GENETIC STUDIES OF A STR AT THE UGB LOCUS

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The so called Short Tandem Repeats (STR) constitute nowadays one of the most interesting sources of information in human genetic studies. This paper deals with the analysis of a tetranucleotide repeat in the Uteroglobin Gene (UGB) (Stöhr & Weber 1994), localised in chromosome 11q (Wolf et al. 1992). Thus, an initial survey in a sample of the population of Galicia is carried out aimed at assessing the technical conditions for UGB phenotyping and evaluate its usefulness in the genetic profiling of human populations.

MATERIALS AND METHODS

Blood samples were obtained from 200 unrelated individuals from the population of Galicia (NW Spain). DNA was extracted by standard chelating resins method (Singer-Sam 1993) or phenol/chloroform (Maniatis et al. 1982).The primer sequences were as described (Stöhr & Weber 1994):
UGB-1: 5'-CAT CTT CCT TGC CCA TTT C-3'.
UGB-2: 5'-TGC ATC CCT CCC CTC TTA-3'.

PCR was carried out from a total volume of 12.5 μL, containing 0.8 μM each primer, 75 μM dNTPs, 0.5 Units of Taq DNA Polymerase (Boehringer Mannheim), in 10 mM Tris-HCl buffer, pH 8.3, containing 50 mM KCl and 1.5 mM $MgCl_2$. PCR was performed in a Perkin Elmer 2400 apparatus according to the following cycling conditions: 94 ^{0}C (30 sec), 52 ^{0}C (30 sec), 72 ^{0}C (30 sec) for 30 cycles, and a final extension at 72 ^{0}C for 5 min. Molecular separation took place in 12 cm wide x 19 cm long x 0.4 mm thick polyacrylamide gels (T=5, C=3, Glycerol 7.1%) in a discontinuous Tris-HCl-Glycine buffer, pH 8.8 according to Ornstein (1964) with modifications. Electrophoresis was conducted at 18 ^{0}C, at a constant 200 V for 3 h, after which the gel was silver stained (Budowle et al. 1991) for UGB band detection.

RESULTS AND DISCUSSION

The abovementioned conditions achieve a good signal of amplification for UGB, $MgCl_2$ being, however, a critical factor; so, non-specific bands or low signal of amplified product were observed respectively at concentrations of 2-3 mM or lower than 1.5 mM (Fig. 1).

Given the size of alleles,(ranging between 387 to 411 bp), the most critical parameters for UGB phenotyping rely on the conditions for molecular separation of alleles. In our experience polyacrylamide matrixes using Tris-HCl-Glycine buffers, followed by silver staining for detection, constitute a reliable modality for UGB phenotyping (Fig. 2).

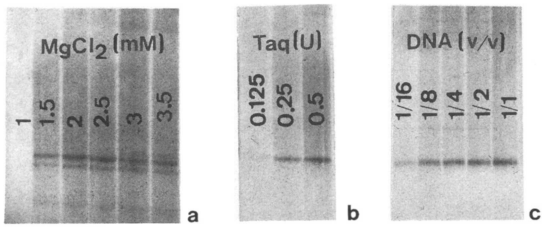

Fig. 1: Effects of $MgCl_2$ (a), Taq DNA Polymerase (b) and amount of DNA (c) in UGB amplification.

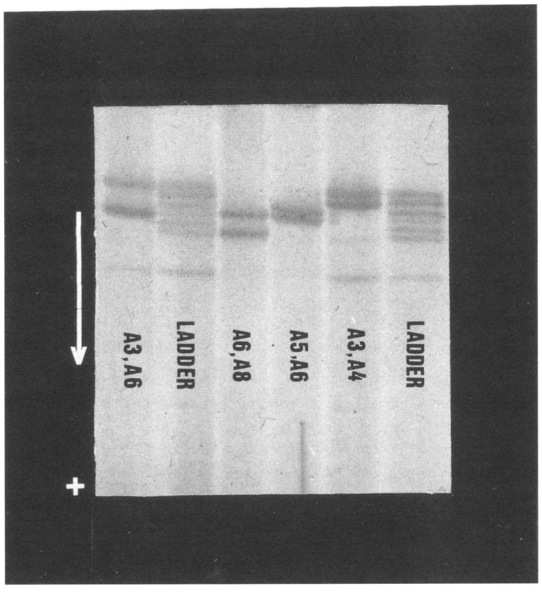

Fig. 2: Phenotype patterns of UGB after electrophoresis of polyacrylamide and Silver Staining. Alleles are denominated according to Stöhr & Weber 1994: A1 (415bp), A2 (411bp), A3 (407 bp), A4 (403 bp), A5 (399 bp), A6 (395 bp), A7 (391 bp) and A8 (387 bp).

Long distance electrophoretic runs and temperature of electrophoresis are crucial factors for an accurate discrimination of the alleles. Preliminary data in family groups (including 25 meioses from 10 informative families) are consistent with an autosomal codominant way of inheritance for this system. Notwithstanding, enlarging the number of cases is advisable in order to estimate its mutation rate.

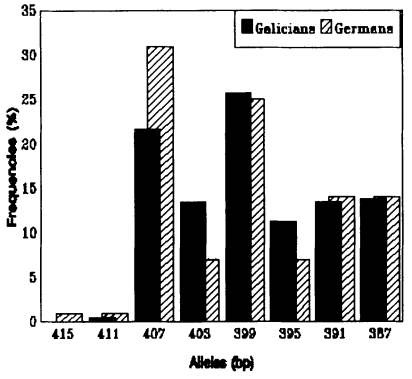

Fig. 3: Allele frequencies of UGB. Galicians (n=200), Germans (from general, n=57).

The distribution of allele frequencies obtained in this study (Fig. 3), configure unbiased values of H= 0.790 and PIC= 0.818, which indicates the degree of variability of this system. Statistical comparisons with the only data so far available, corresponding to unrelated Germans (Stöhr & Weber 1994), does not evidence significant differences after multinomial derived analyses ($G=11.212$, $0.1<p<0.2$, 7 d.f.) (Sokal & Rohlf 1969).

As UGB demands long gels for molecular separation of the alleles, this makes it less suitable than other shorter systems for co-migration of multiplexed loci, unless very log gels (i.e. sequencing gels or similar) are employed. However, the well balanced degree of polymorphism of its alleles, denotes the potential interest of this STR in this type of studies.

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