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Polymorphism of HUMvWA31, HUMTH01, HUMF13A1 and HUMFES/FPS STR Genetic Loci in Jordanians

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ABSTRACT

DNA typing was performed on a sample population consisting of 93 unrelated Jordanians using the ABI Prism™ STR Primer Set for PCR Amplification and Typing of HUMvWA31, HUMTH01, HUMF13A1, and HUMFES/FPS. Genotypic and allelic frequencies for the HUMvWA31, HUMTH01, HUMF13A1, and HUMFES/FPS STR loci were calculated and statistically analyzed. Genotype distribution for all four loci was in agreement with the expectations of the HWE. Our data show that the combined power of discrimination using these four loci (P_D: 0.999953) is reasonably high considering the size of the Jordanian population. Allelic frequencies at the TH01 and FES/FPS loci are significantly different from those observed in African Americans as well as Hispanic Americans. This is significant in light of the fact that forensic laboratories routinely use genetic data for populations to which the individual in question may not necessarily belong. Data presented here have useful applications in forensics, clinical diagnosis and ethnogenetics.

Key words: DNA typing, Polymerase Chain Reaction, short tandem repeats, HUMvWA31, HUMTH01, HUMF13A1, HUMFES/FPS, Jordan.

INTRODUCTION

Short tandem repeat (STR) loci are highly polymorphic consisting of short, repetitive sequence elements of 2 to 7 base pairs each; the overall sequence length is normally less than 400 base pairs (Weber and May, 1989). This, combined with the fact that they are amenable to amplification by the polymerase chain reaction (PCR), makes STR loci especially useful as markers for DNA typing. The number of alleles and consequently the degree of polymorphism is usually high when compared with other non-STR genetic markers (Hayes et al., 1995; Woo and Budowle, 1995; Tahir et al., 1997; Yasin et al., 1999; Hamad et al., 2001). In the last decade, several STR genetic loci have been utilized to develop STR-based DNA typing systems. Some of these systems are now widely used by many forensic laboratories worldwide for DNA typing in criminal settings and identification of human remains among other applications (Edwards et al., 1991; Edwards et al., 1992; Hochmeister et al., 1995). A major drawback in using STR loci as genetic markers is the real possibility that different populations may exhibit considerable levels of polymorphism. Therefore, it is imperative to establish population-based genetic data bases for STR loci, it is also imperative to place strong restrictions on usage of STR data obtained from other populations.

Previously, we have reported on the genetic polymorphism of haptoglobin (Awadallah and Hamad, 2000; Hamad and Awadallah, 2000), PM (Yasin et al., 1999)

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and HLA-DQA1 genetic loci in Jordanians (Hamad et al., 2001). Here, we report on the genetic polymorphism of the STR loci HUMvWA31, HUMTH01, HUMF13A1, and HUMFES/FPS (Lins et al., 1998) in the Jordanian population. Data presented here should be of great value to forensic specialists in Jordan and abroad; it can also be of value to workers in the general fields of clinical diagnosis and ethnogenetics.

MATERIALS AND METHODS

Sample collection

Blood samples from 93 unrelated Jordanians were collected into EDTA vacutainer tubes. All sample donors were informed of the goal of the study; prior to sample collection, each participant was asked to sign a form of consent on the understanding that his / her name will be kept confidential and that the sample will not be used for other purposes.

DNA isolation, amplification and analysis

DNA isolation from human blood samples was performed essentially as previously described (Budowle and Baechtel, 1990). Single locus PCR amplification was done according to the manufacturer's instructions using the ABI Prism™ STR Primer Set Protocol (Applied Biosystems, Foster City, CA, USA). Approximately 2.5 ng template DNA was included in each 50 μl amplification reaction. The amplification product was checked by electrophoresis in 2% agarose gel with ethidium bromide, and the PCR product was then analyzed by capillary electrophoresis with the Genetic Analyzer 310 (Applied Biosystems, USA).

Statistical analysis

Genetic calculations and statistical evaluation was performed as previously described (National Research Council Committee on DNA forensic Science, 1996; Yasin et al., 1999; Hamad et al., 2001). Briefly, allelic frequencies at each locus were calculated from the numbers of each genotype in the sample set and estimates for expected homozygosity and heterozygosity were calculated based on the Hardy-Weinberg Equilibrium (HWE). Perecentage genotype and allele representation was calculated as the number of genotypes or alleles present in the population divided over the total number of possible genotypes or alleles for the specific locus. For each locus, possible departure from the HWE expectations was evaluated by the Chi-Square as well as the G-test statistic where the number of degrees of freedom was calculated as the number of genotypes minus the number of alleles. G-statistic homogeneity test was performed using the STATISTICA for Windows software 1995 version (StatSoft, OK, USA).

