TECHNICAL NOTE

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Genetic Variation at the Short Tandem Repeat Loci HumvWA, HumFXIIIB, and HumFES/FPS in the Egyptian and Yemenian Populations*

REFERENCE: Klintschar M, Al Hammadi N, Lux T, Reichenpfader B. Genetic variation at the short tandem repeat loci HumvWA, HumFXIIIB, and HumFES/FPS in the Egyptian and Yemenian populations. J Forensic Sci 1998;43(4):850–853.

ABSTRACT: The short tandem repeat systems (STRs) HumvWA, HumFXIIIB, and HumFES/FPS were amplified in a triplex polymerase chain reaction (PCR) on blood samples from 100 unrelated Yemenians and 100 unrelated Egyptians. The samples were analyzed by native horizontal discontinual electrophoresis. No deviations from Hardy-Weinberg equilibrium were detected. The mean exclusion chances for Egyptians and Yemenians were 0.634 and 0.591 (vWA), 0.530 and 0.531 (FXIIIB), and 0.573 and 0.583 (FES); the discriminating powers were 0.937 and 0.924 (vWA), 0.900 and 0.899 (FXIIIB), and 0.918 and 0.921 (FES); and the observed heterozygosity rates were 0.84 and 0.72 (vWA), 0.73 and 0.83 (FXIIIB), and 0.81 and 0.80 (FES). No significant differences were found between the two Arab populations, but the differences between both Arab populations and a European population for HumFES and FXIIIB and between the Yemenian sample and a European sample for vWA were significant. No evidence of linkage disequilibrium between any of the three STRs tested was found.

KEYWORDS: forensic science, DNA typing, HumvWA, HumFXIIIB, HumFES/FPS, Egypt, Yemen, population study, short tandem repeat, linkage disequilibrium

The tetrameric short tandem repeat systems (STRs) HumvWA (vWA) (1), HumFXIIIB (FXIIIB) (2) and HumFES/FPS (FES) (3) can be amplified by the polymerase chain reaction (PCR) and subsequently typed using polyacrylamide electrophoresis. The forensic usefulness of STRs has been demonstrated (4). For that purpose, however, it is necessary to obtain population data on these polymorphisms in various populations. As no Arab data on these STRs have been made available to the forensic community so far, the systems HumvWA, HumFXIIIB and HumFES/FPS were tested in both a Yemenian and an Egyptian population sample.

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* This work was supported by grant 6523/1 from the Jubilee Fund of the Austrian National Bank to M. Klintschar and grant EM0075 from the World Health Organization to N. Al Hammadi.

Received 29 July 1997; and in revised form 30 Oct. 1997; accepted 31 Oct. 1997.

Materials and Methods

Sample Preparation

Whole EDTA blood was obtained by venipuncture from 100 unrelated Yemenians from the Sanaa area and 100 unrelated Egyptians from the Cairo area. Bloodstains were prepared on sterilized cotton cloth and subsequently air dried. The DNA was extracted by a slightly modified alkaline lysis protocol (5): A portion of every stain measuring 2 × 2 mm was put into a 1.5 mL centrifuge tube, lysis was accomplished in 10 μL 0.2N NaOH without initial incubation in distilled water, and 90 μL 0.04 M Tris buffer pH 7.5 was used to normalize the pH of the extract.

PCR Amplification and Typing

Aliquots of 2.5 μL of the extracts were used for amplification without prior quantification. The three loci were coamplified in a triplex reaction containing 0.5 μM each vWA primer (1), 0.3 μM each FXIIIB primer (2), 0.5 μM each FES primer (3), 1× PCR buffer II (Perkin Elmer Corporation, Norwalk, CT), 2 mM MgCl2, 200 μM each dNTP, and 1 unit of Amplitaq Gold Polymerase (Perkin Elmer Corporation, Norwalk, CT). The assay was diluted to a total volume of 25 mL with double distilled water. Cycle conditions were 94°C for 9 min followed by 94°C for 1 min, 58°C for 1 min, and 72°C for 1.5 min for 8 cycles followed by 94°C for 1 min, 57°C for 1 min, and 72°C for 1.5 min for 22 cycles in a programmable thermal cycler (TRIO-thermoblock, Biometra, Germany). Electrophoretic separation and typing were performed using native horizontal polyacrylamide gels and silver staining as described (6,7). Sequenced allelic ladders for all STRs were kindly provided by B. Brinkmann, Münster.

Statistical Analysis

The mean exclusion chance (ME) was calculated according to Krüger et al. (8) and the discriminating power (DP) was calculated according to Fisher (9). For checking the Hardy-Weinberg expectations χ^2-tests were performed using a binning approach according

to Rand et al. (10). Comparisons of the allele frequencies between different populations were performed by using two-way contingency tables. The computer program was kindly provided by G. Carmody, Ottawa. For the comparisons at the FES locus the data for alleles 10/10a and 11/11a were pooled when comparing them with samples typed on denaturing gels (11). For linkage analysis the data of both Arab populations were pooled with Austrian data. Linkage testing was performed using an exact tests [GENEPOP software, version 1.2 (M. Raymond and F. Rousset, Montpellier)].

