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FOR THE RECORD

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Allele Frequencies of Penta D and Penta E Loci in Afghanistan Population

POPULATION: Afghanistan (Pashtun, Tajik, and Hazars; $n = 120$).

KEYWORDS: forensic science, Afghanistan population, Pashtun, Tajik, Hazaras, DNA typing, population genetics, Penta D, Penta E

Buccal cells were collected by oral brushes (Sterile Omni Swab, Whatman International Ltd., Maidstone, U.K.) from healthy, random Afghanian individuals deriving from most of Afghanistan provinces. The number and ethnicity of individuals have been chosen in order to obtain a population sample strictly and realistically resembling the Afghanistan population structure and to achieve the highest possible degree of representativeness of the major ethnic groups of the country, mostly represented by Pashtun (more than 40%), Tajik (approximately 25%), and Hazaras (more than 20%). A pondered sampling from these groups has been used to obtain genetic data from the entire Afghanistan population.

DNA was extracted by Chelex method® (1) and following purified by Microcon® YM-100 Centrifugal Filter Units (Millipore Corporation, Bedford, MA) in order to remove potential PCR inhibitors, PCR buffer salts, and excess of PCR primers.

The total amount of human genomic extracted DNA was determined by using AmpF*l*STR® Quantifiler Human DNA Quantification Kit (Applied Biosystems, Foster City, CA) (2), which employs a TaqMan® MGB Probe-based technology (Applied Biosystems) on ABI Prism® 7000 Sequence Detection System (Applied Biosystems).

Reactions were carried out by mixing, in a 25 μL final reaction volume, 10.5 μL of Quantifiler Human Primer Mix, 12.5 μL of Quantifiler PCR Reaction Mix containing AmpErase® UNG (Applied Biosystems), and a 2 μL of sample DNA.

Simultaneous amplifications of 16 short tandem repeats (STR) *loci* (multiplexed PCR) were performed by using PowerPlex® 16 System (Promega Corporation, Madison, WI) (3).

Amplifications of STRs as well as of the gender determination marker, Amelogenin, were conducted in a 25 μL final reaction volume containing 1 ng of genomic DNA, 2.5 μL of Gold ST*R 10 × buffer, 2.5 μL of PowerPlex® 16 10 × primer pair mix, 0.8 μL of AmpliTaq Gold® DNA polymerase (5 U/μL stock solution) (Applied Biosystems), and deionized water to 25 μL final volume.

The reaction mixtures were subjected to a hot start at 95°C for 11 min and 96°C for 1 min to activate the AmpliTaq Gold® DNA polymerase. Amplifications were carried out for 10 cycles by using standard parameters: 94°C for 30 s, 68 s to 60°C (hold for 30 s), 50 s to 70°C (hold for 45 s); then for 22 cycles: 90°C for 30 s, 60 s to 60°C (hold for 30 s), 50 s to 70°C (hold for 45 s). The PCR mixtures were subjected to a single step at 60°C for 30 min and

TABLE 1—*Allele frequencies of Penta D and Penta E in Afghanistan population (n = 120).*

Allele	Penta D	Penta E
2.2	—	—
3.2	—	—
5	—	0.0345
7	—	0.1207
8	—	0.0086
9	0.2458	—
10	0.1864	0.0690
11	0.2203	0.1466
12	0.1441	0.1552
13	0.0847	0.0431
14	0.0508	0.1552
15	0.0678	0.0690
16	—	0.0259
17	—	0.0776
18	—	0.0345
19	—	—
20	—	0.0345
21	—	0.0086
22	—	0.0172
23	—	—
24	—	—
H_o	0.864	0.810
H_e	0.832	0.901
PD	0.935	0.963
CE	0.478	0.646
PIC	0.801	0.884
p	0.860	0.06

H_o, observed heterozygosity; H_e, expected heterozygosity; PD, power of discrimination; CE, chance of exclusion; PIC, polymorphic information content; p, probability value of χ^2 test for Hardy–Weinberg equilibrium.

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then were cooled at 4°C. All amplifications were conducted in a GeneAmp® PCR System 9600 (Applied Biosystems) thermal cycler by using thin-walled 0.2 mL MicroAmp® Reaction Tubes (Applied Biosystems).

The separation and detection of amplified products were conducted with the ABI Prism® 3100 Genetic Analyzer 16-capillary array system (Applied Biosystems) following manufacturer's protocols. Samples were prepared with 9 μL Hi-Di formamide (Applied Biosystems), 1 μL Internal Lane Standard 600 (Promega Corporation), labeled with carboxy-X-rhodamine dye (CXR®), and with 1 μL of amplified sample. Data collection was performed with Data Collection v. 2.0 software (Applied Biosystems), and samples were analyzed with GeneMapper® v. 3.2 software (Applied Biosystems).

Results

Allele frequencies and statistical evaluations of each STR are reported in Table 1.

Several forensic and population parameters of Penta D and Penta E pentanucleotide *loci* were estimated by using the statistical softwares Popgene v. 1.31 (4) and Cervus v. 2.0 (5,6). Each locus was tested for Hardy–Weinberg equilibrium by the χ^2 test.

No evidence of deviation of observed frequencies from those expected according to Hardy–Weinberg equilibrium was detected at any of the two loci, being $p = 0.06$ in Penta E and 0.86 in Penta D. Both *loci* showed a high degree of genetic polymorphism having H_e values of 0.9015 (Penta E) and 0.832 (Penta D). Polymorphism Information Content (PIC) values were 0.884 for Penta E and 0.801 for Penta D demonstrating a good informativeness of both the markers.

The complete data set fulfills requests of this journal for the data set and is available to any interested researcher upon request to berti.an@tiscali.it.

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