when the offspring is female and the alleged father is missing, or in maternity analyses [1]. After an intensive analysis of forensically interesting microsatellite repeats of the human X chromosome in European countries [for example 2–12], the interest shifted to African populations in recent years. Data from larger African populations for X-chromosomal STRs have been published for an Ethiopian population [13], in one investigation including an Afro-American population [14], in another study from the same working group comprising populations from Angola, Mozambique, and Uganda [15], as well as the Karinojong from Uganda [16], in a Somali population from Finland [17], and in our recent study on Ghana [18]. Greater differences in microsatellites and mtDNA between African, Asian, and European populations have been reported [for example 19, 20], showing the importance for further investigations and data collection. Thus also differences in allele frequencies of X-chromosomal STRs could be expected. Especially in paternity analysis, it can be crucial to use frequency data from associated/related populations to obtain reliable results.

Here, we present allele frequencies for 11 X-chromosomal STRs from two African populations, i.e., Morocco and Madagascar.

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Material and methods

Population

Buccal swabs were collected from 50 men and 50 women from Morocco (predominately born and living in Marrakesh) and from 107 men and 62 women from Madagascar. Samples were obtained and analyzed after advice of the Medical Ethics Committees of the University of Duisburg-

Table 1 Allele frequencies for the X-chromosomal STRs DXS6807, DXS8378, DXS7132, DXS6800, DXS9898, DXS7424, DXS101, DXS7133, HPRTB, DXS8377, and DXS7423 in Morroco (n=150 X chromosomes)

DXS6807		DXS8378		DXS7132		DXS6800		DXS9898		DXS7424		DXS101		DXS7133		HPRTB		DXS8377		DXS7423	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
11	0.5267	10	0.1733	11	0.0467	16	0.3533	8.3	0.14	11	0.0067	17	0.02	8	0.0133	10	0.02	41	0.0267	13	0.06
12	0.0133	11	0.3133	12	0.06	17	0.0533	10	0.1	12	0.0267	18	0.1067	9	0.22	11	0.1133	42	0.02	14	0.3467
13	0.0067	12	0.4267	13	0.1267	18	0.14	11	0.16	13	0.0733	19	0.0467	10	0.1733	12	0.3333	43	0.0333	15	0.3867
14	0.2067	13	0.0667	14	0.3467	19	0.3733	12	0.4667	14	0.0733	20	0.02	11	0.54	13	0.2467	44	0.0667	16	0.16
15	0.1933	14	0.02	15	0.2733	20	0.0067	13	0.08	15	0.28	21	0.08	12	0.04	14	0.1933	45	0.0467	17	0.0267
16	0.0533			16	0.0667	21	0.0533	14	0.0476	16	0.3133	22	0.08	13	0.0133	15	0.08	46	0.0333	18	0.02
				17	0.06	22	0	15	0.0067	17	0.14	23	0.04			16	0.0133	47	0.1867		
				18	0.02	23	0.02			18	0.0533	24	0.14					48	0.04		
										19	0.0067	25	0.18					49	0.1067		
										20	0.0267	26	0.1533					50	0.12		
												27	0.0933					51	0.0733		
												28	0.0067					52	0.06		
												29	0.0267					53	0.0867		
												30	0.0067					54	0.04		
																		55	0.0067		
																		56	0.0467		
																		57	0.0067		
PIC	0.59		0.629		0.745		0.661		0.69		0.76		0.876		0.578		0.736		0.901		0.647
MEC_T	0.59		0.629		0.745		0.661		0.68		0.76		0.876		0.578		0.736		0.901		0.647
HET_{exp}	0.639		0.685		0.775		0.71		0.72		0.788		0.886		0.628		0.771		0.907		0.7
HET_{obs}	0.56		0.6		0.6		0.68		0.68		0.74		0.86		0.48		0.76		0.94		0.7
PE	0.341		0.405		0.553		0.444		0.46		0.578		0.767		0.326		0.546		0.811		0.428
PD_f	0.82		0.845		0.919		0.867		0.892		0.927		0.976		0.811		0.912		0.985		0.857
PD_m	0.639		0.685		0.775		0.71		0.72		0.789		0.886		0.628		0.771		0.907		0.7

Freq frequency, *PIC* polymorphism information content, MEC_T mean exclusion chance in trios, HET_{exp} expected heterozygosity, HET_{obs} observed heterozygosity, *PE* power of exclusion, PD_f power of discrimination in females, PD_m power of discrimination in males