Results

The distributions of the observed allele frequencies for the 3 STRs tested were compared with a European population sample from Austria in Table 1. The Austrian database includes already published data (7,11,12) and samples recently typed in our laboratory. The observed and the expected genotype frequencies of the 3 STR loci are given in Tables 2 through 4. Forensically relevant parameters are stated in Table 5.

TABLE 1—*Allelic frequencies for the STRs vWA, FXIIIB, and FES in Arab population samples from Egypt and Yemen compared to Europeans from Austria. The most common alleles are in bold print (n = number of chromosomes analyzed).*

	Egyptians	Yemenians	Austrians
VWA	n:200	n:200	n:932
11	0.000	0.000	0.001
13	0.000	0.000	0.001
14	0.085	0.055	0.089
15	0.095	0.075	0.111
16	**0.270**	**0.205**	0.211
17	**0.240**	**0.345**	**0.276**
18	0.185	0.185	**0.229**
19	0.095	0.105	0.072
20	0.025	0.020	0.011
21	0.005	0.010	0.000
F13B	n:200	n:200	n:810
6	0.170	0.145	0.104
7	0.040	0.050	0.019
8	**0.305**	0.220	**0.258**
9	0.225	**0.310**	0.252
10	**0.260**	**0.275**	**0.360**
11	0.000	0.000	0.007
FES	n:200	n:200	n:874
8	0.035	0.035	0.010
9	0.005	0.010	0.002
10a	0.195	0.215	0.220
10	0.145	0.100	0.053
11a	0.005	0.010	0.014
11	**0.340**	**0.320**	**0.427**
12	**0.205**	**0.225**	**0.227**
13	0.065	0.075	0.047
14	0.005	0.010	0.001

TABLE 2—*Genotype frequencies at the vWA locus in 100 Egyptians and 100 Yemenians.*

	Egyptians		Yemenians	
Genotype	Expected	Observed	Expected	Observed
14 - 14	0.723	0	0.303	1
14 - 15	1.615	2	0.825	1
14 - 16	4.590	5	2.255	0
14 - 17	4.080	4	3.795	4
14 - 18	3.145	4	2.035	3
14 - 19	1.615	1	1.155	1
14 - 20	0.425	1	0.220	0
14 - 21	0.085	0	0.110	0
15 - 15	0.903	1	0.563	1
15 - 16	5.130	3	3.075	1
15 - 17	4.560	3	5.175	8
15 - 18	3.515	5	2.775	2
15 - 19	1.805	3	1.575	0
15 - 20	0.475	1	0.300	1
15 - 21	0.095	0	0.150	0
16 - 16	7.290	7	4.203	4
16 - 17	12.960	15	14.145	14
16 - 18	9.990	10	7.585	9
16 - 19	5.130	4	4.305	8
16 - 20	1.350	2	0.820	0
16 - 21	0.270	1	0.410	1
17 - 17	5.760	5	11.903	14
17 - 18	8.880	8	12.765	7
17 - 19	4.560	7	7.245	5
17 - 20	1.200	1	1.380	2
17 - 21	0.240	0	0.690	1
18 - 18	3.423	3	3.423	6
18 - 19	3.515	4	3.885	3
18 - 20	0.925	0	0.740	1
18 - 21	0.185	0	0.370	0
19 - 19	0.903	0	1.103	2
19 - 20	0.475	0	0.420	0
19 - 21	0.095	0	0.210	0
20 - 20	0.063	0	0.040	0
20 - 21	0.025	0	0.040	0
21 - 21	0.003	0	0.010	0

TABLE 3—*Genotype frequencies at the FXIIIB locus in 100 Egyptians and 100 Yemenians.*

	Egyptians		Yemenians	
Genotype	Expected	Observed	Expected	Observed
6 - 6	2.890	3	2.103	0
6 - 7	1.360	2	1.450	1
6 - 8	10.370	9	6.380	5
6 - 9	7.650	7	8.990	15
6 - 10	8.840	10	7.975	8
7 - 7	0.160	0	0.250	0
7 - 8	2.440	2	2.200	2
7 - 9	1.800	1	3.100	3
7 - 10	2.080	3	2.750	4
8 - 8	9.303	11	4.840	6
8 - 9	13.725	14	13.640	12
8 - 10	15.860	14	12.100	13
9 - 9	5.063	6	9.610	6
9 - 10	11.700	11	17.050	20
10 - 10	6.760	7	7.563	5

At the vWA locus (Table 2), no differences were found between both Arab samples and between the Egyptian and the European sample, but the differences between the Yemenians and the Europeans were significant (Table 6).

At the FXIIIB locus (Table 3), significant differences were found between both Arab samples and the Europeans, but no evidence of population heterogeneity was found between Egyptians and Yemenians (Table 6).

At the FES locus (Table 4), no differences were found between both Arab samples, but the differences between these samples and the Europeans were significant (Table 6).

