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Genetic and population study of a Y-linked tetranucleotide repeat DNA polymorphism with a simple non-isotopic technique

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Abstract. A polymorphic microsatellite (Y-27H39) based on a $(GATA)_n$ repeat was recently discovered on the short arm of the human Y chromosome. We have used a simple technique based on polymerase chain reaction amplification and native polyacrylamide gel electrophoresis followed by highly sensitive silver staining to study the inheritance, the genetic stability and the allele frequency distribution of this polymorphism in the Brazilian population. We have analyzed 100 randomly chosen Caucasian Brazilian father-son pairs with established paternity. Five alleles, four base-pairs apart, were easily distinguishable. Their frequencies were: A (186 bp), 0.19; B (190 bp), 0.49; C (194 bp), 0.24; D (198 bp), 0.07; E (202 bp), 0.01. In all father-son pairs, there was complete allelic concordance. From these data, the probability of discrimination for forensic cases and the average probability of exclusion for paternity cases were both calculated to be 0.66.

Introduction

In contrast to its high degree of size heteromorphism in all populations studied (Schmid 1986), the human Y chromosome appears to have a remarkably low density of DNA polymorphisms (Jakubiczka et al. 1989; Malaspina et al. 1990). Indeed, only a limited number of restriction fragment length polymorphisms has been characterized in the Y chromosome (Ngo et al. 1986) and the few variable number of tandem repeat polymorphisms described so far in this chromosome have such large fragments that they depend on resolution by pulsed-field gel electrophoresis (Oakey and Tyler-Smith 1990).

In 1986, Arnemann et al. identified, in a Y chromosomal cosmid library, clustered GATA repeats in a clone cos27 (subclone pJA2715 = DYS19) that mapped to the short arm. To explore the potential polymorphism of this site, Roewer et al. (1992) designed flanking primers and studied this region (Y-27H39) by the polymerase chain reaction (PCR). The DNA fragments were radioactively labeled during amplification, and the products were resolved in denaturing sequencing polyacrylamide gels. In a study of 33 probands, they identified four alleles, with sizes varying from 186 bp to 198 bp; these were shown to differ by multiples of the basic GATA repeat unit, i.e., by 4 bp.

In the present paper, we report our results on a genetic and populational study of the Y-27H39 polymorphism in 100 Brazilian father-son pairs using a simple non-isotopic technique. The objectives of this study were to evaluate the allele distribution in a larger unselected sample and to study the inheritance and genetic stability of this polymorphism.

Materials and methods

The patients were randomly drawn from paternity cases studied in the Núcleo de Genética Médica de Minas Gerais (GENE/MG) in Belo Horizonte, Brazil. In all father-son pairs studied, the paternity had been previously established by DNA fingerprinting with the multilocal probes F10 (Pena et al. 1990, 1991) and/or $(CAC_5$ (Schafer et al. 1988; Pena et al. 1991). All pairs studied were unrelated and Caucasian, representing a random sample of the Brazilian upper middle class, predominantly with European ancestry, although with some Black, Amerindian and Middle Eastern contributions (Salzano and Freire-Maia 1970). DNA was prepared from peripheral blood as described elsewhere (Pena et al. 1991).

The primers used for PCR were those designed by Roewer et al. (1992) namely, Y-27H39.1: 5′-CTACTGAGTTTCTGTTATAGT-3′ and Y-27H39.2: 5′-ATGGCATGTAGTGAGGACA-3′. As a positive control, we amplified simultaneously (in the same PCR tubes) an autosomal polymorphic region from chromosome 12 (4815LR = D12S67); this is also based on a $(GATA)_n$ repeat (Roewer et al. 1992). For PCR, each sample contained 100 ng DNA template, 1 unit *Taq* DNA polymerase (Promega), buffer provided by the manufacturer, 1.5 m*M* $MgCl_2$, 1 μ*M* of each primer, and 250 μ*M* of each dNTP. The total reaction volume was 25 μl, and each sample was overlaid with 25 μl mineral oil. The PCR was carried out in an MJ Research PTC-100 Thermal Cycler for 25 cycles. Each cycle consisted of 30 s at 94°C for denaturation, 30 s at 51°C for primer annealing, and 90 s at 72°C for primer extension. After amplification, the samples were stored at –20°C until run on electrophoresis gels. The samples were resolved in 16-cm 10% native polyacrylamide gels (9.67% acrylamide, 0.33% bisacrylamide) in TBE buffer (0.09 *M* TRIS-borate, 0.002 *M* EDTA, pH 8.3) for 4 h at 150 V.