Table 2 Allele frequencies for the X-chromosomal STRs DXS6807, DXS8378, DXS7132, DXS6800, DXS9898, DXS7424, DXS101, DXS7133, HPRTB, DXS8377, and DXS7423 in Madagascar (n=231 X chromosomes)

DXS6807		DXS8378		DXS7132		DXS6800		DXS9898		DXS7424		DXS101		DXS7133		HPRTB		DXS8377		DXS7423	
Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq	Allele	Freq
11	0.5108	9	0.013	11	0.0043	16	0.4935	6.3	0.0087	10	0.0173	17	0.0043	9	0.303	6	0.0043	40	0.0043	13	0.0693
12	0.0303	10	0.355	12	0.1255	17	0.0606	8.3	0.1212	11	0.0519	18	0.0087	10	0.2727	9	0.0043	41	0.0216	14	0.4416
13	0.1082	11	0.2727	13	0.1558	18	0.2165	9	0.0043	12	0.0346	19	0.026	11	0.3636	11	0.1342	42	0.0433	15	0.4069
14	0.1861	12	0.303	14	0.2251	19	0.0563	10	0.0216	13	0.1558	20	0.0649	12	0.039	12	0.3377	43	0.0433	16	0.0823
15	0.1039	13	0.0563	15	0.4113	20	0.0952	11	0.1472	14	0.1602	21	0.1342	13	0.0173	13	0.3593	44	0.0909		
16	0.0346			16	0.0563	21	0.0519	12	0.4502	15	0.1905	22	0.0779	14	0.0043	14	0.1039	45	0.1688		
17	0.026			17	0.0173	22	0.026	13	0.1775	16	0.3377	23	0.1385			15	0.0519	46	0.1082		
				18	0.0043			14	0.0693	17	0.0346	24	0.1905			16	0.0043	47	0.0519		
										18	0.0087	25	0.1082					48	0.039		
										19	0.0043	26	0.1212					49	0.0476		
										20	0.0043	27	0.0823					50	0.0823		
												28	0.0346					51	0.0693		
												29	0.0087					52	0.0996		
																		53	0.0476		
																		54	0.0563		
																		55	0.0087		
																		56	0.0013		
																		58	0.0043		
PIC	0.646		0.647		0.699		0.656		0.691		0.767		0.869		0.641		0.692		0.908		0.555
MEC_T	0.646		0.647		0.699		0.656		0.691		0.767		0.869		0.641		0.668		0.895		0.555
HET_{exp}	0.679		0.704		0.737		0.69		0.724		0.794		0.881		0.7		0.723		0.914		0.628
HET_{obs}	0.613		0.661		0.645		0.484		0.597		0.774		0.823		0.741		0.778		0.855		0.742
PE	0.397		0.435		0.487		0.416		0.467		0.588		0.757		0.428		0.742		0.824		0.326
PD_f	0.864		0.855		0.893		0.87		0.891		0.931		0.974		0.851		0.871		0.986		0.789
PD_m	0.679		0.704		0.737		0.69		0.724		0.794		0.881		0.7		0.717		0.913		0.628

Freq frequency, *PIC* polymorphism information content, MEC_T mean exclusion chance in trios, HET_{exp} expected heterozygosity, HET_{obs} observed heterozygosity, *PE* power of exclusion, PD_f power of discrimination in females, PD_m power of discrimination in males

Table 3 Frequency of DXS101-DXS7424 haplotypes in the two populations from Morocco and Madagascar

