GENETIC STUDIES OF A STR AT THE vWF LOCUS AND ITS APPLICATION TO INDIVIDUALISATION

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INTRODUCTION

The feasibility of DNA analysis from minute and even partially degraded samples, may explain in some extent the increasing interest in PCR technology in the characterization from human biological remains [1,2]. The introduction of the STR ("Short Tandem Repeats") [3,4] is substantially enlarging the battery of possible DNA markers susceptible to application in these types of studies with this finality. This work concerns preliminary formal and population genetic studies on a tetranucleotide repeat in the intron 40 (between nt 1640–1750) in the von Willebrand Factor locus, vWF [5]. Further analyses in human biological remains are carried out with the objective of evaluating the usefulness of this STR in genetic individual profiling.

MATERIALS AND METHODS

The DNA was extracted from 20 family groups, 110 unrelated healthy individuals from Galicia, and in minute biological samples: whole blood, hair (roots and shafts), whole saliva, bloodstains, using standard phenol/chloroform [6] and chelating resins [7] depending on the case.

For vWF PCR–amplification, the primers were synthesised according to [5]:

vWA1: 5´ CCC TAG TGA ATG ATA AGA ATA ATC 3´
vWA2: 5´ GGA CAG ATG ATA AAT ACA TAG GAT GGA TGG 3´

The PCR reaction parameters (total 25 µl) consisted of: 20–50 ng DNA, 200 µM dNTP´s, 0.25 µM each primer, 0.5 units of Taq DNA Polymerase, $MgCl_2$ 1.5 mM, in 10 mM Tris–HCl buffer pH 9, containing Triton X–100 (1%) and KCl 50 mM.

The temperature cycling conditions were as follows: denaturation 45 s, 94°C; annealing 30 s, 50°C; extension 30 s, 72°C; 30 cycles.

Amplified vWF was electrophoresed in horizontal gel electrophoresis in a Tris–HCl/Glycine buffer pH8.8, according to [8] with modifications. Electrophoresis was accomplished in horizontal polyacrylamide plates, at 12.5 V/cm for 3 h, at constant cooling at 6 C, followed by a specific DNA Silver Staining [2].

RESULTS AND DISCUSSION

Polyacrylamide gel electrophoresis, in non–denaturing thin layer gels achieves a reliable separation between the bands of vWF (Fig 1). Unlike other AMPLPs (such as COL2A1) [9], urea/formamide denaturing and non denaturing gels lead to the same genotype diagnosis. The inclusion of composite vWF allelic ladder is of crucial importance for an accurate interpretation. Non–radiactive modalities of detection such as Silver Staining, achieve sufficient sensitivity for a clear visualisation of vWF phenotypes.

An initial population survey in 110 individuals from Galicia gave the allele frequencies displayed in Figure 2. Up to 6 allelic variants have been detected with frequencies ranging between (0.05 and 0.291). The heterozygosity index configures a value of h=0.816 and the PIC was 0.771.

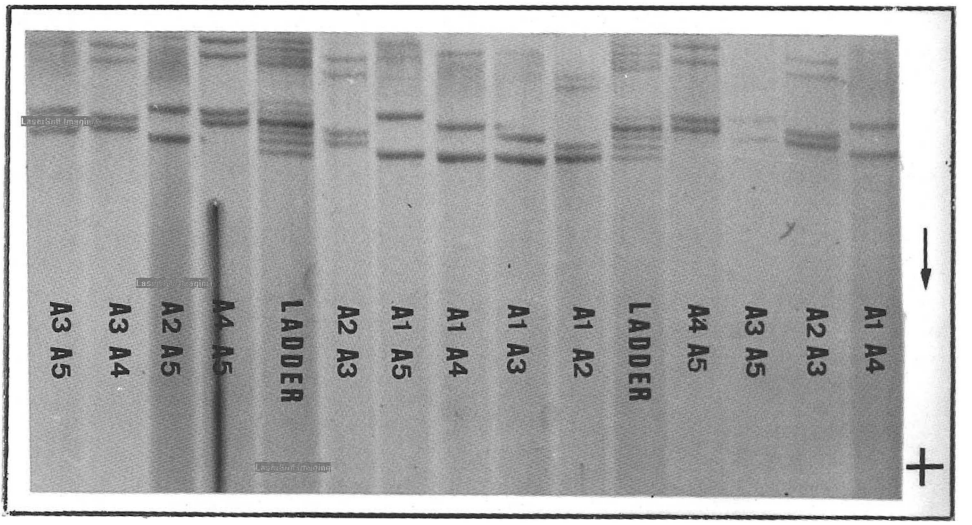

Fig.1.– Electrophoretic patterns of the vWF phenotypes.

