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# Early Detection of Hereditary Medullary Thyroid Cancer with Polymorphic DNA Probes

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*We have performed linkage analysis on 32 families with hereditary medullary thyroid cancer (MTC) with seven polymorphic DNA probes situated near the centromere of chromosome 10. Nineteen of these families were affected with multiple endocrine neoplasia type 2A (MEN 2A), and the remainder had MTC without pheochromocytoma. There were no instances of recombination between the MEN 2A susceptibility gene and the IRBP.H4 marker. Two other probes, TB14.34 and MCK2, are also tightly linked ( $\theta = 0.00$  and  $\theta = 0.02$ , respectively). Because TB14.34 and IRBP.H4/MCK2 are situated on opposite sides of the MEN 2A gene, screening with flanking DNA markers is now feasible. (Henry Ford Hosp Med J 1989;37:106-8)*

Several families have been identified in France in which medullary thyroid cancer (MTC) is transmitted in an autosomal dominant fashion. A national organization, the Groupe d'Etude des Tumeurs a Calcitonine (GETC), has been formed to coordinate various research efforts and to advise protocols for the screening and detection of these tumors (1). For the majority of these families, one or more members are affected with MTC and pheochromocytoma (the multiple endocrine neoplasia type 2A [MEN 2A] syndrome), but several families in which the adrenal tumor does not appear have been registered.

Linkage studies have localized the gene predisposing to MEN 2A to chromosome 10 (2,3), and this association has been confirmed in the French families (4). Genetic linkage provides a novel means for detecting the carrier state of MEN 2A prior to the onset of symptomatic disease (5). As part of an ongoing program of the International Agency for Research on Cancer, we are evaluating the usefulness of several recently developed chromosome 10 probes for the screening for the syndrome. We present the results of these investigations and discuss the application to the screening program.

## Methods

Through the efforts of the GETC and other interested physicians, 32 families with two or more cases of MTC suitable for linkage analysis were identified. Pheochromocytomas were diagnosed in members of 19 of these families; for the remaining 13 families, only MTC was inherited.

Blood samples were collected from available key family members and lymphoblastoid cell lines established when required. DNA was isolated and digested with the appropriate restriction enzymes and tested with the seven probes listed in the Table (6-11). Genetic distances were estimated with the

LINKAGE program (12) using published age-of-onset curves (13), equal male:female recombination, and a gene frequency of  $2 \times 10^{-5}$ .

## Results

Each family was tested with between two and seven of the available probes. The polymorphisms and the results of the linkage analysis are summarized in the Table. For IRBP.H4, polymorphisms are detectable with two restriction enzymes, BglII and MspI. The IRBP.H4, MCK2, and TB14.34 probes were closely linked to the MTC gene and proved to be the most valuable for screening. A single recombinant was seen with MCK2 in a family with MTC without pheochromocytoma. One patient, from a family without pheochromocytoma, was recombinant (or a nonpenetrant carrier) with both MEN203 and TB14.34. Recombination was seen in the MEN 2A families with TB10.16 and 48.11 only. The recombination fractions and confidence intervals are compatible with those reported elsewhere (14).

## Discussion

The availability of a group of closely linked polymorphic DNA markers permits the early diagnosis of the carrier state of the MEN 2A syndrome within an acceptable degree of uncer-

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**Table**  
**Summary of Linkage Results of GETC/IARC Families**

Probe/Restriction Enzyme*	Families Tested	Number Informative	PIC†	Maximum lod score	$\theta$	Upper Confidence Interval of $\theta$ ‡
IRBP.H4/BglIII (6)	32	21	0.22	11.23	0.00	0.07
IRBP.H4/MspI (7)	7	3	0.36	1.44	0.00	0.31
TBIRBP9/TaqI§ (8)	12	6	0.36	—	—	—
MCK2/MspI (9)	27	18	0.32	14.28	0.02	0.08
TB10.163/MspI (10)	22	20	0.48	2.54	0.12	0.27
MEN203/BglIII	19	13	0.35	2.71	0.06	0.25
48.11/TaqI¶	19	8	0.36	4.38	0.06	0.19
TB14.34/TaqI (8)	13	8	0.37	2.77	0.00	0.16

\*Probes were digested with restriction enzymes listed.

†PIC = polymorphism information content.

‡The upper confidence interval is based on the one-lod method (11).

§For the TBIRBP9 probe, the cumulative lod score was negative at all recombination fractions tested (lod = -0.93 at  $\theta = 0.01$ ).

||Y. Nakamura, personal communication, 1989 (unpublished data).

¶The 48.11 probe is a cDNA encoding mannose-binding protein, localized to 10q11.2 (R.A.B. Ezekowitz, personal communication, 1989 [unpublished data]).

tainty (5). The number of probes suitable for screening is expanding, and the consultant geneticist has several alternatives for diagnostic testing. Because DNA testing is time-consuming and relatively expensive, it is worthwhile to attempt to define a strategy that will permit the diagnosis to be made with high accuracy at the lowest cost. The following general principles are useful in selecting an initial set of screening probes.

