

Segregation of Single-Locus DNA-Fragments in a Large Family

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Material and Methods

The family tested is of Caucasian origin and lives in all parts of West Germany. DNA was extracted from EDTA-blood by either phenol-chloroform extraction or the salting out method (1). After quantification and control of high molecular weight 5 µg DNA digested with Hinf I was loaded onto a 0.7 % agarose gel. After Southern transfer on Hybond N (Amersham) the hybridisation was performed in a hybridisation oven. Prehybridisation was carried out for 1 h in 10x Denhardt's and 3 h in hybridisation solution (10x Denhardt's, 6 % PEG 6000, 0.1 % SDS, 1x SSC, 50 µg/ml herring sperm DNA) followed by the hybridisation in respective solution and the probe labelled with ³²P by oligo-labelling (2). Two washing steps were performed at room temperature (2x SSC, 1.5 % SDS) for 10 min followed by a washing step (15 min, 1x SSC, 1 % SDS and 1-2 washing steps (0.1 x SSC, 0.1 % SDS) at 62° C for 10 min each. DNA from some family-members were run on several gels to achieve an overlapping of the different parts of the pedigree. DNAs showing one fragment only were reinvestigated on a gel with 500 bp at the bottom of the gel in order to establish the state of zygosity.

Results

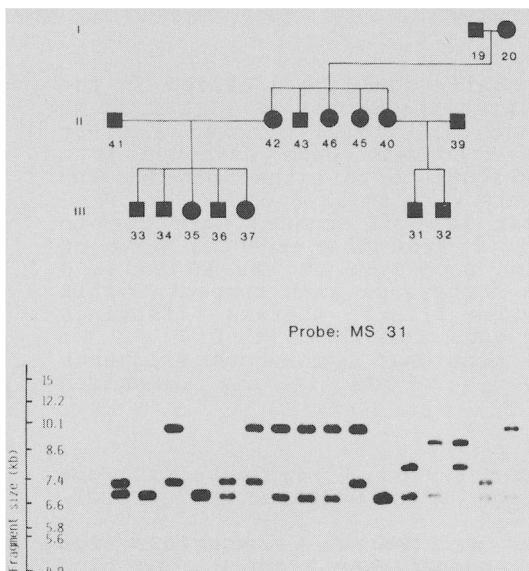


Fig. 1
Excerpt from the
pedigree. Homozygo-
sity of members
III,33 and III,35
at locus D7S21 is
proved

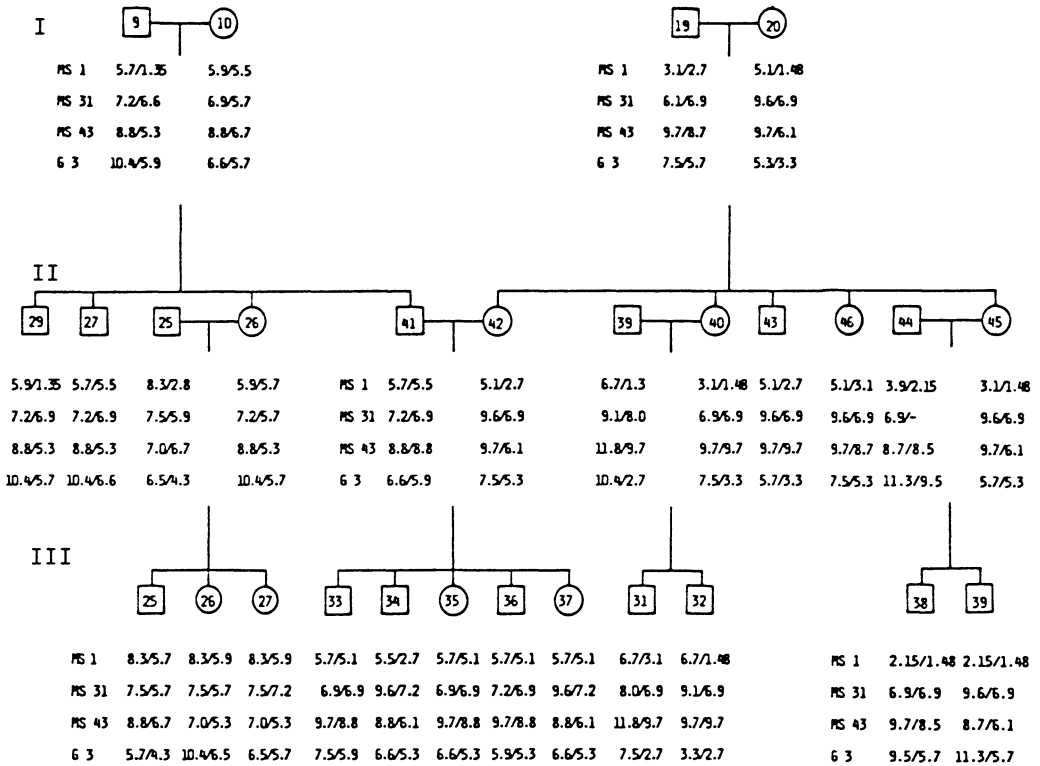


Fig. 2

Excerpt from the pedigree. Given are the respective kb sizes of restriction fragments as revealed by probing with MS1, MS31, MS 43 and G 3, respectively.

93 members of a 3 generation family could be included in the study. The fragments obtained with the probes MS 1 (D1S7), MS 31 (D7S21), MS 43 (D12S11) and G 3 (D7S22) showed a clear segregation. In two children fragments were detected with probe MS 1 which were not attributable to either parent. The differences between the fragments were small (4.8-5.1 kb, 5.8-5.9 kb). Thus we believed that the differences were due to mutational events. No deviations from the expected mode of inheritance were observed, when probes MS 31, MS 43 and G 3 were used. Offspring II/41 is homozygous with respect to his Hinf I 8.8 kb fragment at locus D12S11, whereas offsprings III/33 and III/35 are homozygous for their Hinf I 6.9 kb fragment at locus D7S21. The mentioned homozygous fragments are quite common (frequency ~ 3.5 %) in the Caucasian population.

Literature

1. Miller S, Dykes D, Polesky H (1988), A simple salting out procedure for extracting DNA from human nucleated cells. Nucl. Ac. Res. 16,3
2. Feinberg A, Vogelstein B (1983), A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity, Anal. Biochem. 132: 6-13