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# Application of Minisatellite DNA Probes to Linkage in MEN-2

Bruce A.J. Ponder,\* Alec J. Jeffreys, Nicola E. Hartley, Clare Carter, Douglas F. Easton, Hakan Telenius, and Margareta Telenius-Berg

*We describe the potential benefits and the limitations of the use of highly polymorphic minisatellite DNA probes for genetic linkage analysis of multiple endocrine neoplasia type 2A (MEN-2A). The advantage of these probes is that up to 34 loci can be examined in a single experiment, and since the loci are highly polymorphic, almost every individual in every family is informative. The disadvantage is that the DNA fragment lengths of the alleles at any given locus differ from one family to another, so that families cannot be combined, and large single sibships are needed to obtain significant linkage data. A variable DNA fragment which appears to show linkage in an initial screen of a single sibship must therefore be purified and cloned before chromosomal assignment and extension to further families is possible. These features of the probes are illustrated by a tentative linkage obtained in a large sibship with MEN-2A. (Henry Ford Hosp Med J 1987;35:161-3)*

Individuals differ in the precise nucleotide sequence of their DNA. Some of these differences can be revealed by restriction enzymes, which cleave DNA at specific sequences and thus generate different patterns of DNA fragments from different individuals. These "restriction fragment length polymorphisms" (RFLPs) have provided an extensive set of markers for genetic linkage studies needed for locating the genes responsible for inherited cancers such as multiple endocrine neoplasia type 2 (MEN-2).

A practical difficulty is that most RFLPs are useful in only a minority of families, and examine linkage at only a single genetic locus, so that many markers must be tested in several families to have a reasonable chance of success. Jeffreys et al (1) described two DNA probes which each recognize a sequence which occurs at 60 or more loci scattered through the genome. At each locus, the sequence is present in a different number of copies in the form of end-to-end (tandem) repeats. If the DNA is digested with a restriction enzyme which cuts outside the tandem repeat sequence, but not within it, the result is a series of DNA fragments of different lengths, the lengths depending on the number of repeats at each locus. At the majority of loci the degree of polymorphism is such that about 95% of individuals are heterozygous in their alleles. Almost every individual is therefore informative for linkage, and it is possible to examine linkage at many loci simultaneously. The probability that the locus for any given inherited syndrome is closely linked to within 10 centiMorgans (cM) of one of the loci recognized by the probes is about 0.2 (2). We have therefore applied these probes to the search for linkage in a large MEN-2A family.

## Methods

The analysis was performed on a sibship of nine and their affected mother, from the Swedish MEN-2A family reported

by Telenius-Berg et al (3). The diagnosis of MEN-2A was confirmed histologically in each case; all unaffected individuals had normal pentagastrin screening results at age 30 or older. DNA was extracted from EDTA anticoagulated blood samples, digested with Hinf I restriction enzyme, electrophoresed on agarose gels, and filter hybridized with the minisatellite probes 33.15 and 33.6 as described by Jeffreys et al (1).

## Results

Hinf I digests of DNA from the affected mother and from each of her nine children were Southern blot hybridized with <sup>32</sup>P-labeled minisatellite probes 33.15 and 33.6. The resulting DNA fingerprints detected by probe 33.15 are shown in the Figure. Using both probes, a total of 26 maternal DNA fragments were sufficiently well resolved to enable their segregation into offspring to be followed.

