IMPROVED CONDITIONS FOR GENOTYPE DIAGNOSIS OF A STR OF THE hTPO LOCUS

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INTRODUCTION

The human genome contains numerous multidisperse non-coding regions composed by core units repeated in a variable number of times. The so called Short Tandem Repeat (STRs) correspond to tandem repetitions of only 1-5 nucleotides units [1,2,3]. Given the condition of non-coding DNA, these regions usually exhibit a high degree of variability which confers them an increasing interest in human genetic studies.

This work deals with the analysis of a tetranucleotide repeat (AATG) in the intron 10 of the human thyroid peroxidase gene (hTPO) [4] placed on the chromosome 2p. Thus, in an initial step, a reliable protocol for routine hTPO genotyping is proposed, paying particular attention to PCR-amplification and electrophoretic conditions. Formal and population genetic studies have also be carried out.

MATERIAL AND METHODS

Biological samples were obtained from whole blood from autochthonous individuals from the Galician population (NW Iberian Peninsula). The DNA was extracted by standard methods: (Phenol/chloroform)[5] or chelating resin [6]. The statistical sample consisted of 125 unrelated healthy Galicians, for genotype and gene frequencies analyses, and 20 family groups.

The hTPO amplification took place under the following conditions:

Primer sequences [4]:
AATG strand: 5'CAC TAG CAC CCA GAA CCG TG 3'
TTAC strand: 5'CCT TGT CAG CGT TTA TTT GCC 3'
Temperature profile: 30 cycles
Denaturing: 94°C for 1 min. Annealing: 63°C for 30 s. Extension: 72°C for 90 s.

The amplification mixture consisted of 20-50 ng of DNA, 200 μM dNTPs, 1 μM of both primers, 0.5 units of Taq DNA Polymerase (Promega), $MgCl_2$ 1.5 mM, in 10 mM Tris-HCl, pH 9.0, containing 50 mM KCl and 1% Triton X-100, in a total volumen of 25 μl.

Electrophoretic separation of amplified fragments was made in polyacrylamide gels (T=10, C=5, 0.4 mm thickness and 12 cm long). 0.375 M Tris-HCl pH 8.8 was used as gel buffer and 0.125 M Tris-Glycine pH 8.8 for the bridge. Electrophoresis was carried out at constant 11 V/cm for 4 h, and the detection of the hTPO bands was accomplished by Silver Staining [7].

RESULTS AND DISCUSSION

Electrophoresis.- The electrophoretic patterns of hTPO following the above conditions are displayed in Figure 1. Electrophoresis under non-denaturing and 6% urea denaturing gels lead to an identical interpretation of the hTPO genotypes (data not shown). Bands differing in 4 nucleotides can readily be distinguished. 12 cm long gel plates display sufficient separation between bands. As compared with longer gels (25 cm) a higher separation is obtained but simultaneously lesser definition of the bands is observed . Thus, although running in sequencing gels are not particularly necessary, electrophoretic separations in miniaturized gels are not advisable.

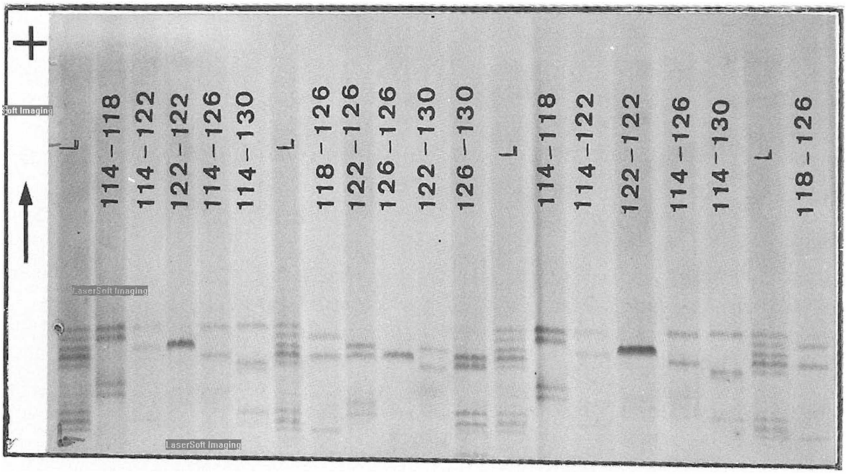

Fig.1.- hTPO phenotypes in semi-dry polyacrylamide gel electrophoresis.