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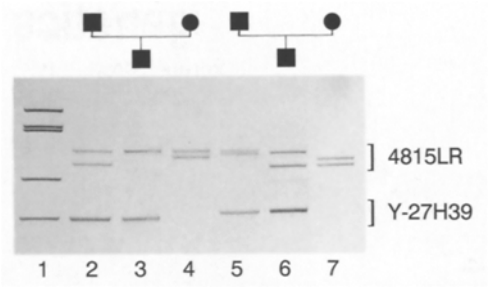

Fig. 1. Two father-son-mother trios with paternity established by multilocus DNA fingerprinting. The Y-27H39 region and the autosomal marker 4815LR (D12S67) were co-amplified by PCR. The products of amplification of Y-27H39 are only seen in males (■), whereas the 4815LR products are seen in both males and females (●). The Y-27H39 alleles seen are A *(lanes 2, 3)* and B *(lanes 5, 6)*. *Hae*III fragments of ϕ X174 with sizes 310, 281, 271, 234 and 194 bp are shown in *lane 1*

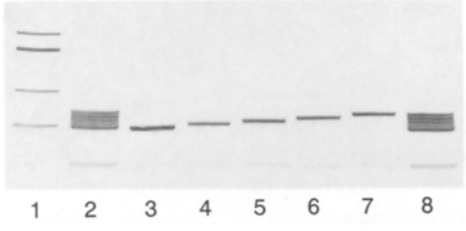

Fig. 2. Five probands displaying different Y-27H39 alleles in increasing order of size: A (186 bp, *lane 3*), B (190 bp, *lane 4*), C (194 bp, *lane 5*), D (198 bp, *lane 6*) and E (202 bp, *lane 7*). Allelic ladders are shown in *lanes 2 and 8*. *Hae*III fragments of ϕ X174 with sizes 310, 281, 271, 234 and 194 bp are shown in *lane 1*. The faint band observed under the main Y-27H39 amplification product is invariant and thus appears to be a PCR artifact

Following electrophoresis, the fragments were visualized using a simple, highly sensitive silver staining, extensively modified from Herring et al. (1982) as follows: the gels were fixed for 20 min at room temperature with 300 ml aqueous solution of 10% (v/v) ethanol and 0.5% (v/v) acetic acid, after which they were incubated with 300 ml 0.17% (w/v) silver nitrate for 25 min with agitation. The gels were then rinsed in 300 ml deionized water for 3 min, and developed in an aqueous solution of 3% (w/v) sodium hydroxide 0.1% (w/v) formaldehyde until the bands were well visualized, at which point the staining reaction was stopped with fixative solution.

Results and discussion

In males, Y-27H39 was amplified as a single band with a variable size around 190 bp. No amplification product was seen in females (Fig. 1). In the 100 pairs studied, we could resolve 5 alleles, 4 bp apart, with sizes varying from 186 to 202 bp (Fig. 2). The frequencies of these alleles were: A (186 bp), 0.19; B (190 bp), 0.49; C (194 bp), 0.24; D (198 bp), 0.07; E (202 bp), 0.01. In all cases, there was complete agreement of the alleles among fathers and sons. These allele frequencies are similar to those obtained in Germany by Roewer et al. (1992), with a much more limited population sample.

Because of their holandric inheritance and lack of recombination, Y-linked polymorphisms are extremely useful in evolutionary studies (Oakey and Tyler-Smith 1990; Spurdle and Jenkins 1991). They are also applicable in paternity testing (Chakraborty 1985), especially in deficiency cases in which the proband is male (F. R. Santos, S. D. J. Pena and J. Epplen, in preparation). Finally, they are of great usefulness in forensic identification, particularly in rape cases where their utilization avoids confusion between the victim's and the assailant's DNA (Roewer and Epplen 1992). With the non-isotopic methodology that we describe here, the Y-27H39 polymorphism is simple to study. Inheritance is holandric, and no mutations have been observed in the 100 father-son pairs studied. In Y-linked polymorphisms, the average probability of exclusion in paternity testing and the probability of discrimination in criminal identification are the same, and are given by:

$$P(Y) = 1 - \sum (P_i)^2.$$

For Y-27H39, in the population studied, these probabilities are equal to 0.66.

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