TECHNICAL NOTE

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The Distribution of the vWF Alleles and Genotypes in the Palestinian Population

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ABSTRACT: Short tandem repeat (STR) loci amplified by PCR are known as a useful tool for individual identification and paternity testing. Direct PCR amplification from small amounts of whole blood is a rapid and convenient method for population screening for STR and VNTR markers. The allele frequencies of the vWF locus were determined for 127 unrelated Palestinians. Co-dominant segregation was observed in 20 mother/child pairs. Nine alleles were observed, with frequencies ranging from 0.004 to 0.327. Heterozygosity was 79%, and discrimination power was 0.927.

KEYWORDS: forensic science, DNA typing vWF, population genetics, direct polymerase chain reaction, short tandem repeat, Palestinians

Short tandem repeats (STRs) or microsatellites, which are composed of tandemly repeated sequences of 1 to 6 bp in length, are abundant in the human genome (1). The rapid analysis time and minimal DNA requirements make STR analysis an attractive methodology for paternity and forensic studies (2). A tetranucleotide polymorphism in the region between nucleotides 1640 and 1794 in intron 40 of the vWF gene was reported by Kimpton (3). We report here the results of an analysis of allele and genotype frequencies of this locus in the Palestinian population using direct PCR from whole blood.

Materials and Methods

Blood samples in vacutainer tubes from 127 unrelated Palestinians living in Jerusalem, and another 20 mother/child pairs were obtained from Almakassed Hospital in Jerusalem. Fifty μL of whole blood were added to 500 μL of 50 mM NaOH and boiled for 15 min. Then 100 μL of Tris-HCl 1M (pH 8.0) were added to neutralize the solution. Three μL of the neutralized solution were amplified in a final volume of 25 μL in a Perkin-Elmer 480 thermocycler. The amplification mixture was modified from that reported by Kimpton et al. (3). Each reaction contained 75 μM Tris-HCl pH 9.0, 2.0 μM $MgCl_2$, 0.1% Tween 20, 20 μM $(NH_4)_2SO_4$, 200 μM of each dNTP and 80 ng of each primer. The primers were (3):

vWA1 = 5′ CCCTAGTGGATGATAAGAATAATC 3′ and

vWA2 = 5′ GGACAGATGATAAATACATAGGATGGATGG 3′

Primers were synthesized using a DNA synthesizer model 394 (Applied Biosystems inc., California).

The amplification product was labeled by S^{35} end-labeling of 5′ vWA1 primer. Primer was end-labeled in a reaction mixture containing 1 μg primer, 2 μL of 10× kinase buffer (USB, Ohio), 2 μL of spermidine (10 mM), 5 μL of [γ-^{35}S]dATP (10 μci/μL), 10 units T4 DNA polynucleotide kinase (USB, Ohio) and water to 20 μL. The reaction mixture was incubated at 37°C for 1 h. The reaction was terminated by heating to 100°C for 2 min. For labeled PCR amplification, the reaction mixture contained 0.5 μL of 1μg/20 μL solution of the labeled primer reaction mixture plus 0.5 μL of unlabeled primer (80 ng total primer) and 80 ng of the other unlabeled primer. The temperature cycling conditions were as follows: denaturing at 94°C for 60 s, annealing at 55°C for 60 s, extension at 72°C for 30 s. The reaction was carried out for 30 cycles. The final extension was at 72°C for 7 min. Three μL of reaction product were subjected to electrophoresis, using 6% denaturing 40 by 21 cm polyacrylamide gel (5%T, 3%C, 0.4-mm thickness) in a sequencing gel apparatus (Bio-Rad, California). The gel was run at 2000 V for 2 h in 1XTBE buffer and exposed to X-Ray film.

The alleles were designated 7–16, according to the number of the tandem repeats per allele. One allele was sequenced using the ABI Sequencer, Model 377 (Applied Biosystems Inc., California) and used as a standard for determination of allele repeat number.

A standard Chi-square analysis of the observed and expected genotypes was carried out in order to test for Hardy-Weinberg equilibrium. The power of discrimination was calculated using Fisher's formula (4).