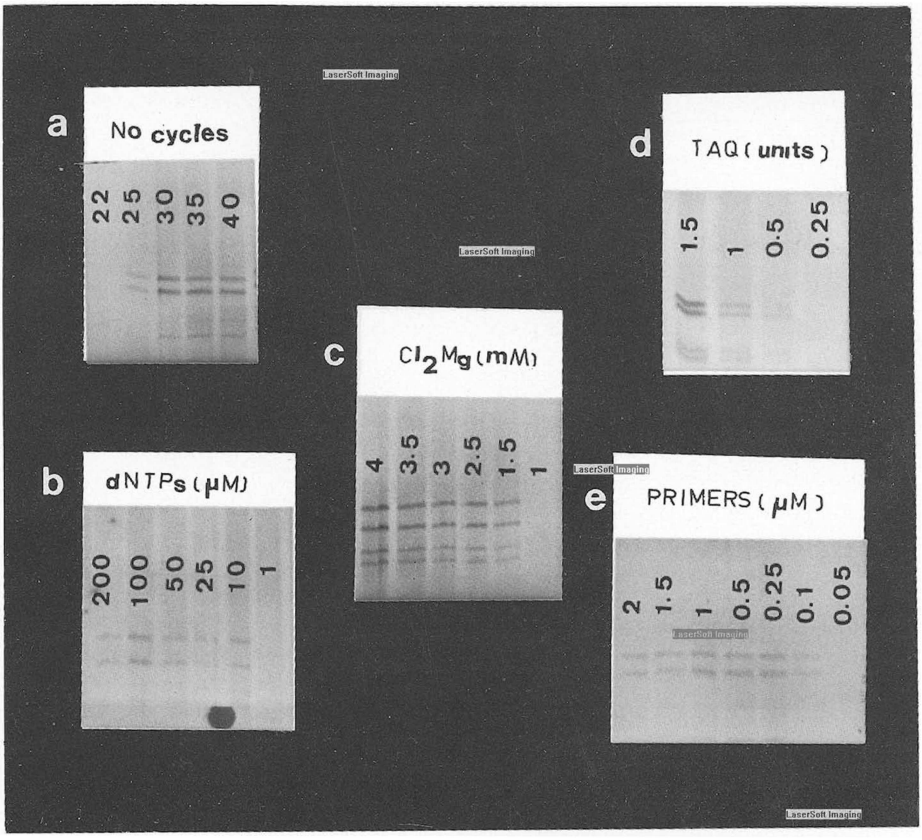

Fig. 2a-2e.- Effectiveness of amplification parameters for hTPO phenotype analysis. Fig.2a: No. cycles. Fig.2b: d-NTPs concentration. Fig.2c: $MgCl_2$ concentration. Fig.2d: Taq Polymerase (Units/25μl). Fig.2e: Primers concentration.

Amplification.-
- Cycles: Good results are obtained between 30-40 cycles. Below 25, weak or negative results are observed (Fig 2a).
- d-NTPs: Positive results in a range comprised between 10 μM to 200 μM. Higher or lower concentrations lead to negative results (Fig 2b).
- $MgCl_2$: Positive results are observed in a relatively high range (1.5 mM-4 mM). Concentrations of 1 mM or less, gave negative results (Fig 2c).
- Taq DNA Polymerase: Unlike standard higher concentrations, 1 unit of DNA polymerase/50 μl of total amplification mixture is sufficient to achieve reliable reproducible results (Fig 2d). In our experience, many of the commercially available Taq DNA polymerases allow a substantial reduction of the concentration without an appreciable reduction of the yield of amplification.
- Primers: Concentration ranging between 2-0.25 μM displayed efective amplifications. Lower concentrations lead to poor results.

Population and formal genetic studies.- Provisional results in 125 individuals (250 chromosomes) of the Galician population are summarised in Table I. This STR configures thus a value of h=0.655 and PIC=0.605. Genetic studies in 20 family groups show a codominant manner of inheritance for the hTPO alleles without exceptions.

TABLE 1.- Genotype and allele frequencies distribution of the hTPO.

GENOTYPE FREQUENCIES	
GENOTYPE	OBS (EXP)
106bp-114bp	1 (0.54)
114bp	31 (36.45)
114bp-118bp	16 (11.34)
114bp-122bp	9 (10.26)
114bp-126bp	42 (34.56)
114bp-130bp	5 (5.40)
118bp-126bp	5 (5.38)
122bp	1 (0.72)
122bp-126bp	6 (4.86)
122bp-130bp	2 (0.76)
126bp	4 (8.19)
126bp-130bp	3 (2.56)

ALLELE FREQUENCIES
106bp = 0.004
114bp = 0.540
118bp = 0.084
122bp = 0.076
126bp = 0.256
130bp = 0.040

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