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Nine chromosome 10 DNA markers (FNRB, D10S34, D10Z1, MEN203, D10S94, RBP3, D10S15, MBP [48.11], D10S22) were typed in two large Canadian pedigrees with multiple endocrine neoplasia type 2A (MEN 2A). These markers and the gene for MEN 2A (MEN2A) are believed to be in one linkage group spanning approximately 15 cM (male). MEN203 and D10S94 were informative and tightly linked to MEN2A with no recombinants observed in 26 meiotic events. D10S15 (MCK2), widely used in DNA genotyping predictions, demonstrated two recombinants in these two families. The use of multiple flanking markers increases both the likelihood of informativeness and the accuracy of risk assessments for predictive testing. We were able to assign a risk estimate for all 10 at-risk individuals. (Henry Ford Hosp Med J 1992;40:224-6)

Multiple endocrine neoplasia type 2A (MEN 2A) is the association of three tumors: medullary thyroid carcinoma (MTC), pheochromocytoma, and parathyroid tumors. Early recognition and removal of occult MTCs significantly reduces the risk of subsequent metastases, and the screening of at-risk relatives of affected individuals for early neoplastic changes is encouraged. MEN 2A is a dominantly inherited genetic disorder which has been mapped to chromosome 10 [see (1) and references therein]. Screening can be selectively targeted to individuals at high risk of developing cancer through the use of closely linked DNA markers (2,3). In countries where systematic screening programs for MEN 2A have been instituted (France, Germany, Great Britain, the Netherlands, Sweden), 25% of MTC cases have been found to be familial.

We present a DNA analysis for screening two such families. These pedigrees contain members located in various provinces of Canada. Nationwide collaborations which assemble various branches of MEN 2A families can help in the early detection of individuals at high risk for MTC.

Methods

Strategy for linkage analysis

The MEN 2A gene (MEN2A) was first assigned to chromosome 10 tightly linked to the interstitial retinol binding protein (RBP3) locus ($\theta = 0.02$) and to the anonymous MCK2 sequence ($\theta = 0.05$) at 10q11.2 (4,5). More distal markers on the long arm of chromosome 10 include the mannose binding protein cDNA probe 48.11 ($\theta = 0.6$) and the anonymous probe TB10.16 ($\theta = 0.10$). Two flanking markers have been identified on 10p: the gene for the beta subunit of the fibronectin receptor (FNRB) ($\theta = 0.06$) and the anonymous sequence TB14.34 ($\theta = 0.07$). Recently, a number of centromeric markers have been found to be informative and show no recombination with the MEN 2A locus. These include the centromeric alphoid sequence D10Z1; MEN203 and D10S94 (Fig 1).

By using a combination of flanking and centromeric restriction fragment length polymorphisms (RFLPs), almost all individuals will be heterozygous and all families potentially informative. If carrier probabilities approaching 0% or 100% can be obtained in at-risk individuals, those identified at high risk can be targeted for selective screening for early symptoms. We have used nine chromosome 10 markers to analyze the two Canadian families [Table 1, (6-15)].

DNA analysis

DNA was extracted from peripheral blood lymphocytes with a modified phenolchloroform method. Five µg aliquots of DNA were digested with each of six enzymes: BanII, PvuII, HinfI, Bg1II, TaqI, and MspI. The DNA fragments were separated by electrophoresis on 0.8% agarose gels with TPE buffer. The DNA was denatured and blotted onto charged nylon membranes (Nytran) by the passive Southern method and baked at 80 °C for 1 to 2 hours before being hybridized with [^{32}P]-dCTP radiolabeled probes. Blots were washed under high stringency conditions and exposed to Kodak X-AR Omat film with two High Plus intensifying screens at −85 °C for one to seven days. The blots were then subjected to high temperature/low salt stripping and rehybridized repeatedly. See Table 2 for the order of probes

