Minisatellite DNA Probe MZ1.3: Band Sharing Rates Among Siblings and the Part of Informative Bands Among Children

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INTRODUCTION

After Vassart et al. (1987) had demonstrated the possibility to detect hypervariable fragments in human DNA utilizing the wildtype bacteriophage M13, Schacker et al. (1990) managed to isolate a minisatellite probe, called MZ1.3 by screening a human library with a DNA probe, which comprised the complete bacteriophage M13mp18. The 27bp repetetive sequence of MZ1.3 shows variable homology of 53% - 73% to the repetitive sequence of the protein III gene of the M13 genome, whereas it does not indicate a clear homology to the probes 33.6 and 33.15. Yet, it is suitable for producing highly informative fingerprints from human DNA (Schacker et al. 1990). In 1989/90 a collaborative study of eleven European laboratories MZ1.3 (BIOTEST; Art. No. 825020) was initiated in order to test the reproducibilty by different laboratories and to extend the population genetic data (Schneider et al., in preparation). As part of this study we analysed the fingerprints of Caucasian families with three or more children, with respect to the number of informative bands among 186 children and the band sharing rates among siblings.

MATERIAL AND METHODS

Human genomic DNA was isolated from 10 ml EDTA blood with a salting out procedure (saturated NaCl solution) and precipitated with ethanol (Miller et al. 1988). For Southern blot analysis, 10 μg of DNA were digested with 30 units of restriction endonuclease Hinf I (BIOLABS) at 37°C over night. Restriction fragments were separated on a 0.7% agarose gel in TBE buffer at a constant voltage (40 V) for 27 hours. Gel dimensions were 20x25 cm. After electrophoresis and depurination with 0.3M HCl for 30 min, Southern blotting was performed in 0.4M NaOH. The transfer of DNA onto a nylon membrane (Nytran Ny 13, SCHLEICHER & SCHÜLL) was completed after 4 - 6 hours. Alternatively a vacuum blotter with 0.5M NaOH and 1.5M NaCl as transfer buffer was used. Blotting was completed after 2 hours. The membrane was washed in neutralization buffer (1.5M NaCl, 0.5M Tris, pH 8) for 5 min. and followed by a second wash with 6xSSC for 2 min. The dried membrane was baked for 2 hours at 80°C. Nylon filters were prehybridized for 4 hours in 20 ml of hybridization solution (5xSSC, 0,1% N-lauroylsarcosine, Na-salt, 0.02% SDS) and hybridized for 15 hours at 60°C in 20 ml hybridization solution containing the digoxigenin labeled probe MZ1.3. After hybridization the membranes were washed twice in 2xSSC, 0.1% SDS for 5 min, and twice with 0.2xSSC, 0.1% SDS for 15 min. For color development a digoxigenin labeled nucleic acid detection kit (BOEHRINGER MANNHEIM) was used.

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RESULTS AND DISCUSSION

For calculation of band sharing rates as 0.5x(n/a + n/b) (n = no. of common bands; a, b = total no. of bands per individual) 222 sibling pairs were compared, which yielded an average band sharing rate for siblings of 63.0% +/- 11.2%. On the other hand 391 parent/ child comparisons yielded a band sharing rate of 59.4% +/- 11.8%, which is in the same order and nearly identical to the rate given by Schacker et al. (1990) (59.9% +/- 7.8%). Accordingly, band sharing rates between parents and children are very similar to the band sharing rates between siblings (Fig. 1 and 2). However it is of interest that the band sharing rates are not proportional to the total number of bands (Fig. 3).

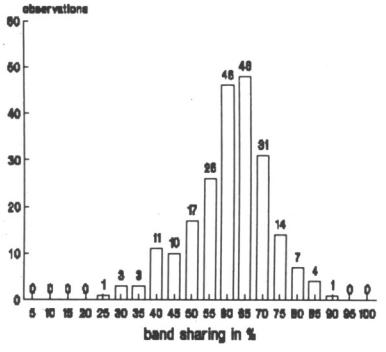

Figure 1: Distribution of all possible band sharing rates (n=222) among 186 children

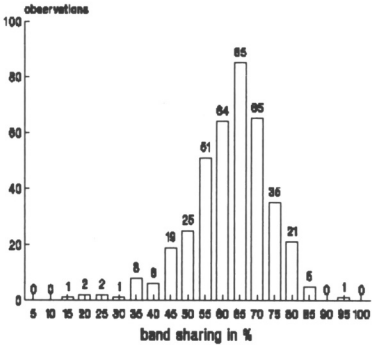

Figure 2: Distribution of band sharing rates (n=391) between parents and children

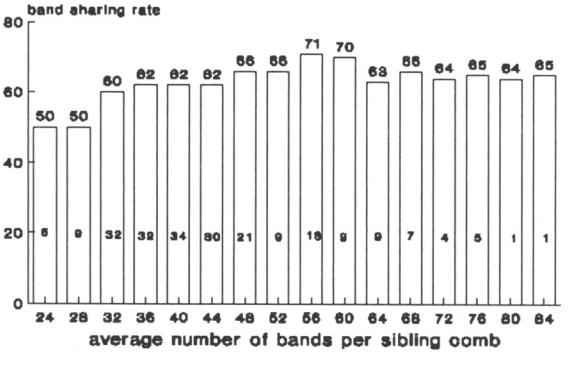

Figure 3: Relations between band sharing rates (n=189) among siblings and total number of bands. The number of observations are given in the columns

All fragments found in 186 children could be associated with parental fragments without finding a new mutation. The average total number of bands was 20.5±6.3, the average number of informative maternal plus paternal bands only 13.4±4.0 (65.4%). Noteworthy bands of all mother-child-father trios within a distance of 0.5 mm consequently were considered to be identical. With regard to the distribution of informative bands (Fig. 4) it is obvious that the number of informative bands is directly proportional to the total number of bands per child. Accordingly it is important to achieve a large number of total bands to reach a maximum of information. Nevertheless, despite of an increased number of informative bands (up to 26 per child) in cases with large numbers of total bands, the relative amount of informative bands decreased from 83.5% to 61.9% (Fig. 5). Further analysis may show whether this observation is significant.

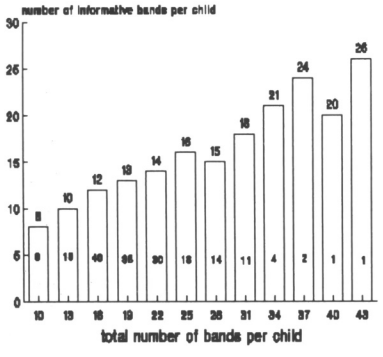

Figure 4: Number of informative bands among 189 siblings dependent on the total number of bands per child. The number of observations are given in the columns

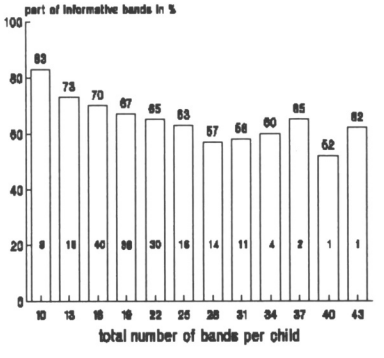

Figure 5: Part of informative bands among 189 siblings dependent on the total number per child. The number of observations are given in the columns

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