DXS101	DXS7424	Frequency in Morocco (%)	Frequency in Madagascar (%)
17	17	1 (2%)	0
18	13	1 (2%)	0
18	15	1 (2%)	0
18	16	1 (2%)	0
18	17	2 (4%)	0
19	13	1 (2%)	1 (0.9%)
19	14	0	2 (1.9%)
19	16	1 (2%)	1 (0.9%)
19	17	1 (2%)	0
20	10	0	1 (0.9%)
20	13	1 (2%)	3 (2.8%)
20	15	0	3 (2.8%)
20	18	1 (2%)	0
21	12	0	1 (0.9%)
21	13	0	1 (0.9%)
21	14	1 (2%)	1 (0.9%)
21	15	1 (2%)	6 (5.6%)
21	16	2 (4%)	5 (4.7%)
22	13	1 (2%)	1 (0.9%)
22	14	1 (2%)	0
22	15	1 (2%)	0
22	16	2 (4%)	7 (6.5%)
23	13	0	1 (0.9%)
23	14	0	4 (3.7%)
23	15	1 (2%)	5 (4.7%)
23	16	0	4 (3.7%)
24	11	0	1 (0.9%)
24	13	0	1 (0.9%)
24	14	0	5 (4.7%)
24	15	4 (8%)	2 (1.9%)
24	16	1 (2%)	8 (7.5%)
24	17	1 (2%)	3 (2.8%)
24	18	1 (2%)	1 (0.9%)
25	11	0	1 (0.9%)
25	12	1 (2%)	0
25	13	1 (2%)	7 (6.5%)
25	14	0	1 (0.9%)
25	15	3 (6%)	0
25	16	3 (6%)	3 (2.8%)
26	12	1 (2%)	1 (0.9%)
26	13	0	2 (2.8%)
26	14	1 (2%)	1 (0.9%)
26	15	3 (6%)	0
26	16	3 (6%)	6 (5.6%)
26	17	1 (2%)	1 (0.9%)
27	11	0	3 (2.8%)
27	12	0	1 (0.9%)
27	13	0	1 (0.9%)
27	14	0	1 (0.9%)
27	15	2 (4%)	1 (0.9%)
27	16	2 (4%)	4 (3.7%)
28	14	0	2 (1.9%)
28	15	0	3 (2.8%)
29	15	1 (2%)	0

Essen and University of Schleswig-Holstein in accordance with the declaration of Helsinki. The anonymity of the individuals investigated was preserved corresponding to the rules of data protection of the Human Medical Faculties Essen and Kiel. Every person investigated gave informed consent. Additionally, four girls and five boys have been analyzed, whose parents were included in the 41 males and 32 females from Morocco and gave permission for investigation.

DNA extraction

DNA was extracted from buccal swabs using the innuPrep DNA Mini Kit (Analytik Jena, Jena, Germany) and the Qiagen Blood Mini Kit (Qiagen, Hilden, Germany).

DNA amplification and fragment analysis

Two hexaplex amplification reactions were performed as described previously [18]. The first comprised the STRs DXS6807, DXS7132, DXS6800, DXS9898, DXS101, and HPRTB; the second included the STRs DXS8378, DXS7132, DXS7424, DXS7133, and DXS8377. All primer sequences have been taken from http://www.chrx-str.org/. DXS7132 was included in both hexaplex amplification reactions (with the same primer sequences) as an internal control against mix-up of samples. PCR fragments were analyzed using an ABIPrsim310 Genetic Analyzer (Applied Biosystems, Weiterstadt, Germany), and fragment sizes were determined using the Genemapper ID v3.2 software with especially self-defined panels and bin sets. The ladder was composed of cell line DNAs and individuals from a German population as described before [18]. Allelic nomenclature used for typing was mostly according to Szibor et al. [21, 22] with the exception of DXS9898 in cell line 9948. Here, we found 13 repeats instead of 14 repeats, which is in line with results from Gomes et al. [14].

Table 4 Genetic distances between the population from Morocco and European, Asian, and African populations (F_{ST} values)

