TWO DIFFERENT MECHANISMS ARE INVOLVED IN THE POLYMORPHISM SHOWN BY THE LOCUS D2S44

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INTRODUCTION

Tandemly repeated sequences (VNTRs) represent a rich source of highly polymorphic markers useful in genetic linkage analysis, genome mapping and personal identification. These VNTRs are hotspots of homologous recombination and are present throughout the human genome (Wahls et al., 1990). Mutations in the flanking region can generate or delete new restriction sites, hence contributing to increase the polymorphism.

The polymorphic locus D2S44, recognized by the YNH24 probe, was first described by Nakamura et al (1987). D2S44 locus polymorphism has been demonstrated using different restriction enzymes, e.g. MspI (Nakamura et al., 1987), BamHI (Henke et al., 1990), HaeIII (Budowle et al., 1990), HinfI (Gill et al., 1991), etc. In our study we have analyzed the databases of the D2S44 alleles in the spanish population, using two different restriction enzymes, HaeIII and HinfI, over a sample of 177 individuals. The distribution profiles obtained have been compared, in order to observe the correlation between the fragment lengths in both samples. From 354 analyzed alleles, 10 have shown a different size with regard to those expected. In 5 of these 10 individuals, the new alleles are likely generated by the creation of a new HinfI site.

MATERIAL AND METHODS

SAMPLE ANALYSIS: DNA from 177 Spanish unrelated individuals were extracted from whole blood. Two alliquots of 5-10 μg of DNA each were digested with HaeIII or HinfI, and the resultant fragments electrophoresed and blotted following the EDNAP protocol recommendations. The YNH24 probe was labelled with ^{32}P by random priming or with alkaline phosphatase.

DATA ANALYSIS: Band profiles were analyzed using a densitometer (Elscript 400, Hirschmann), as well as a computerized scanner system (EquiDNA, Pharmagen/Filosoft). The values obtained were pooled into a database that was used for the statistical analysis. A simple regression analysis was performed, taking arbitrarily the HaeIII values as the dependent variable. Those values that were out of the confidence intervals for the straight line fitted were rejected. Finally, we performed a comparison between the two equations obtained.

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RESULTS AND DISCUSSION

1.- Comparing the two data sets studied, the HinfI fragments are longer than the corresponding HaeIII ones. The average value of the difference is 1063 bp (SD = ±7.74), (Figure 1).

2.- The regression analysis suggests a linear relationship between the two data sets (Figure 2). This fact is in agreement with the existence of a VNTR polymorphism. However, some data pairs show a marked divergence with respect to the average value, and lie out of both confidence and prediction limits for the straight line fitted. When these data (3.03% of the total) are removed from the plot, the regression analysis gives significant differences respect to the formerly made, and the parameters defining the relationship are closer to the linearity (Figure 3), as could be expected in a pure VNTR polymorphism.

3.-The divergent data are listed in Table 1. In all cases, only one of the two alleles shows a significant difference from the average value (Figure 4). There are two reasons that justify the existence of these "rare" alleles:

- Loss of small HaeIII fragments (Chakraborty and Jin, 1992).
- Creation/elimination of restriction sites for the used enzymes (Waye and Fourney, 1990).

4.- The loss of small HaeIII fragments is possible in three individuals, which are homozygous for HaeIII and have HinfI alleles with length close to 2 kb (# 1, 2 and 6). The corresponding HaeIII allele must have around 1 kb in length, and the limit of the detection, using the standardized electrophoretic protocols, is in this range.

5.- Mutation of a restriction site could explain the divergence in almost all cases. Significantly, half of the divergent alleles have similar lengths (2-2.2 kb for HinfI) and show similar differences (≈500 bp). This suggests that a linkage between the mutated restriction site and a VNTR allele must exist.

6.- The analysis of the HinfI database showed that the only alleles in the range 2127-2086 bp were "rare" alleles. The alleles found in the HaeIII database in the range 1450-1750 bp, were a mixture of normal and mutant alleles. These data support the existence of a new HinfI site. However, this fact will be only proved by sequencing the 5′ and 3′ flanking regions of the D2S44 VNTR locus.

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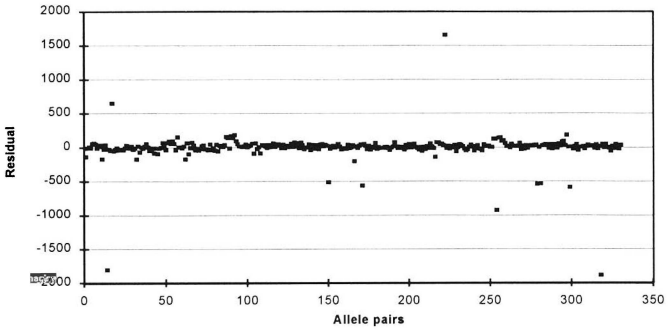

Figure 1. Plot of residuals from the average difference in the analyzed population (0 in Y axis is equivalent to 1063 bp).

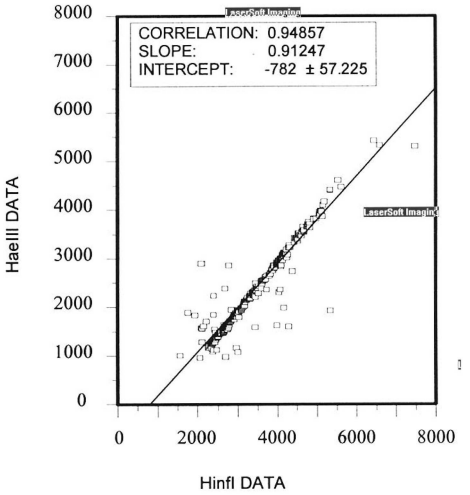

FIGURE 2. Regression of *HaeIII* data over *HinfI* data (whole population).

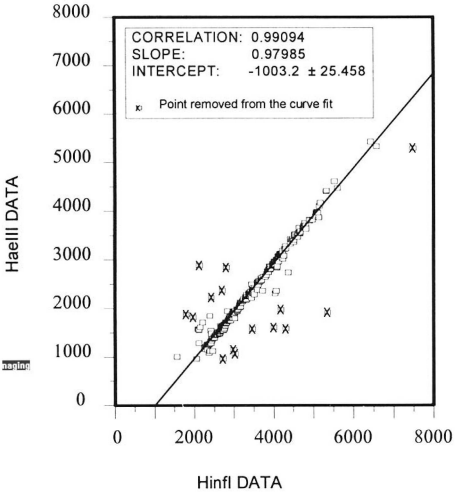

FIGURE 3. Regression of *HaeIII* data over *HinfI* data (modified population).

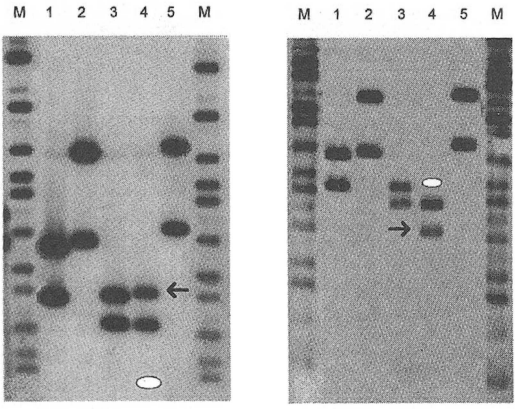

FIGURE 4. Lane 4 shows an individual carrying a "rare" allele (arrows). Blank ellipses indicate the expected positions for the "normal" alleles.

5. DNA polymorphisms in paternity testing