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Practical Guidelines for DNA-Based Testing in Multiple Endocrine Neoplasia Type 1

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Multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominant predisposition to neoplastic lesions of the parathyroid glands, the neuroendocrine pancreas, and the anterior pituitary gland. The predisposing genetic defect was localized to the long arm of chromosome 11 by genetic linkage analysis in three affected families. By analyzing six MEN 1 families with 14 DNA marker systems located close to the MEN 1 gene, we have developed a method to identify carriers of the MEN 1 predisposition. We describe practical aspects of such DNA-based diagnostic procedures. (Henry Ford Hosp Med J 1992;40:173-6)

It is generally believed that morbidity and mortality in neoplastic diseases may be reduced by prevention, early detection, and by identifying individuals "at increased risk." In multiple endocrine neoplasia type 1 (MEN 1) the two latter goals are approached by offering biochemical screening programs to the members of these families. Since the MEN 1 predisposition has a genetic origin, the utilization of such screening programs is largely dependent on factors such as mode of inheritance, penetrance, expression, and age of onset. The familial and genetic nature of the disease was first pointed out by Wermer (1) in 1954 who suggested that an autosomal dominant gene with high penetrance controlled the trait. The clinical picture is variable, depending largely on the glands involved and whether or not the lesions hypersecrete symptom-causing hormones. The parathyroid glands are most frequently involved, followed by the neuroendocrine pancreas and the anterior pituitary gland. It has been shown that the age of diagnosis may be lowered by approximately two decades by screening (2). On the other hand, the reported ages of onset range from 6 to 81 years (3,4) which makes it difficult to exclude family members from being at risk. Thus, children of unaffected family members at risk are sometimes also included in the screening programs. Furthermore, the much more common sporadic counterparts of MEN 1-associated neoplastic lesions may mimick the MEN 1 phenotype. Hence, the diagnosis of the MEN 1 predisposition would benefit from a method to detect carriers of the gene (MEN1).

Methods for DNA Testing

Clinical cases

The five Swedish families with MEN 1 have been described elsewhere (5,6). The sixth family is a large pedigree from Tasmania, first reported by Shepherd (7) in 1985 in which over 150 affected individuals from 2,300 family members spanning eight generations had been traced back to a woman who came to Tasmania in the middle of the last century (8). The description of this patient recorded by the ship's surgeon is consistent with the diagnosis of MEN 1.

The scoring for MEN 1 was conservative in order to avoid incorrect diagnoses. Unaffected individuals at risk were scored as "normal" only if they had passed the age of 35 years and had undergone repeated and extensive biochemical screenings (2) with no signs of the disease, while offspring of MEN 1 cases who did not fulfill these criteria were scored as "unknown."

DNA probes and Southern analysis

A set of DNA probes suitable for DNA testing is described in the Table. D11S288/pTHH26, D11S149/p3C7, PYGM/pMCMP1, and D11S146/pHB159 are available from ATCC. CD20 was obtained from T. F. Tedder, pcAGP9/PGA from B. Zelle, D11S97/pMS51 from ICI, INT2/SS6 from N. Spurr, clone 8/D11S751 from T. Rabbits, and the newly isolated cosmid clones for D11S480, D11S427, and D11S449 from Y. Nakamura. New allele systems were identified through locus expansion in cosmids for PGA (cCLPGA12), PYGM (cCL15), D11S146 (cCL59), and D11S751/clone 8 (cCL8). Two new cosmid clones, D11S750/cCLGW4 and D11S807/cCLGW39, were isolated from a library constructed from radiation-reduced somatic cell hybrids containing the 11q12-q13 region (9).

Three marker systems (D11S146, D11S751, and INT2) with two separate 2-allele restriction fragment length polymorphisms (RFLPs) can be combined to 4-allele systems by haplotyping. The markers D11S288 and D11S149 can also be combined by haplotyping to increase their relative information con-

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Table
DNA Markers Linked to the MEN 1 Locus