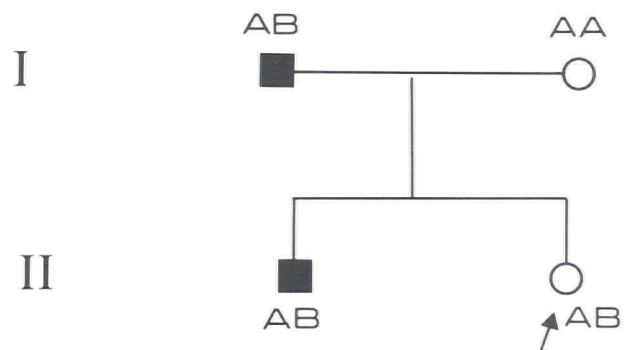
### Recombination fraction

The closer the linkage between the restriction fragment length polymorphism (RFLP) and the MTC gene, the more precise the assigned risk. The principle is illustrated in Fig 1. The parents of the girl II-2, age 15, inquire about her risk of being a carrier. If DNA testing proves to be informative, the risk assigned to the girl will be a function of the recombination rate between the marker system employed and the susceptibility gene. For example, if the haplotypes pictured were those of the MCK2 probe ( $\theta = 0.02$ ), her risk estimate would be 96.1%. The same results with either MEN203 or I48.11, which are more distantly linked ( $\theta = 0.06$ ), yield a risk of 88.8%. Were the girl to inherit the AA haplotype instead, her risk of being affected would be 3.9% ( $\theta = 0.02$ ) and 11.2% ( $\theta = 0.06$ ). The large cumulative lod scores obtained with the probes RBP3 and MCK2 reflect their proximity to the MTC gene.

The ability to provide a precise risk figure also depends on the accuracy to which the recombination fraction is known. The upper confidence limits bounding the recombination fractions for IRBP.H4 and MCK2 are acceptably narrow, but the chromosomal locations of some more recently available probes are not precisely known.

### Probe information content

To permit the genetic diagnosis of a child, an affected parent must be heterozygous for at least one marker of interest. The expected value of a probe can be measured in terms of the probability of a carrier being heterozygous at the marker allele. The polymorphism information content (PIC) is a figure that predicts



*Fig 1—Hypothetical pedigree of nuclear family informative with RFLP analysis. Solid figures are affected members (squares = males, circles = females), and letters represent marker alleles.*

the probability that a child of a random mating will be informative at the marker locus (15). Twenty of the 22 families analyzed with the TB10.163 polymorphism were informative to some extent. This system of three polymorphic alleles had the highest PIC of any probe used, but unfortunately the marker is only loosely linked.

### Flanking markers

A pair of informative markers located on opposite sides of the MTC gene may be used jointly in calculating a very precise recurrence risk. For example, consider the situation illustrated in Fig 2. The affected mother II-2 has inherited both the TB14.34 allele B and the MCK2 allele C from her affected father. Therefore, these two markers and the disease gene must be located on

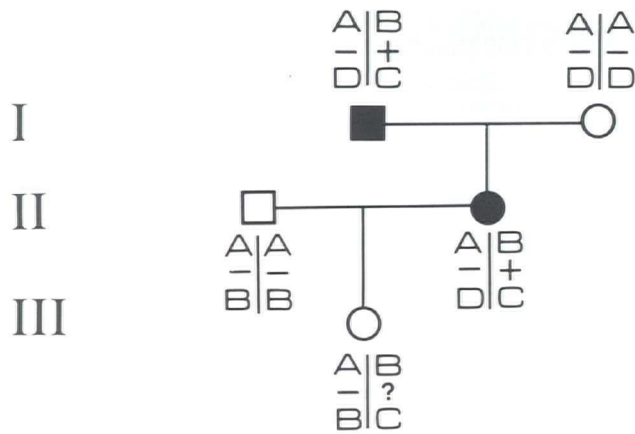


Fig 2—Hypothetical pedigree of family informative with flanking markers. (Upper letter represents *TB14.34* allele, lower letter represents *MCK2* allele, and phase is indicated by the vertical divider.)

the same chromosome. Her child has inherited both linked markers. Barring a double crossover (an extremely unlikely event), the child will have inherited the disease gene as well. Based on current map distances (14), this risk is above 99%.

#### Linkage disequilibrium

When polymorphic marker alleles are in genetic equilibrium with a disease gene, a marker haplotype will not, by itself, provide information about carrier risk. This is not the case when the marker and the susceptibility gene are in linkage disequilibrium. If a susceptibility gene is positively associated in a population with a rare allele, those carrying the rare allele will be at increased risk for carrying the mutation as well. This principle has been exploited for the prenatal screening of cystic fibrosis (16). Our early results show the *MTC* gene to be associated with the rare allele of the *IRBP.H4/BgIII* polymorphism (17). This association helps explain the high lod score achieved with *IRBP.H4* despite its lower degree of informativity. Our findings must be confirmed in other *MTC* families and in diverse populations before the application of linkage disequilibrium to risk estimation can be practical.

#### Ease of interpretation

Two polymorphic systems are available for the *IRBP.H4* probe, with restriction enzymes *BgIII* and *MspI*. In our experience the alleles of the *MspI* polymorphism have not always been clearly distinguishable. Currently, we reserve this probe for families that are not informative with *RBP3/BgIII*.

We have not yet observed genetic heterogeneity among our group of 19 *MEN 2A* families studied. Linkage results of the families with *MTC* without pheochromocytoma, although suggestive, are not yet definitive (17), and we await additional data

before we can be confident in screening this subgroup of patients.

The optimal strategy for choosing probes for screening of patients for *MEN 2A* will be determined by considering these factors. Based on our experience of 32 families, we feel that initial screening efforts should employ the *MCK2/MspI*, *IRBP.H4/BgIII*, and *TB14.34/TaqI* polymorphisms. This limited selection provides a high degree of anticipated heterozygosity as well as the potential for exploitation of markers flanking the *MTC* gene.

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