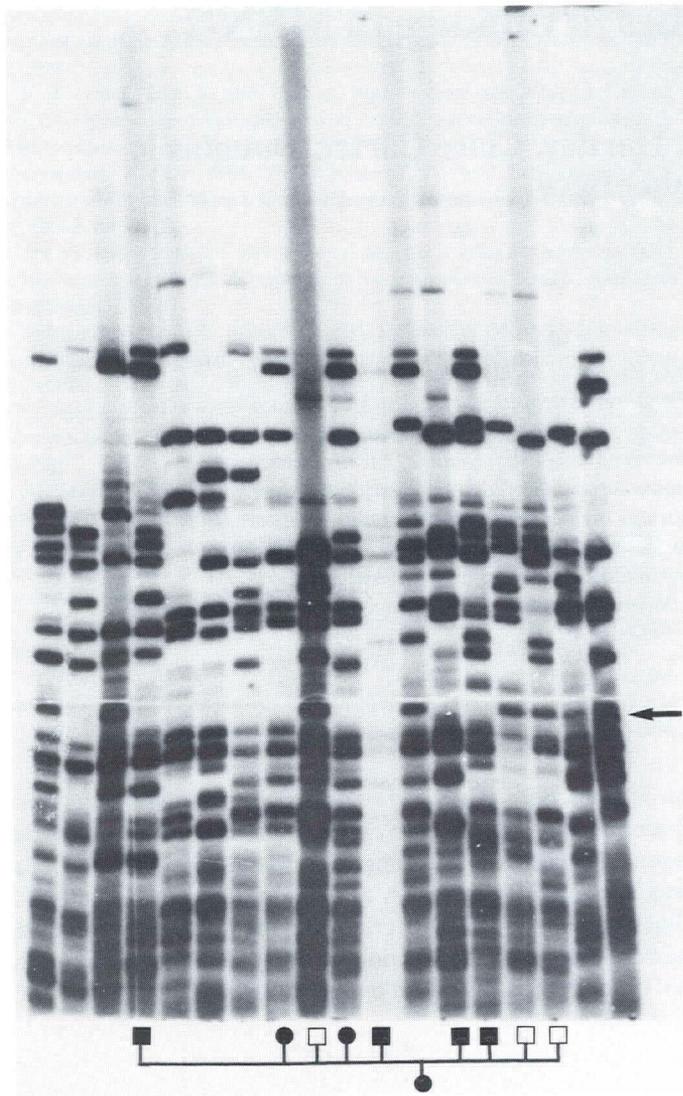
Pairwise analysis of these segregation patterns revealed only one instance of apparent allelism between two maternal bands and three instances of apparent linkage in coupling of pairs of maternal bands. The 26 maternal bands are therefore derived from 22 distinct and recombinationally separable loci.

One fragment recognized by probe 33.15 showed complete cosegregation in repulsion with the MEN-2A phenotype in nine siblings: the fragment, which was present in the affected mother, was inherited by her three children who did not have MEN-2,

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Figure—Fingerprint detected by probe 33.15. The affected mother and her nine children are shown underneath the corresponding tracks of the gel. The pedigree is inverted for easier alignment. The arrow indicates the maternal DNA fragment present in the three unaffected children (□), but not in the six affected children (■, ●). The other tracks contain samples from adjacent branches of the family, which are uninformative here because the putative linked fragment is in repulsion.

and not by her six children who did (Figure). The allelic fragment, which would show cosegregation in coupling, cannot be detected and is presumably either concealed behind other fragments, or is small and has run off the gel.

### Discussion

The finding of a DNA fragment which shows cosegregation with the MEN-2 phenotype in nine siblings is suggestive evidence that the locus for the DNA fragment is linked with the

locus for the MEN-2 gene, but by no means proof. The next step is to consider how good the evidence is, before setting out to purify the DNA fragment and clone it for further investigation of linkage.

If we ignore the slight nonpenetrance of the MEN-2 gene, the observation is that no recombinations have occurred between the DNA fragment and the MEN-2 gene in nine independent meioses. The probability that this result reflects genuine linkage can be calculated from the likelihood of obtaining zero out of nine recombinants for any given recombination fraction between zero and 0.5 (unlinked) and the prior distribution of the genetic distance between the marker and the MEN-2 gene, by application of Bayes' theorem. The calculations are set out in the Table. In the particular case described here, the probability that the locus defined by the DNA fragment lies within 10 cM of the locus for the MEN-2A gene is 0.39, and that it lies on the same chromosome (ie, a recombination fraction of less than 0.5) is 0.7. If the penetrance of the MEN-2 gene is assumed to be only 95%, adjustments to the likelihoods reduce these probabilities only slightly (to 37% and 67%, respectively). These probabilities seem high enough to justify the work involved in purification and cloning the putative linked DNA fragment. Note, however, that if only eight siblings had been available for analysis, 0/8 recombinants would have given a probability of 57% that the MEN-2 locus was on the same chromosome; and if in the sibship of nine, one individual had been wrongly categorized with respect to MEN-2A, giving 1/9 recombinants, the probability would have fallen to 24%.