Locus Name	Probe Name	Enzyme	Constant Bands	Allele Sizes (kb)	Allele Frequency	Electrophoresis* (% agarose, length)
D11S288	p3C7	MspI		5.7	0.72	
D11S149	pTHH26	PvuII		3.1	0.28	
				5.2	0.14	
				3.2	0.84	
CD20	pB1-21	MspI	2.8	9.0	0.47	
				6.0	0.53	
D11S480	cCI11-319®	PstI	several	3.0	0.50	
				1.4	0.50	
PGA	pcAGP9	EcoR1		19.0	0.06	
				16.6+15+3.5	0.37	
				16.6+15+12+3.5	0.54	0.5-0.6%, 30 cm
	cCLPGA12®	PvuII	several	11	0.37	
				6+5	0.63	
PYGM	pMCMP1®	Msp1		2.2 – 2.5	5 alleles	1.1%, 30 cm
	cCL15®	EcoR1	14; 6.6; 5.4	19.0	0.11	
				17.5	0.89	0.6%, 20-30 cm
D11S427	cCI11-4®	EcoR1	21; 16	9.0 – 9.6	4 alleles	0.5-0.6%, 30 cm
D11S750	cCLGW4®	SacI	several	3.6	0.80	
				2.4	0.20	
D11S807	cCLGW39®	SacI	several	5.4	0.70	
				4.8	0.30	0.9%, 20 cm
D11S449	cCI11-219®	PvuII	several	3.7	0.33	
				3.0	0.67	
D11S97	pMS51	HaeIII/TaqI		1.3 – 4.3	9 alleles	1%, 20 cm
D11S146	pHBI59	TaqI		1.2	0.55	
				0.8	0.45	
	cCL59®	EcoRV	24; 18; 7.4	7.0	0.22	
				6.4	0.78	0.9%, 20 cm
D11S751	cCL-8®	EcoRV	20; 5.2; 2.2	6.6	0.50	
				5.8	0.50	0.8%, 20 cm
		SacI	16; 12; 4; 3	10.0	0.50	
				9.2	0.50	0.8%, 20 cm
	clone 8	EcoR1		7.4	0.89	
				6.7	0.11	0.6%, 20-30 cm
INT2	SS6	TaqI		4.1	0.60	
				2.3	0.40	
		BamHI		8.4	0.63	
				5.6+2.8	0.37	

*Electrophoresis conditions are only indicated when special conditions are needed.
Note: Loci in bold indicate "anchor markers" previously mapped on reference families. Loci in italics indicate those located closest to the MEN 1 gene.
® indicates probes with repetitive elements for which human DNA was included in the hybridization.

tent. Haplotyping of the two markers for the PYGM locus and the combined D11S97 + D11S146 markers is also possible since these markers are closely linked. Details for isolation and restriction enzyme cleavage of DNA, Southern analysis, and hybridization to radioactively-labeled probes have been described (10).

Discussion

Localization of the MEN 1 gene to chromosomal region 11q11-q13

Localization of tumor predisposing genes has been facilitated in several conditions such as retinoblastoma and familial polyposis coli by identification of constitutional chromosomal aberrations. This has not been possible in MEN 1. Our working hypothesis was that MEN 1-associated tumors may result from unmasking of a recessive mutation according to the two-mutation model originally postulated by Knudson (11). Genetic rearrangements in such tumors would indicate on which chromosome MEN1 is situated. Such rearrangements would be expected in all tumor cells, restricted to a specific chromosome (12), and would serve to eliminate the wild type allele of MEN1.

We compared constitutional and tumor genotypes at different chromosomal loci in two brothers with insulinomas who had inherited the disease from their mother (5). Markers on 17 chromosomes showed retained genotypes, but both tumors had lost one of the constitutional alleles at all informative loci on chromosome 11. Combined pedigree and genotype analysis revealed that the lost alleles were always derived from the unaffected father (5). These findings fit the hypothesis that the tumors resulted from elimination of the two MEN1 alleles (13). Subsequently, the tentative localization was confirmed by demonstration of linkage between MEN1 and the PYGM locus at 11q13 in three families (5). No meiotic recombinations were observed and the odds in favor of linkage were $10^{4.37}$:1, which is well above the level of significance accepted for linkage analy-

sis. The localization was later confirmed in four other families (14,15).

The linkage map around the MEN1 locus (16,17) includes 7 polymorphic "anchor markers" covering approximately 12 cM; 11p- (D11S149-D11S288) -PGA-PYGM- (D11S97-D11S146-INT2). These markers were analyzed in five families and meiotic crossovers were identified for markers at both ends of this region (16).