		Madagascar [this study]	Ghana [18]	Afro-American [14]	Angola [15]	Mozambique [15]	Uganda [15]	Karimojong [16]	Somali [17]	Korea [28]	Spain [2]	Portugal [5]	Germany [4]	Latvia [7]
DXS6807	F_{ST}	0.00962	0.01969	ND	ND	ND	ND	ND	ND	0.03052	ND	ND	−0.00177	0.00335
	p value	0.06306±0.0237	0							0			0.65766±0.0430	0.15315±0.0139
DXS8378	F_{ST}	0.02972	0.01105	0.01058	0.00214	0.01306	0.00236	0.00869	0.02752	0.31094	0.01871	0.00717	0.02727	0.03915
	p value	0	0.03604±0.0148	0.14414±0.0242	0.31532±0.0474	0.02703±0.0139	0.27027±0.0429	0.05405±0.0201	0	0	0	0.09009±0.0235	0	0
DXS7132	F_{ST}	0.02238	0.01653	0.01279	0.00356	−0.00060	0.05798	0.03092	0.00125	0.00941	0.01580	0.02116	0.01611	ND
	p value	0	0.00901±0.0091	0.02703±0.0139	0.25225±0.0227	0.45045±0.0271	0	0	0.19820±0.0264	0.00901±0.0091	0	0	0	
DXS6800	F_{ST}	0.08314	0.19331	ND	ND	ND	ND	ND	ND	0.33592	ND	ND	0.00245	ND
	p value	0	0							0			0.13514±0.0339	
DXS9898	F_{ST}	0.00623	0.00550	0.00910	0.00031	0.02240	0.03658	0.00456	ND	0.04997	0.04905	0.03725	0.03637	0.03246
	p value	0.04505±0.0203	0.07207±0.0182	0.04505±0.0152	0.39640±0.0388	0.02703±0.0139	0	0.05405±0.0309		0	0	0	0	0
DXS101	F_{ST}	0.01399	0.02466	0.01621	0.01307	0.02521	0.00459	ND	ND	0.02505	0.00614	0.00245	0.00794	0.00926
	p value	0	0	0	0.03604±0.0148	0	0.22523±0.0529			0	0.04505±0.0152	0.14414±0.0473	0	0.00901±0.0091
DXS7133	F_{ST}	0.02924	0.03291	ND	ND	ND	ND	0.04524	ND	ND	ND	ND	0.07256	0.03697
	p value	0	0					0		0			0	0
HPRTB	F_{ST}	0.01388	0.00613	−0.00253	0.00526	−0.00435	0.01204	ND	0.00271	0.04547	0.01996	0.00744	0.00377	0.01039
	p value	0.01802±0.0121	0.08108±0.0316	0.60360±0.0508	0.19820±0.0402	0.70270±0.0466	0.09009±0.0271		0.13514±0.0244	0	0.00901±0.0091	0.03604±0.0201	0	0.02703±0.0194
DXS8377	F_{ST}	0.02104	0.00503	0.01101	0.01013	0.01296	0.02124	ND	ND	0.00921	0.00901	0.00578	0.01550	0.01608
	p value	0	0	0	0.01802±0.0121	0.00901±0.0091	0			0	0	0.02703±0.0194	0	0
DXS7423	F_{ST}	0.00708	0.02886	0.03897	0.01923	0.02208	0.00767	0.00256	0.01184	0.03835	−0.00075	−0.00355	−0.00263	ND
	p value	0.07207±0.0297	0	0	0.03604±0.0201	0.00901±0.0091	0.15315±0.0305	0.18919±0.0394	0.00901±0.0091	0	0.36937±0.0370	0.79279±0.0265	0.70270±0.0265	

p values less than 0.05 were considered statistically significant

ND not done

Table 5 Genetic distances between the population from Madagascar and European, Asian, and African populations (F_{ST} values)

		Morocco [this study]	Ghana [6]	Afro-American [14]	Angola [15]	Mozambique [15]	Uganda [15]	Karimojong [16]	Somali [17]	Korea [28]	Spain [2]	Portugal [5]	Germany [4]	Latvia [7]
DXS6807	F_{ST}	0.00962	0.02028	ND	ND	ND	ND			0.04024	ND	ND	0.01376	0.00662
	p value	0.06306±0.0237	0							0			0	0.06306±0.0194
DXS8378	F_{ST}	0.02972	0.01326	0.02429	0.02025	0.00088	0.01627	0.02846	0.00042	0.25514	0.00286	0.00486	0.00247	−0.00162
	p value	0	0.00901±0.0091	0	0.01802±0.0121	0.28829±0.0539	0.09910±0.0212	0	0.28829±0.0402	0	0.19820±0.0264	0.09910±0.0252	0.13514±0.0365	0.54054±0.0606
DXS7132	F_{ST}	0.02238	0.04704	0.04843	0.03633	0.03054	0.07454	0.03591	0.02489	0.03019	0.03826	0.04851	0.04745	ND
	p value	0	0	0	0.00901±0.0091	0	0	0	0	0	0	0	0	
DXS6800	F_{ST}	0.08314	0.16766	ND	ND	ND	ND			0.19693	ND	ND	0.07161	ND
	p value	0	0							0			0	
DXS9898	F_{ST}	0.00623	0.00988	0.00724	0.00281	0.01544	0.03240	0.00396		0.02061	0.03705	0.03176	0.02898	0.03043
	p value	0.04505±0.0203	0	0.05405±0.0242	0.18919±0.0370	0	0	0.02703±0.0194		0	0	0	0	0
DXS101	F_{ST}	0.01399	0.01573	0.01652	0.00937	0.00712	−0.00149			0.02309	0.02240	0.01312	0.01662	0.02797
	p value	0	0	0	0.03604±0.0201	0.00901±0.0091	0.48649±0.0364			0	0	0	0	0
DXS7133	F_{ST}	0.02924	0.08758	ND	ND	ND	ND	0.14176		ND	ND	ND	0.03063	0.00538
	p value	0	0					0					0	0.06306±0.0194
HPRTB	F_{ST}	0.01388	0.01989	0.02896	0.01862	0.00032	0.02697		0.01499	0.02185	0.04330	−0.00370	0.00318	−0.00455
	p value	0.01802±0.0121	0	0	0.05405±0.0201	0.44144±0.0438	0.00901±0.0091		0	0.01802±0.0121	0	0.84685±0.0338	0.14414±0.0364	0.81982±0.0345
DXS8377	F_{ST}	0.02104	0.01157	0.00726	0.00461	0.00652	0.01211			0.01282	0.00685	0.01768	0.01241	0.02070
	p value	0	0	0.01802±0.0182	0.11712±0.0333	0.02703±0.0139	0.02703±0.0139			0	0	0	0	0
DXS7423	F_{ST}	0.00708	0.01433	0.02140	0.00701	0.00397	0.01428	0.00067	0.00096	0.03274	0.01769	0.00490	0.01448	ND
	p value	0.07207±0.0297	0.00901±0.0091	0	0.13514±0.0152	0.21622±0.0338	0.14414±0.0201	0.27928±0.0550	0.21622±0.0364	0	0.00901±0.0091	0.09009±0.0332	0	