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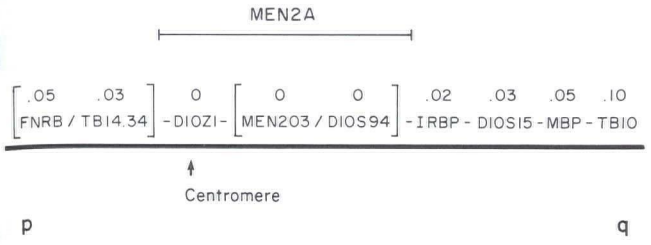

Fig 1—Chromosome 10: A genetic map of nine markers flanking the MEN 2A locus. Recombination fractions from the centromere *(D10Z1) (combined male and female) are shown above each marker as a percentage.*

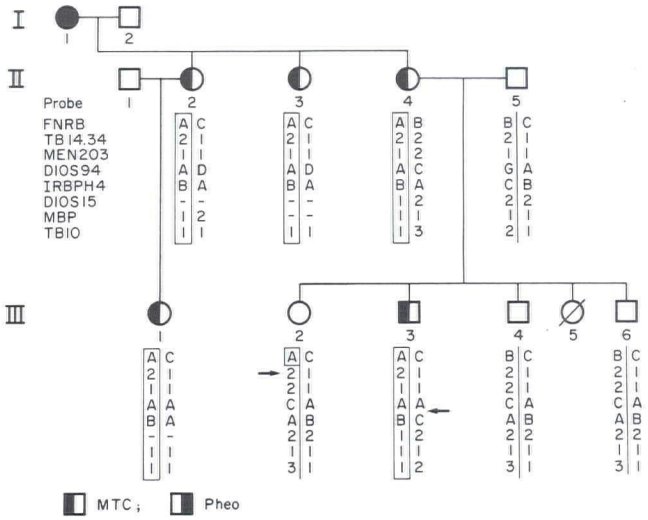

Fig 2—Family 1 pedigree and haplotype analysis with chromosome 10 RFLP loci. FNRB, D10S94, and IRBP.H4 alleles represent individual marker haplotypes using multiple RFLPs for each probe. The boxed haplotype is that segregating with the MEN 2A gene. The arrows indicate the positions of recombinations in affected individuals.

used. Blots probed with TB14.34 and MEN203 were hybridized in the presence of 100 µg/mL of sheared human placental DNA. LOD scores were calculated with the LINKAGE program (16) using a gene frequency of 1:50,000 and published penetrance figures (2).

Results and Discussion

Individuals in these extended families are highly motivated, and participation of both affected and unaffected members has been good. The two families consist of 26 sampled individuals, including 12 affected individuals. Eight had MTC only and four had both MTC and pheochromocytomas.

Family 1 (Fig 2) was referred to us for DNA analysis for three individuals in generation IV with a 50% a priori risk (at birth) of inheriting the MEN 2A allele from their mother II-4. Markers FNRB, D10S34, MEN203, D10S94, RBP3, D10S15, and D10S22 are all informative. Segregation of these is such that all at-risk individuals have been assigned considerably lowered risk of inheriting the mutant allele (Table 3). Individuals III-2, III-4, and III-6 are assessed at < 1%, given both flanking and centromeric informative meioses. There has apparently been a recombination between markers FNRB and MEN203 in individual III-2, but the MEN 2A gene is more likely to have segregated with the closely linked centromeric marker MEN203.

Family 2 is somewhat more complex. Fig 3 shows the haplotypes most likely segregating in this family. Flanking markers FNRB and D10S15 are informative. MEN2A segregates with the haplotype B21A1121 in the second generation. Therefore, individuals II-5, III-6, III-7, III-8, III-10, IV-1, and IV-2 all have a lowered risk for inheriting the MEN 2A allele. Individuals III-1 and III-4 each show a recombination between FNRB and D10S15. MEN2A segregates with D10S15 in III-1 and with FNRB in III-4. Individual III-1's children are at low risk of having inherited the mutant MEN 2A allele since III-1 is informative with the centromeric probe D10S94 and with D10S15.

