Characterisation of 98 alleles in 105 unrelated individuals in the F8VWF gene

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Source/Description: A variable number of tandem repeats (VNTR) of an ATCT sequence was described in the intron 40 of the von Willebrand factor gene (F8VWF) gene (1). The whole VNTR region was amplified and then digested by AluI restriction endonuclease.

Polymorphism: Based on the presence of two AluI restriction sites (1) in the amplified sequence, each allele was characterized by the combination of three restriction fragments. Their length ranged between 228 and 308 bp and one of the three fragments was always larger than 264 bp. At this time, 98 alleles designated by the length of the corresponding three restriction fragments, have been detected.

Allele Frequencies: This method has allowed us to characterize 98 alleles in 105 unrelated Caucasians. The alleles frequency ranges between 1/210 for the 46 less frequent (example: 296 + 260 + 252 bp) and 9/210 for the most frequent (272 + 240 + 236 bp). According to these data, the calculated heterozygosity rate is 0.98.

Chromosomal Localization: 12p12–12pter (2).

Mendelian Inheritance: Co-dominant segregation was demonstrated in 45 families. Our data did not show any recombination event in all the meioses in 3 generations of 9 families and in 2 generations of 36 families.

Methods: Two primers: W31 = GCCCTAGTGGATGATAA-GAATAATCAG and W32 = GCAGACTTCTACTGTTTGA-GGTAAGTG, corresponding respectively to NT 1639–1665 and NT 2404–2378 (1), were used to amplify the whole VNTR region. PCR amplifications (3) (30 cycles: 1 min at 94°C, 1 min at 60°C, 1 min at 72°C) were performed in a 100 µl reaction

mixture containing 50 mM KCl, 10 mM Tris-HCl pH 8.0, 1.5 mM MgCl₂, 0.1 mg/ml gelatin, 15 µM dNTPs, 50 pmoles of each primer, 5 µCi of α³⁵S dATP and 0.5 µg of genomic DNA. Direct amplification from whole blood, without DNA extraction, can also be performed (4). Following direct digestion by AluI restriction endonuclease, the labelled samples were electrophoresed for 5 h at 2000 V on a 6% sequencing gel and autoradiographed overnight. A control plasmid sequencing reaction was used as size marker.

Other Comments: Considering only partial sequences of the intron 40 VNTR, Peake *et al.* (5) identified, in a panel of 53 individuals, 8 alleles in the 5' region while Ploos van Amstel and Reitsma (6) identified 6 alleles of the 3' region among 24 individuals. The present report describes the identification of the AluI digested fragments of the whole polymorphic region which allowed us to distinguish between some amplified fragments of identical lengths. The multi-allelic polymorphism thus characterized may be useful for the study of the inheritance of rare subtypes of von Willebrand's disease and antenatal diagnosis of its severe forms. Furthermore, other applications of this very polymorphic sequence may be of interest: paternity testing, detection of samples contamination and even forensic studies as the results can be obtained in less than 48 h.

References: 1) Mancuso, D.J. *et al.* (1989) *J. Biol. Chem.* **264**, 19514–19527. 2) Ginsburg, D. *et al.* (1985) *Science* **228**, 1401–1426. 3) Saiki, R.K. *et al.* (1985) *Science* **230**, 1350–1354. 4) Mercier, B. *et al.* (1990) *Nucl. Acids Res.* **18**, 5908. 5) Peake, I.R. *et al.* (1990) *Blood* **76**, 555–561. 6) Ploos van Amstel, H.K. *et al.* (1990) *Nucl. Acids Res.* **18**, 4957.