Panel of polymorphic DNA markers

We then attempted to develop a reliable and accurate DNA-based diagnostic procedure for the MEN 1 predisposition (6). To be of clinical use such procedures should fulfill the following criteria:

1. Meiotic crossing-over between the disease and the marker loci must be rare and should be detectable. This can be achieved by using markers close to, as well as flanking, the locus on both sides.

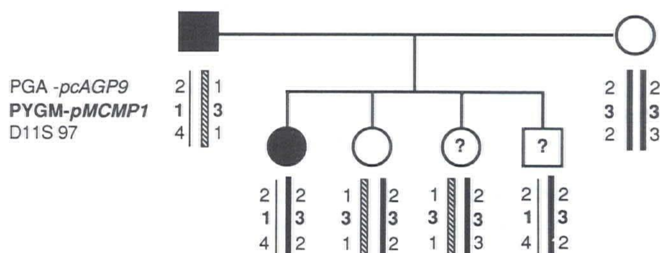
2. Incorrect diagnosis of an individual, nonpaternity, or mixing of samples are possible causes of incorrect results from DNA-based testing of inherited diseases. Nonpaternity or sample mixups can sometimes be recognized if several markers are used in the linkage analysis.

DNA-based testing procedures can make use of the detailed genetic mapping data recently generated for the chromosomal region 11q11-13 (9,18,19). This involved isolation of new genetic markers which have been mapped physically utilizing somatic cell hybrids or pulsed-field gel electrophoresis. Using different polymorphic DNA markers, we genotyped 124 members of six MEN 1 families, including 59 affected individuals, and determined the genetic linkage between MEN1 and these marker systems (6). Fourteen marker systems (18 DNA probes) were found to be linked to MEN1. Four of the marker systems were on the centromeric side of MEN1 while the others were either telomeric or close to MEN1. The markers, their alleles, and experimental conditions are listed in the Table. The order from centromere (top) to telomere is based on combined data from linkage and physical mapping (9,16-19) and the markers closest to MEN1 are italicized (Table).

DNA-based diagnosis of MEN 1

DNA-based predictive testing for MEN1 utilizes markers located close to the disease gene on chromosome 11. In a kindred with at least two affected members it is possible to follow the disease gene by the segregation of the marker alleles thereby identifying the haplotype for this chromosomal region carrying the MEN1 mutation. In most clinical situations, it is possible to identify a haplotype with the mutant MEN1 allele in the middle if the described set of markers is used (Figure). With two flanking marker systems informative, the calculated predicted accuracy would be greater than 99.5%. The practical considerations of such DNA-based testing are described in the following examples:

1. A large family where several members can be genotyped: This is the "easiest" situation, where the family alone is large enough to show linkage between MEN1 and the RFLP markers.



Figure—Pedigree illustrating DNA-based predictive testing for MEN 1. Filled symbols indicate affected family members. Numbers below each individual indicate the genotypes for the informative marker systems listed next to the symbol of the carrier parent. The haplotypes for each individual are depicted along an illustrated chromosome segment with the inferred mutant chromosome indicated by a thin line and the wild type chromosome of the carrier parent hatched. As illustrated, the two younger children have not shown any signs of MEN 1. The haplotypes illustrate that the first daughter (II-1) and the brother (II-4, age 25) carry the disease gene, while the second (II-2, age 36) and third daughters (II-3, age 26) are not gene carriers.

2. A family where only a few persons can be genotyped: There is a theoretical possibility of genetic heterogeneity between MEN 1 families which could be overlooked in this type of family. This issue will be clarified by genetic analysis of additional MEN 1 families although the disease gene has been linked to 11q13 in all 11 reported families (5,6,14-16).

3. A family where only one affected individual can be analyzed: Generally, linkage analysis can only be applied when the haplotypes of at least two affected individuals can be compared. However, allele losses in MEN 1-associated pancreatic and parathyroid tumors serve to eliminate the wild type allele (5,20). Tumor studies could therefore be used to identify which haplotype carries the mutated MEN1 allele. However, pituitary and adrenocortical tumors could not be used for this purpose since the former are rarely operated on in MEN 1 patients and the latter have not shown allele losses for chromosome 11 markers (21).

4. A single patient with MEN 1 syndrome: This patient most certainly has a constitutional predisposition to MEN 1. However, the mutational event may be prezygotic or postzygotic, and in the last situation it is not necessarily heritable. Hence, the offspring may all be without risk of developing MEN 1.