Results and Discussion

Because alleles are codominant, and all phenotypes can be distinguished, frequencies were estimated by the traditional count method. Table 1 shows the allele frequencies of the tandem repeat region between nucleotides 1640 and 1794 in intron 40 of the

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TABLE 1—*vWF allele frequencies in a sample of 127 unrelated Palestinians according to allele repeat number.*

Allele	Size(bp)	Frequency
7	130	0.004
9	138	0.090
10	142	0.150
11	146	0.220
12	150	0.327
13	154	0.134
14	158	0.055
15	162	0.015
16	166	0.004

human vWF locus. There were nine alleles detected in 127 unrelated Palestinians, with frequencies ranging from 0.004 (alleles 7 and 16) to 0.327 (allele 12). The observed heterozygosity was 79%. Table 2 shows the genotype distribution of the vWF marker in the population. The genotype distribution meets Hardy-Weinberg expectations. The discrimination power was 0.927.

Figure 1 shows a sample of nine individuals typed with the vWF marker. Nonspecific amplified bands, so-called "shadow-bands" were observed in all the PCR reactions shown in Fig. 1. They did not interfere with the determination of the size of the marker alleles.

Two alleles (11 and 12) were relatively common in the Palestinian population, having frequencies of 0.220 and 0.327, respectively.

TABLE 2—*vWF genotypes in a sample of 127 unrelated Palestinians.*

Genotype	Observed	Expected
7, 15	1	0.010
9, 10	5	3.420
9, 11	4	5.029
9, 12	11	7.475
9, 13	4	3.063
10, 10	1	2.857
10, 11	9	8.382
10, 12	12	12.459
10, 13	3	5.105
10, 14	2	2.095
10, 15	2	0.571
10, 16	1	0.152
11, 11	8	6.147
11, 12	18	18.273
11, 13	9	7.488
11, 14	1	3.073
12, 12	14	13.580
12, 13	6	11.129
12, 14	7	4.568
12, 15	1	1.245
13, 13	4	2.280
13, 14	4	1.871
Others	0	6.858
Total	127	127.000

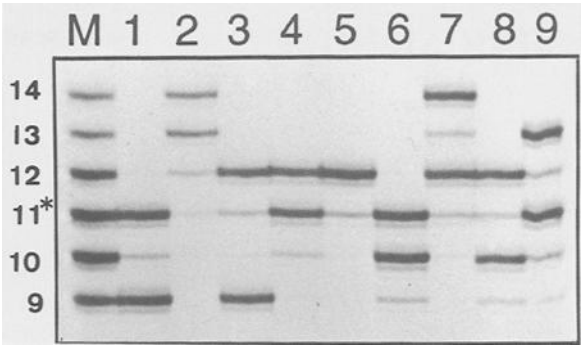

FIG. 1—*vWF genotypes obtained with polyacrylamide electrophoresis. M, allele ladder produced by amplification of DNA from three heterozygous individuals; Lanes 1–9, amplification of DNA from nine different individuals. Allele repeat numbers according to sequenced allele, shown by an asterisk.*

These two alleles were also found as the most frequent in the British Caucasian population (3). The two rare alleles (7 and 16) were not detected in the British population. Mendelian segregation for the vWF was observed in 20 mother/child pairs (data not shown).

In conclusion, the vWF locus appears to be an informative genetic marker for individual identification and paternity testing in the Palestinian population. In our study, we have introduced a simple and rapid method for carrying out the amplification reaction directly from whole blood without DNA purification, using as little as 0.23 μL of whole blood. This method is suitable and convenient for population screening programs of STR markers. We currently are investigating other markers in the Palestinian population and studying their forensic utility.

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References

1. Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1983;44:388–96.
2. Alford R, Hammond H, Coto I, Caskey C. Rapid and efficient resolution of parentage by amplification of short tandem repeats. Am J Hum Genet 1994;55:190–5.
3. Kimpton C, Walton A, Gill P. A further tetranucleotide repeat polymorphism in the vWF gene. Hum Molec Genet 1992;1:287.
4. Fisher R. Standard calculations for evaluating a blood group system. Heredity 1951;5:95–102.

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