

MATCHING CRITERIA FOR PATERNITY TESTING WITH VNTR SYSTEMS

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ABSTRACT

The variability in duplicate testings and in mother/child comparisons of RFLP VNTR data for paternity testing was analyzed. *HinfI* digested DNA was separated by electrophoresis in agarose gels and hybridized with radiolabelled probes detecting the VNTR-systems D7S22 (g3), D5S43 (MS8), D7S21 (MS31), D12S11 (MS43), and D2S44 (YNH24). The band positions on autoradiographs were measured with a ruler with 0.5 mm resolution. Initial analyses demonstrated that, when the samples which should be compared were investigated on the same gel, the absolute difference in migration distance was the parameter with the lowest variability. Comparisons of 445 duplicate investigations on the same gel of DNA from 108 individuals showed no differences exceeding 1.25 mm. This matching criterion was used for the comparisons of 1,012 differences in 215 mother-child pairs. All mother-child differences were less than 1.25 mm except for an assumed mutation in D7S21 (MS31), and this matching criterion has been chosen for the evaluation of Danish paternity cases. The allele distributions of the five VNTR systems in 530 unrelated Danes are presented.

INTRODUCTION

We wanted to establish simple exclusion/inclusion (matching) criteria for paternity testing by means of analysis of the Restriction Fragment Length Polymorphism (RFLP) of Variable Numbers of Tandem Repeats (VNTR) regions with the single locus probe DNA technique. It was important for us (i) that the DNA investigations in paternity cases were performed with the technique which we presently use for DNA investigations in crime cases, i.e. the technique agreed upon by the European DNA Profiling (EDNAP) Group, and (ii) that the background for the matching criteria and their documentation could be understood intuitively. Here, we present our matching criteria and the allele distributions of the VNTR systems investigated in Danes.

MATERIAL AND METHODS

Individuals: Blood samples were obtained in families and paternity cases from 215 mother/child pairs, 42 men who had been excluded from paternity with conventional tests, and from further 273 unrelated, random Danes.

Isolation and digestion of DNA: Genomic DNA was isolated with phenol or 6 M NaCl. The DNA was digested with the restriction enzyme *HinfI* according to the manufacturer's specifications.

Electrophoresis: Separation of the DNA fragments was performed by electrophoresis in 0.7 % agarose gels (20 cm x 20 cm). The DNA samples from the mother, child, and

the putative father(s) in a case were investigated on the same gel. At least three lanes with Amersham ^{35}S molecular weight ladder were included on each gel. The gels were run over night until the 2.39 kb band of the ladder had migrated 14.0 cm.

Hybridization: After Southern blotting, hybridization with ^{32}P radiolabelled probes was performed as sequential reprobing on the membrane. The probes detected the VNTR-systems D7S22 (g3), D5S43 (MS8), D7S21 (MS31), D12S11 (MS43) (Wong et al 1987), and D2S44 (YNH24) (Wyman & White 1980). Autoradiography was performed on X-ray films.

Band sizing: The migrations of the bands were measured with a ruler with 0.5 mm resolution, and the kilobase values were calculated by local, hyperbolic approximation (Elder & Southern 1987).

Normalized migration length: Initial investigations demonstrated that the absolute difference in migration distance was the parameter with the lowest variability. Although the variability expressed in per cent of the kb-values was almost constant for low kb-values, the variability increased with increasing kilobase values above 6 - 8 kb. When the gels were run under standardized conditions, the kb-values could be transformed into normalized migration length (NML) values:

$$\text{NML} = \frac{800}{3.8 + \text{KB}^{1.5}} + 33.75$$

The NML-value can conceptually be understood as the migration in mm on a standard (or average) gel. The NML-values fitted the normal distribution.

Calculation of upper and lower limits of matching windows: The actual kb-value of a non-maternal band of the child matching a band in the putative father was transformed into an NML-value (NML_0), and the upper (NML_U) and lower (NML_L) values of the window were defined as $\text{NML}_U = \text{NML}_0 - 1.25$ mm and $\text{NML}_L = \text{NML}_0 + 1.25$ mm. The NML_U and NML_L values were retransformed into upper (KB_U) and lower (KB_L) kb-values of the window.

Calculation of allele frequencies in matching windows: The number of alleles in the matching window ($\text{KB}_L - \text{KB}_U$) in 530 random, unrelated Danes was counted and this number was divided by the total number of alleles investigated.

RESULTS AND DISCUSSION

Variability when DNA from a person was tested twice on the same gel: DNA was isolated with (i) phenol and (ii) 6 M NaCl from the same blood sample from 108 unrelated individuals. The two DNA preparations from each person were investigated on the same gel and hybridized with five probes giving 445 bands for the analysis. No migration differences (D) exceeded 1.25 mm. The frequencies of the differences were: D = 0.0 mm: 80.4 %, D = 0.5 mm: 18.7 %, D = 1.0 mm: 0.9 %, D = 1.5 mm: 0.0 %.

Preliminary matching criteria: Based on these results, preliminary matching criteria in paternity testing were established. Thus, the hypothesis of paternity was not rejected if the migration difference between the non-maternal band in the child and the nearest band in the putative father was less than 1.25 mm in electrophoresis on the same gel.

Test of the validity of the matching criteria: The differences between the migrations of the bands identical by descent in the mothers and their children were analyzed in 215 mother/child pairs investigated with five probes giving 1,012 bands (fig. 1). All

differences were less than 1.25 mm, except for a mutation in D7S21 (MS31). The migration differences between the non-maternal band of the child and the nearest band of the putative father were analyzed in 42 cases (210 bands) with a man who had been excluded as father by investigations with well established, conventional systems. In 17.2 % of the cases, the migration differences were less than 1.25 mm, but the majority of the differences (68.6 %) were above 2.5 mm (fig. 1). Although the matching criteria has not yet been tested critically on a larger group of father/child pairs, we have no reason to believe that the matching criteria cannot be used for father/child comparisons.