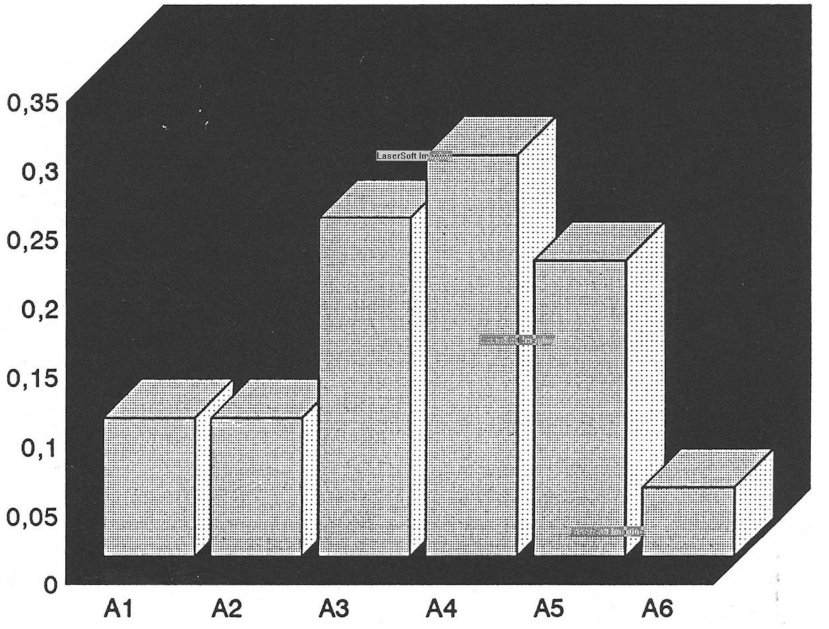

Fig.2.– Allele frequencies distribution at the vWF locus.A1: 0.1, A2: 0.1, A3: 0.245, A4: 0.291, A5: 0.214, A6: 0.05

Family analyses are underway. Preliminary results in 20 groups are in agreement without exception with an autosomal codominant model of inheritance.

In the Figures 3a–3d the vWF phenotypes from different biological sources and conditions are presented.

- Bloodstains: vWF genotype was easily identified even from a 1 mm^2 bloodstain kept at room temperature for 5 years. (Fig. 3a). Other stains and enviromental conditions are under study.
- Whole blood: positive identification from 0.01 microliter of whole fresh blood (Fig. 3b).
- Whole saliva: positive results from 1 microliter (Fig. 3c).
- Plucked hairs: positive identification from 1 root, and 3 cm hair shafts (Fig. 3d).

Without doubt the promising possibilities od this DNA marker merit further studies in order to asses its exact usefulness in individual characterization, paternity testing and population profiling.

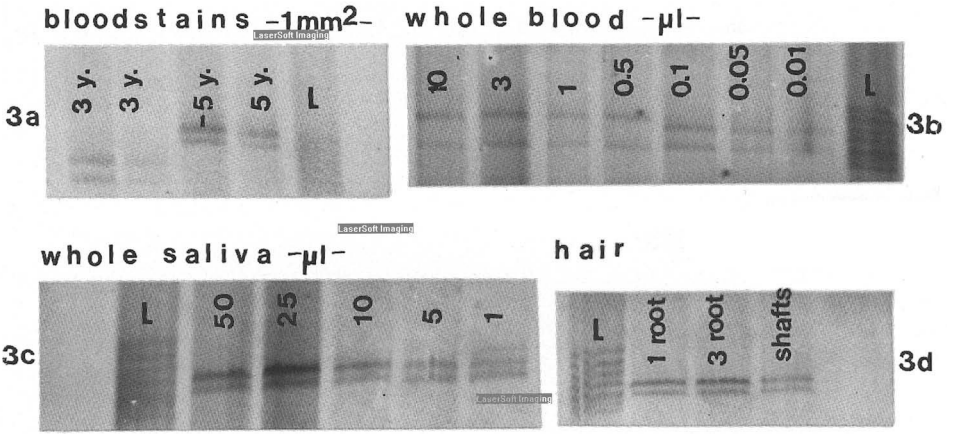

Fig.3a–3d.– vWF phenotypes in minute biological samples.

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