Ten unaffected individuals at 50% risk at birth were offered risk assessment (Table 3). For all of these, DNA analysis resulted in risk assessments sufficiently low which appear to justify their removal from the screening program. Baseline pentagastrin tests have all been normal. There were no equivocal results.

Table 1
Chromosome 10 Pericentric DNA Markers

Locus	Probe	Location	Res. Enzyme	PIC
FNRB (6)	pGEM32	10p11.2	BanII Hinf	0.71
D10S34 (7)	TB14.34		TaqI	
D10Z1 (8)	p10RP8	10 CEN-q11.2	HinfI	0.50
MEN203 (9)		10 CEN	Bg1II	
D10S94 (10,11)	EC0350	10 CEN	MspI TaqI	
	c23	10 CEN	PvuII	
RBP3 (4,12)	IRBP-H4	10q11.2	Bg1II MspI	0.67
D10S15 (13)	MCK2	10q11.2	MspI	0.35
MBP (14)	48.11	10q11.2-21	TaqI	
D10S22 (15)	TB10.163	10q21.1	MspI	

PIC = polymorphic information content.

Table 2
Strategy for DNA Screening Analysis

Rest. Enzyme	Probes			
BanII	c23			
PvuII	FNRB			
HinfI	D10Z1	FNRB		
Bg1II	IRBP-H4	MEN203	FNRB	
TaqI	48.11	EC0350	TB14.34	
MspI	MCK2	IRBP-H4	ECO350	TB10.163

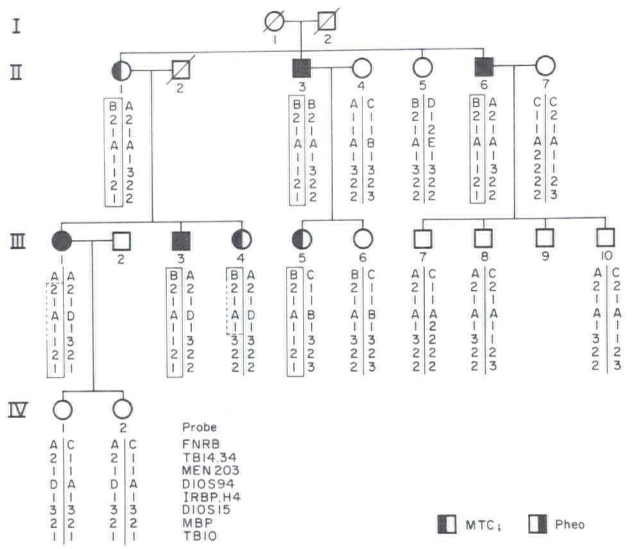

Fig 3—Family 2 pedigree and haplotype analysis with chromosome 10 RFLP loci. The boxed haplotype is that segregating with the MEN 2A gene. Recombinations are evident in individuals III-1 and III-4. These occurred in II-1 within the boxed areas bounded by the dashed lines.

The use of both flanking and centromeric RFLPs in MEN 2A families ensures that preclinical diagnoses are as accurate as possible. The strategy employed here provides a comprehensive analysis using as little as 30 μg of DNA. As more centromeric markers are identified, and as the mapping of MEN 2A improves, the accuracy of diagnosis will improve.

Acknowledgments

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Table 3
Risks for MEN 2A Family Members to be Gene Carriers

Family	Individuals	Clinical Risk (%)	Risk with DNA Markers (%)
1	III-2	23	< 1
1	III-4	23	< 1
1	III-6	41	< 1
2	II-5	9	< 1
2	III-6	23	1.3
2	III-7	33	< 1
2	III-8	41	< 1
2	III-10	23	< 1
2	IV-1	33	< 1
2	IV-2	33	< 1

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