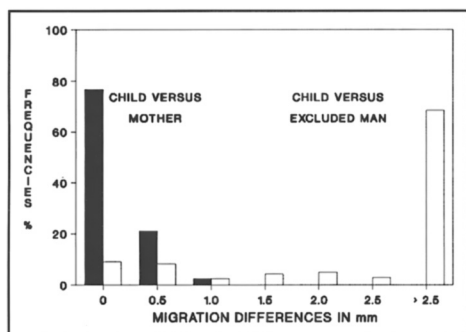


Fig. 1. Migration differences between mother/child pairs and excluded man/child pairs

Allele distributions of the VNTR systems in Danes: Figure 2 shows the allele distributions of D7S22 (g3), D5S43 (MS8), D7S21 (MS31), D12S11 (MS43), and D2S44 (YNH24) in 530 random, unrelated Danes. The frequencies were calculated in consecutive matching windows (i.e. 2.50 NML mm intervals, cf. above). The allele distribution of D7S22 (g3) was based on the analysis of 248 maternal alleles in mother/child pairs because a number of bands representing D7S22 alleles were lost for the analysis due to the size of the fragments or due to weak hybridizations with the g3 probe.

Paternity Index: Based on the estimates of the population frequencies in Danes of the paternal VNTR alleles in the child, the Paternity Index in a Danish paternity case may be calculated as previously described (Henningsen 1983).

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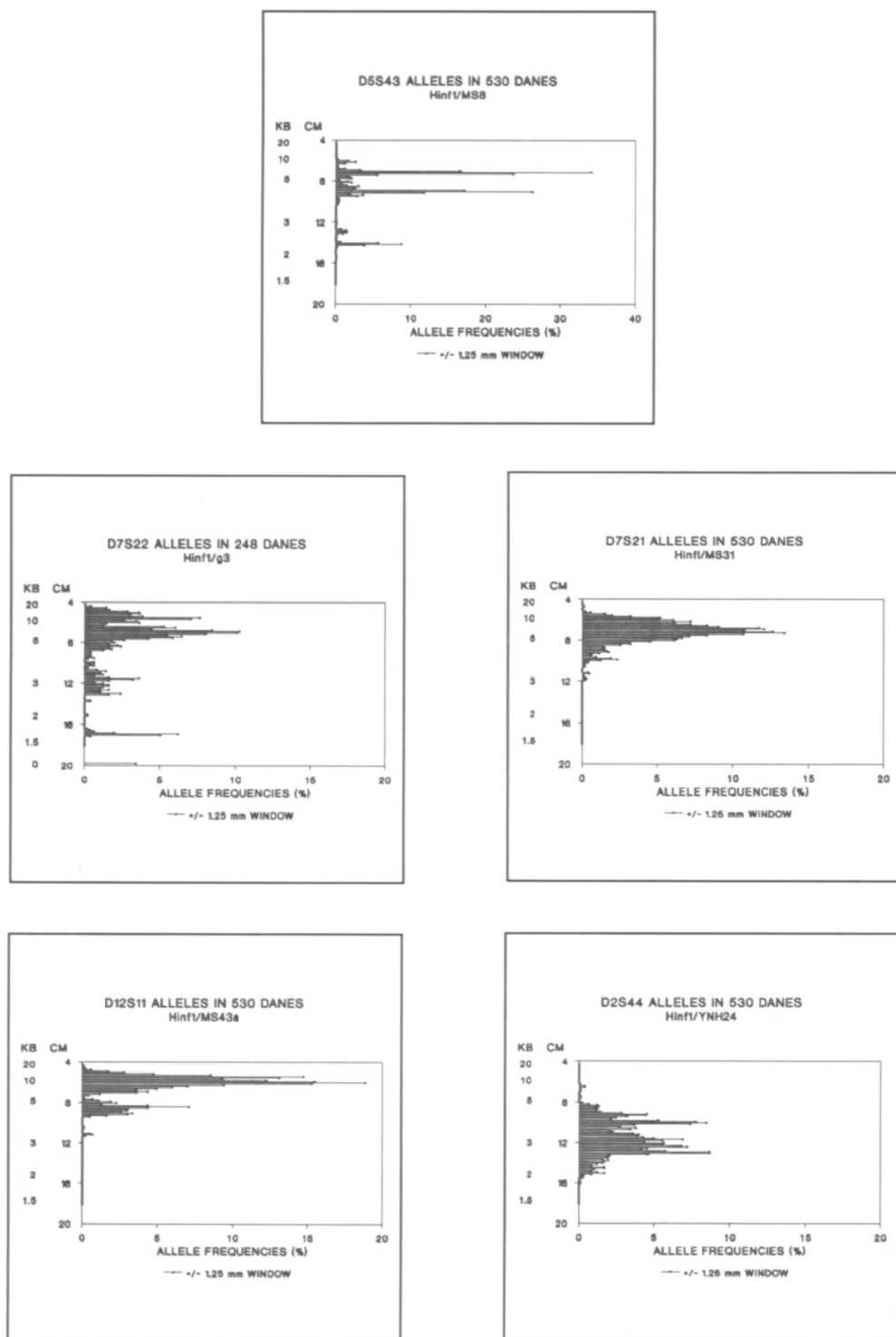


Fig. 2. Allele distribution of *D5S43* (MS8), *D7S22* (g3), *D7S21* (MS31), *D12S11* (MS43), and *D2S44* (YNH24) in Danes