RESULTS AND DISCUSSION

The genotypic and allelic frequencies for four STR loci (HUMvWA31, HUMTH01, HUMF13A1 and HUMFES/FPS) in Jordanians are shown in tables 1 and 2 respectively. For vWA31, genotype 16-17 was the most predominant occurring at a frequency of 0.17204 followed by genotype 15-16 occurring at a frequency of 0.12903. Of 55 possible genotypes for this locus, only 23 genotypes were observed; percentage genotype representation was 41.8%. The number of alleles for the HUMvWA31 locus present in the Jordanian population was 9 out of 10; in other words, percentage allelic representation was 90%. The most predominant allele was 17 with a frequency of 0.30108 followed by alleles 16 and 15 with a frequency of 0.23656 and 0.15054 respectively; allele 11 was not represented in Jordanians. Genotypes 6-8 and 7-9 were the most common with regard to TH01 locus occurring at a frequency of 0.10869 and 0.09783, respectively. Percentage genotype representation was calculated at 69.4% and percentage allelic representation was 87.5%. Allele 9 was the most prevalent with a frequency of 0.22043 while allele 11 was absent. For F13A1 locus, the most common genotype was 5-6 occurring at a frequency of 0.13684. Percentage genotype representation was 14%. Allele 5 was the most common occurring at a frequency of 0.34946 and alleles 14, 15, 17, 18 and 19 were not represented. Percentage allelic representation for the HUMF13A1 locus in Jordanians was 72.2%. Most common Genotypes for the FES/FPS STR locus were 10-11, 11-11 and 9-10 with a frequency of 0.20213, 0.17021 and 0.13829 respectively. Percentage genotype representation was 44.4%. Alleles 11 and 10 were very prevelant with a frequecny of 0.40323 and 0.28495 respectively; alleles 7 and 14 were absent; percentage allelic representation was 75%.

Possible departure from the expectations of the HWE at each locus was evaluated using the Chi-Square and G-statistic tests at 99.5% level of significance and a number of degrees of freedom equal to the number of genotypes minus the number of alleles. As shown in table 2, all loci were in agreement with the expectations of the HWE ($p < 1.000$). Also shown in table 2 is the power of discrimination (P_D) and power of identity (P_I) for each locus. It should be noted that percentage genotype and allele representation for these

Table 1. Genotypic Frequency Distribution at Four STR Genetic Loci in Jordanians.

STR Locus							
vWA31		TH01		F13A1		FES/FPS	
Gen.[a]	Freq.[b]	Gen.	Freq.	Gen.	Freq.	Gen.	Freq.
12-15	0.01075	5-5	0.04348	3.2-3.2	0.03158	8-9	0.01064
13-14	0.02151	5-6	0.04348	3.2-4	0.01053	8-11	0.02128
13-16	0.02151	5-7	0.03261	3.2-5	0.11579	8-12	0.01064
13-17	0.03226	5-8	0.02174	3.2-6	0.07368	9-9	0.01064
13-18	0.03226	5-9	0.04348	3.2-7	0.01053	9-10	0.13829
13-20	0.01075	6-6	0.06522	3.2-8	0.02105	9-11	0.09574
14-15	0.07527	6-7	0.02174	4-4	0.01053	9-12	0.02128
14-16	0.03226	6-8	0.10869	4-5	0.09474	10-10	0.06383
14-17	0.02151	6-9	0.06522	4-6	0.08421	10-11	0.20213
14-18	0.03226	6-9.3	0.01087	4-7	0.01053	10-12	0.05319
14-19	0.01075	6-10	0.03261	5-5	0.10526	10-13	0.04255
15-15	0.01075	7-7	0.02174	5-6	0.13684	11-11	0.17021
15-16	0.12903	7-8	0.05435	5-7	0.09474	11-12	0.11702
15-17	0.04301	7-9	0.09783	5-8	0.02105	11-13	0.02128
15-19	0.02151	7-9.3	0.04348	5-12	0.01053	12-12	0.01064
16-16	0.03226	7-10	0.02174	6-6	0.02105	12-13	0.01064
16-17	0.17204	8-8	0.03261	6-7	0.03158	-	-
16-18	0.03226	8-9	0.05435	6-12	0.01053	-	-
16-19	0.02151	8-9.3	0.01087	6-16	0.01053	-	-
17-17	0.10753	8-10	0.02174	7-7	0.04211	-	-
17-18	0.08602	9-9	0.05435	7-10	0.02105	-	-
17-19	0.03226	9-9.3	0.03261	7-13	0.01053	-	-
18-19	0.01075	9-10	0.04348	9-11	0.01053	-	-
-	-	9.3-9.3	0.01087	20-20	0.01053	-	-
-	-	10-10	0.01087	-	-	-	-
%GR[c]	**41.8%** (23/55)	**69.4%** (25/36)		**14%** (24/171)		**44.4%** (16/36)	

[a] Gen.: Genotype.
[b] Freq.: Frequency.
[c] %GR: Percentage Genotype Representation.