5. A family with an "MEN 1-related" clinical picture: Again, the possibility of genetic heterogeneity must be taken into account.

DNA-based diagnosis and biochemical screening provide a tool to detect individuals at risk of developing MEN 1 and to identify the neoplastic lesion at an early stage. A future goal will be to use these tools to decrease morbidity and mortality in MEN 1.

References

1. Wermer P. Genetic aspects of adenomatosis of endocrine glands. Am J Med 1954;16:363-71.

2. Skogseid B, Eriksson B, Lundquist G, et al. Multiple endocrine neoplasia type 1: A 10-year prospective screening study in four kindreds. J Clin Endocrinol Metab 1991;73:281-7.

3. Eberle F, Grun R. Multiple endocrine neoplasia, type I (MEN I). Ergeb Inn Med Kinderheilkd 1981;46:75-149.

4. Lips CJ, Vasen HF, Lamers CB. Multiple endocrine neoplasia syndromes. Crit Rev Oncol Hematol 1984;2:117-84.

5. Larsson C, Skogseid B, Oberg K, Nakamura Y, Nordenskjold M. Multiple endocrine neoplasia type 1 gene maps to chromosome 11 and is lost in insulinoma. Nature 1988;332:85-7.

6. Larsson C, Shepherd J, Nakamura Y, et al. Predictive testing for multiple endocrine neoplasia type 1 using DNA polymorphisms. J Clin Invest 1992; 89:1344-9.

7. Shepherd JJ. Latent familial multiple endocrine neoplasia in Tasmania. Med J Aust 1985;142:395-7.

8. Shepherd JJ. The natural history of MEN1 which may be highly unrecognised rather than highly uncommon. Arch Surg 1991 (in press).

9. Larsson C, Weber G, Kvanta E, et al. Isolation and mapping of polymorphic cosmid clones used for sublocalization of the MEN1 locus. Hum Genet 1992; 89:187-93.

10. Bergerheim U, Nordenskjold M, Collins VP. Deletion mapping in human renal cell carcinoma. Cancer Res 1989;49:1390-6.

11. Knudson AG Jr. Mutation and cancer: Statistical study of retinoblastoma. Proc Natl Acad Sci USA 1971;68:820-3.

12. Larsson C, Bystrom C, Skoog L, Rotstein S, Nordenskjold M. Genomic alterations in human breast carcinomas. Genes Chromosom Cancer 1990;2: 191-7.

13. Larsson C, Nordenskjold M. Multiple endocrine neoplasia. Cancer Surv 1990;4:703-23.

14. Bale SJ, Bale AE, Stewart K, et al. Linkage analysis of multiple endocrine neoplasia type 1 with INT2 and other markers on chromosome 11. Genomics 1989;4:320-2. (Published erratum appears in Genomics 1989;5:166.)

15. Thakker RV, Bouleoux P, Wooding C, et al. Association of parathyroid tumors in multiple endocrine neoplasia type 1 with loss of alleles on chromosome 11. N Engl J Med 1989;321:218-24.

16. Nakamura Y, Larsson C, Julier C, et al. Localization of the genetic defect in multiple endocrine neoplasia type 1 within a small region of chromosome 11. Am J Hum Genet 1989;44:751-5.

17. Julier C, Nakamura Y, Lathrop M, et al. A detailed genetic map of the long arm of chromosome 11. Genomics 1990;7:335-45.

18. Tokino T, Takahashi E-I, Mori M, et al. Isolation and mapping of 62 new RFLP markers on human chromosome 11. Am J Hum Genet 1991;48:258-68.

19. Janson M, Larsson C, Werelius B, et al. Detailed physical map of human chromosomal region 11q12-13 shows high meiotic recombination rate around the MEN1 locus. Proc Natl Acad Sci USA 1991;88:10609-13.

20. Bystrom C, Larsson C, Blomberg C, et al. Localization of the MEN1 gene to a small region within chromosome 11q13 by deletion mapping in tumors. Proc Natl Acad Sci USA 1990;87:1968-72.

21. Skogseid B, Larsson C, Lindgren PG, et al. Clinical and genetic features of adrenal lesions in multiple endocrine neoplasia type 1. J Clin Endocrinol Metab 1992;75:76-81.