Cloning the fragment is necessary because each of the loci revealed by the minisatellite probes is highly polymorphic, with many alleles represented by DNA fragments of different lengths. The same locus will be represented by different alleles in different families and in different branches of the same large family. Until the putative linked DNA fragment can be identified with a particular locus, it is not possible to extend the linkage analysis, because it is impossible to tell which DNA fragments represent that locus, and are therefore to be tested for linkage, in another family or branch. If the DNA fragment is cloned, however, this problem can be overcome. This is because the tandemly repeated DNA sequence which is recognized by the minisatellite probes has two elements: a "core," common to all loci, and a "flanking region," which differs from locus to locus in both length and sequence and is therefore specific for each locus (2). Cloned DNA fragments from a human DNA fingerprint, when used as hybridization probes under stringent conditions, therefore act as locus-specific probes for highly variable loci and can be used for linkage analysis in several families.

Minisatellite probes may provide a "shortcut" to the detection of linkage in an inherited cancer syndrome such as MEN-2A. If one family gives a negative result, it may be worth examining a second or even third family because every allele at every locus is not resolved on any single gel. It is essential, however, to have a large group of close relatives for analysis to be able to generate a result with a sufficiently high probability of reflecting genuine linkage to justify the considerable effort involved in cloning the cosegregating DNA fragment to confirm the linkage. Misclassification of even one of the scored individuals with respect to disease phenotype in a single, rela-

**Table**  
**Calculation of Probability of Genuine Linkage from Observation of Cosegregation of Minisatellite DNA Fragment with MEN-2 in Nine Siblings**

	Recombination fraction ( $\theta$ )					
	0	0.1	0.2	0.3	0.4	0.5
Relative likelihood of getting 9/9 given that marker locus is within given distance of disease locus, given by $L(\theta) = 256[(1 - \theta)^9 + \theta^9]$	256	99	34	10	2.9	1
	rest of genome					
Averaged over 0 $\rightarrow$ 0.1 etc:	168	63	21	6	1	
Prior probability that marker locus is within specified range of locations*	.22/30	.29/30	.41/30	.58/30	.19/20	
Therefore: The posterior probability that the marker locus is within 10 cm of the MEN-2A locus is:	$168 \times .22/30 \Big/ [19/20 + (168 \times .22/30) + \sum \left( \begin{array}{l} \text{likelihood} \times \text{prior probability terms} \\ \text{for all other positions on the} \\ \text{same chromosome (ie, recombination)} \\ \text{fraction less than 0.5} \end{array} \right) ] = 0.39$					

\*The genome is assumed to be 30 Morgans in length, and the MEN-2 gene is assumed for simplicity to be located on a chromosome of length 1.5 Morgans (ie, 1/20th of genome) and to be genetically equidistant from either end. Recombination fractions are converted to map units using Haldane's mapping function. Altering these assumptions within reasonable limits does not greatly change the estimated probability.

tively small pedigree is likely to be disastrous in its effect on determining statistical significance of an apparent linkage.

### Addendum

The putatively linked minisatellite DNA fragment has now been cloned and localized to chromosome 11 using a somatic cell hybrid panel (Jeffreys et al, unpublished data).\*

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\*Editors' note: See later reference to chromosome 10 linkage provided in the guest editorial, p 93.

*"On principle, it is quite wrong to try founding a theory on observable magnitudes alone. In reality, the opposite happens. It is the theory which decides what we can observe."*

*Albert Einstein—1926*