loci in Jordanians permits their use as powerful genetic markers for DNA typing purposes. This, notwithstanding the fact that HUMF13A1 locus percentage genotype representation was low (14%); which argues against its use as genetic marker in the Jordanian population. In other words, where the HUMTH01 STR locus (8 alleles) has a P_I of 0.05411, the HUMF13A1 locus (18 alleles) has a P_I of 0.08382. Assuming that percentage genotype representation was 10-20% higher, the P_I value for this locus would be significantly lower resulting in greater power of discrimination. A mathematical model for choosing population-tailored sets of genetic loci for DNA typing purposes based on percentage allelic and genotypic representation in combination with the probability of matching (P_M) is currently under investigation (unpulished data). The least powerful marker in the set was HUMFES/FPS with P_I of 0.12510; again considering the number of alleles of this locus (8 alleles), percentage genotype representation is not high enough (44.4%) to yield a reasonable P_I relative to the absolute P_I which approaches 0.02778. The combined power of identity using the four STR loci in Jordanians was calculated at 0.000047 and hence the power of discrimination calculated at 0.999953. The combined P_I using these STR loci in addition to the PM system (Yasin et al., 1999) and the HLA-DQA1 locus (Hamad et al., 2001) was 0.000000019, the combined P_D was therefore 0.999999981. Combining these three DNA typing systems should yield statisfactory levels of population resolution and individual identification in Jordanians considering the size of the Jordanian population which does not exceed 5 million according to the

Table 2. Observed Allele Frequencies for Four STR Loci in the Jordanian Population and Statistical Parameters for Forensic Testing.

Allele	vWA31 (N: 93)	TH01 (N: 93)	F13A1 (N: 93)	FES/FPS (N: 93)
3.2	-	-	0.15054	-
4	-	-	0.11290	-
5	-	0.11290	0.34946	-
6	-	0.20430	0.19892	-
7	-	0.15591	0.13441	0.00000
8	-	0.16666	0.02151	0.02151
9	-	0.22043	0.00538	0.14516
9.3	-	0.05914	-	-
10	-	0.06989	0.01075	0.28495
11	0.00000	0.00000	0.00538	0.40323
12	0.00538	-	0.01075	0.11828
13	0.05914	-	0.00538	0.03763
14	0.09677	-	0.00000	0.00000
15	0.15054	-	0.00000	-
16	0.23656	-	0.00538	-
17	0.30108	-	0.00000	-
18	0.09677	-	0.00000	-
19	0.04839	-	0.00000	-
20	0.00538	-	0.01075	-
H_{obs} [a]	0.84946	0.76087	0.77895	0.74468
H_{exp} [b]	0.80610	0.83646	0.78437	0.71927
χ^2	0.58546	0.26974	3.34389	0.12799
G-test	0.50037	0.29931	0.56569	0.10124
P_I [c]	0.08290	0.05411	0.08382	0.12510
P_D [d]	0.91710	0.94589	0.91618	0.87490

[a] H_{obs}: observed heterozygosity.
[b] H_{exp}: expected heterozygosity.
[c] P_I: Power of identity.
[d] P_D: Power of discrimination.

most recent Jordanian census bureau data.

Discrepancy in allelic distribution for each locus between Jordanians and 3 distinct American ethnic groups was investigated using the G-statistic (homogeneity) test. Lack of data on Arab and neighboring populations as well as most populations of the world limited the comparison to Jordanians versus Caucasian Americans, African Americans and Hispanic Americans (table 3). There was considerable discrepancy between Jordanians and all three American populations at the TH01 locus. Additionally, statistical differences between Jordanians and African Americans as well as Hispanic Americans at the FES/FPS locus were observed. This is significant in light of the fact that a good percentage of forensic laboratories around the world use population data included in the manufacturer's kit for data interpretation. These findings confirm the need to establish

Table 3. G-statistic Test for Homogeneity in Jordanians versus Caucasian Americans, African Americans and Hispanic Americans.

Locus	*p* value		
	Jordanians vs. Caucasians Americans	Jordanians vs. Hispanic Americans	Jordanians vs. African Americans
vWA31	< 0.8181	< 0.9696	< 0.9727
TH01	< 0.0017*	< 0.0026*	< 0.0283*
F13A1	< 0.8755	< 0.9999	< 0.9993
FES/FPS	< 0.3488	< 0.0866*	< 0.0492*

* Significant difference.

genetic databases for populations where individual identification in forensic settings, criminal proceedings or paternity testing relys on DNA typing procedures. It is worth noting that the degree of discrepancy between seemingly unrelated populations is much less when coding sequences or sequences other than STR are the subject of comparison (Hayes et al., 1995; Woo and Budowle, 1995; Tahir et al., 1997; Yasin et al., 1999; Awadallah and Hamad, 2000; Hamad and Awadallah, 2000; Hamad et al., 2001). No clear answer is readily available to explain this observation. It is likely however, that genetic pressures may have some bearing on the pattern of dispersion of STR alleles in different populations.

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