

ALLELE FREQUENCIES OF THE vWA STR LOCUS IN ITALY

Buscemi L., *Tagliabracci A., Cucurachi N.,*Mencarelli R.,Porro D., Giorgetti R. and Ferrara S.D.

Istituto di Medicina Legale e delle Assicurazioni, Università di Parma, Italy.

*Istituto di Medicina Legale e delle Assicurazioni, Università di Ancona, Italy.

INTRODUCTION

The tetrameric (TCTA) tandem repeat system vWa, located in intron 40 of the vWF gene, was first described by Kimpton et al. (1992). The polymorphism of this locus has been analyzed in two population samples of North and Central Italy, by the polymerase chain reaction (Saiki et al. 1985). The aim of the present study was to set up a data base, to check whether allelic frequencies show inter-regional differences and to evaluate this system for forensic purposes.

MATERIALS AND METHODS

Sample preparation: DNA was extracted from blood samples of 211 unrelated healthy subjects according to the phenol-isoamyl alcohol method (Budowle and Baetchel 1990).

PCR reaction mix: 50 ng template DNA, 1 U Taq polymerase (Promega, USA), 2 μ l buffer (Promega), 0.6 μ M each primer, 100 μ M each dNTP, made up to total volume of 25 μ l, with the addition of 1-2 drops of oil.

Primer sequences (Kimpton et al. 1992)

vWA1= 5' CCCTAGTGGATGATAAGAATAATC3'

vWA2= 5' GGACAGATGATAAATACATAGGATGGATGG3'

Temperature cycling conditions:

denaturation 94°/1min

annealing 50°/1min

extension 72°/2min

cycles 30

Electrophoresis: denaturing polyacrylamide gel (6% T, 3% C, 7mM urea, thickness 750 μ m), piperazine diacrylamide as crosslinker, 100mM formate; 20 cm. separation distance, 2% agarose plugs (Allen et al. 1989; Budowle et al. 1991); running time: 1000 V, 40 mA, 3 W, ramping every 10 min up to 15 W as suggested by Wiegand et al (1993).

The bands were visualised by silver staining (Budowle et al. 1991) and phenotyped by side-to-side comparison with an allelic ladder -Fig. 1-.

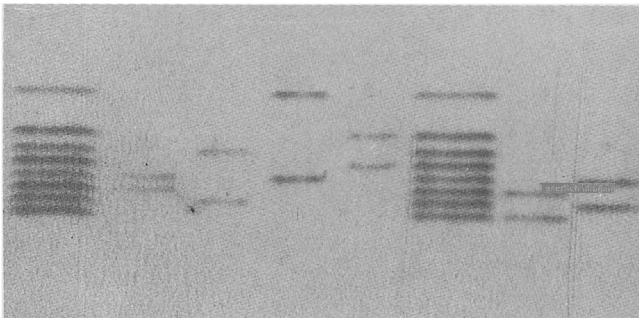


Fig. 1 Silver stained vWA system polyacrylamide gel of the amplified fragments. From left to right: allelic ladder is in lane 1,6. vWA phenotypes: lane 2: 11-12; lane 3: 9-13; lane 4: 5-11; lane 5: 8-10; lane 7: 12-14; lane 8: 11-13.

RESULTS AND DISCUSSION

The 7 common alleles, identified in the range 138 to 162 bp and classified according to the number of repeat sequences, were found. A new smaller allele, tentatively designated as vWA*5, was discovered. No significant differences between allele frequencies in the 2 Italian samples were found. The most frequent alleles were vWA*11, vWA*12, vWA*10, in line with the frequencies found in previous studies on Caucasians (Kimpton et al.1992; Brinkmann, pers. comm.1992). The frequency distribution of vWa system alleles is shown in figure 2. Observed and expected genotypes were compared assuming the Hardy-Weinberg law ($\chi^2 = 22.166$ $0.75 < P < 0.90$; 28 d.f.). The Italian sample was then statistically compared with previous studies on Caucasians (Kimpton et al.1992; Brinkmann, pers. comm.1992) and no deviation from homogeneity was found: $\chi^2 = 8.1436$ $P = 0.9030 \pm 0.0094$; G statistic = 8.7207 $P = 0.8820 \pm 0.0094$. The results of the present study suggest that this STR system could be a powerful tool for forensic purposes-Table 1-.

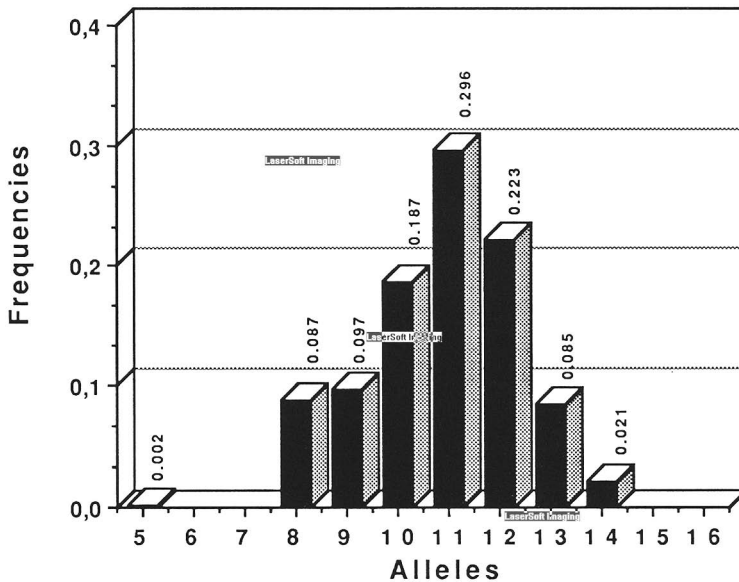


Fig. 2 Frequency distribution of vWa system alleles in an Italian population sample.

Table 1. Statistical evaluations

	Mean excl. chance	DI	Heterozy.
vWA	0.61	0.93	0.82

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