p values less than 0.05 were considered statistically significant

ND not done

Statistical analysis

Allele frequencies, haplotype and gene diversities, exact test of population differentiation population pairwise genetic distances F_{ST}, Hardy–Weinberg equilibrium in females, analysis of molecular variance, and pairwise exact test of linkage disequilibrium were all performed using the Arlequin ver. 3.0 software [23]. Genetic distance analysis was performed for all X-STRs using F_{ST}, since comparison of F_{ST} and R_{ST} values in sub-Saharan African populations studied by Caglia et al. [24] showed a better performance for F_{ST} values in an African context. Forensic efficiency parameters were assessed for each locus following Desmarais et al. [25].

Results and discussion

Genotyping of both population samples for the 11 X-chromosomal STRs resulted in allele frequencies and forensic efficiency parameters as shown in Tables 1 and 2. DXS6807 and DXS7133 were the least informative markers in the population sample from Morocco, DXS6800 and DXS7423 in the population sample from Madagascar. DXS101 and DXS8377 demonstrated the highest values in both population samples. Combined forensic efficiency parameters were calculated for all STRs with the exception of DXS7424, for which linkage disequilibrium to DXS101 has previously been detected [26], as follows: combined power of discrimination for males 0.9999999 (Morocco) and 0.999999 (Madagascar), combined power of discrimination for females 0.999999999 (Morocco) and greater than 0.999999999 (Madagascar), and combined mean exclusion chance for trios 0.9999996 (Morocco) and 0.999991 (Madagascar). The frequency of DXS101–DXS7424 haplotypes is shown in Table 3 for both population samples. Linkage disequilibrium between DXS101 and DXS7424 has not been tested for our samples because the number of investigated individuals was too small. The investigation of four father–daughter and five mother–son meioses from Morocco revealed no mutations in any STR analyzed in this study.

In the locus DXS9898 the allelic repeat 6.3 was detected and verified via sequencing analysis. Concerning the locus HPRTB, different nomenclatures have been used [for example 2, 15, 21]. This issue has been addressed in a recent letter by Szibor et al. [22]. They proposed the use of the old nomenclature—resulting in a homozygote 14 in the cell line NA9947A, since this nomenclature is already established in the forensic field, even if it is not exactly in accordance with the ISFG nomenclature recommendations. Therefore, we employed the old nomenclature and adjusted the results from studies using the other nomenclature for comparison purposes.

Genetic distances were estimated for each STR marker with the exception of DXS7424—due to the linkage disequilibrium to DXS101—to different European [2, 4, 5, 7, 27], Asian [28], Afro-American [14], and African [15–18] populations as available (see Tables 4 and 5 for all results). Significant genetic distances are distributed rather heterogeneous between different STRs and different populations. European and Asian influences seem to be much higher in Morocco and in Madagascar than in other African populations.

The X-chromosomal STRs employed in this work proved to be highly discriminating. Combined values of forensic efficiency parameters were high in the population samples from Morocco and Madagascar. Therefore, the two hexaplex assays showed to be very useful for identification studies involving people from Africa, for example in applications of political asylum in complex deficiency cases. These investigations can be rather difficult, since in many cases the analysis has to be done only with two persons, e.g., father and child. In such deficiency cases, it could be shown that the possibility to include a relative of the biological father or even a nonrelated man as father is not to be neglected [29, 30].

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