TABLE 4—*Genotype frequencies at the FES locus in 100 Egyptians and 100 Yemenians.*

	Egyptians		Yemenians	
Genotype	Expected	Observed	Expected	Observed
8 - 8	0.123	1	0.123	0
8 - 9	0.035	0	0.070	0
8 - 10a	1.365	1	1.505	1
8 - 10	1.015	1	0.700	1
8 - 11a	0.035	0	0.070	0
8 - 11	2.380	1	2.240	2
8 - 12	1.435	2	1.575	2
8 - 13	0.455	0	0.525	1
8 - 14	0.035	0	0.070	0
9 - 9	0.003	0	0.010	0
9 - 10a	0.195	0	0.430	0
9 - 10	0.145	0	0.200	0
9 - 11a	0.005	0	0.020	0
9 - 11	0.340	1	0.640	1
9 - 12	0.205	0	0.450	1
9 - 13	0.065	0	0.150	0
9 - 14	0.005	0	0.020	0
10a - 10a	3.803	3	4.623	5
10a - 10	5.655	6	4.300	6
10a - 11a	0.195	0	0.430	1
10a - 11	13.260	9	13.760	16
10a - 12	7.995	14	9.675	7
10a - 13	2.535	3	3.225	2
10a - 14	0.195	0	0.430	0
10 - 10	2.103	1	1.000	2
10 - 11a	0.145	0	0.200	1
10 - 11	9.860	13	6.400	4
10 - 12	5.950	5	4.500	3
10 - 13	1.885	2	1.500	0
10 - 14	0.145	0	0.200	1
11a - 11a	0.003	0	0.010	0
11a - 11	0.340	1	0.640	0
11a - 12	0.205	0	0.450	0
11a - 13	0.065	0	0.150	0
11a - 14	0.005	0	0.020	0
11 - 11	11.560	13	10.240	8
11 - 12	13.940	13	14.400	17
11 - 13	4.420	3	4.800	7
11 - 14	0.340	1	0.640	1
12 - 12	4.203	1	5.063	5
12 - 13	2.665	5	3.375	5
12 - 14	0.205	0	0.450	0
13 - 13	0.423	0	0.563	0
13 - 14	0.065	0	0.150	0
14 - 14	0.003	0	0.010	0

TABLE 5—*Forensically relevant parameters for the three STRs in the three populations studied (H obs. = observed Heterozygosity; DP = Discriminating power; MEC = Mean Exclusion Chance).*

	Population	VWA	FXIIIB	FES
H. obs.	Egyptians	0.84	0.73	0.81
	Yemenians	0.72	0.83	0.80
	Austrians	0.81	0.79	0.74
DP	Egyptians	0.937	0.900	0.918
	Yemenians	0.924	0.899	0.921
	Austrians	0.932	0.883	0.861
MEC	Egyptians	0.634	0.530	0.573
	Yemenians	0.591	0.531	0.583
	Austrians	0.621	0.496	0.483

Deviations from Hardy-Weinberg expectations were found in any of the populations in neither of the three STRs ($p > 0.05$). Furthermore, no evidence of linkage disequilibrium between any of the three STRs tested was found ($p > 0.05$). The combined ME of the 3 STRs was 0.928 for the Egyptian sample and 0.920 for the Yemenian one; the combined DP was 0.9995 and 0.9994, respectively.

Discussion

No significant differences between two Arab populations, one from the Arab peninsula, the other one from northern Africa, were found in the three STRs tested in this study. Although differences might be found in larger samples, it can thus be supposed that, as for other DNA polymorphisms (13,14,15), there would be no substantial differences in DNA profile frequency estimates if either database or the pooled data of both databases were used as reference in casework involving Arabs. Significant differences, however, were found between both Arab populations and a European sample from Austria at the FXIIIB and at the FES locus, while a significant heterogeneity at the vWA locus was found only between the Yemenians and the Austrians. The forensically relevant parameters for the Arabs studied herein were higher at the FES locus, while comparable to those found in the Austrians at the vWA and FXIIIB loci. The differences for the FES data can be attributed to the relatively low frequency of allele 11 in the Arab populations and to the high frequency of allele 10 compared with allele 10a, a subvariant which can be detected only under native conditions at electrophoresis (16). This variant was found to be common in other Caucasian populations studied, but extremely rare in non-Caucasians (17).

In conclusion, the three STRs vWA, FXIIIB, and FES proved to be valuable for differentiation of individuals of Arabic descent. Particularly FES proved to be of higher polymorphicity in Arabs than in other populations, provided an electrophoretic setup which allows for differentiation between the variants 10 and 10a is used.

Acknowledgments

The authors are grateful to Ms. M. Langer for improving the style of the manuscript.

TABLE 6—*χ^2 comparisons of the Egyptian (Egy.), the Yemenian (Yem.), and the Austrian (Austr.) populations samples.*

Populations	VWA	p	FXIIIB	p	FES	p
Egy-Yem.	χ^2 7.85	0.344	χ^2 6.56	0.199	χ^2 3.29	0.927
Austr.-Yem.	χ^2 21.62	0.020	χ^2 16.83	0.007	χ^2 26.99	0.003
Austr.-Egy.	χ^2 13.98	0.136	χ^2 17.26	0.005	χ^2 34.148	<0.001

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