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Tumorigenesis in multiple endocrine neoplasia type 1 (MEN 1) involves the unmasking of a recessive mutation at the MEN 1 locus which has been mapped to chromosomal region 11q11-13. By analyzing 58 DNA markers on a panel of radiation-reduced somatic cell hybrids, the region encompassing the MEN 1 gene was divided into nine subregions. Pulsed field gel electrophoresis analysis of markers within subgroups showed that the recombination rate around the MEN 1 locus is high. Combined linkage analysis in MEN 1 families and deletion mapping in MEN 1-related tumors suggest the MEN 1 gene is located centromeric to D11S807 and telomeric to PYGM. (Henry Ford Hosp Med J 1992;40:159-61)

Multiple endocrine neoplasia type 1 (MEN 1) is a syndrome in which neoplastic lesions in the parathyroids, the neuroendocrine pancreas, and the pituitary gland are associated. The MEN 1 susceptibility locus was mapped to the centromeric part of the long arm of chromosome 11 by genetic linkage utilizing restriction fragment length polymorphisms (RFLPs) in three affected families (1). Furthermore, combined family and genotype analysis have shown that tumorigenesis in MEN 1 involves the unmasking of a recessive mutation at the MEN 1 locus (1-4). This is likely accomplished by mitotic recombination or deletion or loss of one chromosome 11 complement (1,3-6). These findings suggest the possibility of further localization of the gene by deletion mapping in tumors as well as by identification of meiotic crossovers in families.

Several strategies are being used to identify the MEN 1 gene (MEN1). A useful start is to identify flanking markers for the gene. If the putative gene-containing region can be restricted to less than a few million base pairs, it can be cloned and screened for expressed sequences. The present linkage map around the MEN 1 locus includes seven RFLP markers covering approximately 12 cM (7,8), a region too large to be cloned by conventional methods.

Methods

Physical mapping and linkage analysis

The DNA markers available for linkage analysis and physical mapping include polymorphic and nonpolymorphic plasmid markers, cosmid clones isolated for locus expansion of the polymorphic plasmid markers, and cosmid clones isolated from somatic cell hybrids containing different parts of human chromosome 11 on a hamster background (9-11).

The DNA markers were localized to different parts of chromosome 11 using a panel of Chinese hamster x human somatic cell hybrids. J1-C14 carries one intact chromosome 11 while MC-1, J1-44, R28-4E, and J1-11 contain only part of this chromosome (12). The Goss–Harris radiation-reduced hybrids, R131-33B1, R184-5D1, R184-4C2, R184-3A1, R184/7C1, and R184-1A2, were derived from a hybrid cell line containing all of 11q and a small part of 11p; after gamma-irradiation, the cells were fused with Chinese hamster cells and selected with a monoclonal antibody corresponding to a gene encoding a human cell surface antigen (MDU1) mapped to 11q13 (13).

Methods for isolation and restriction enzyme cleavage of DNA, Southern analysis, hybridization to radioactively labeled probes, and analysis by pulsed field gel electrophoresis (PFGE) have been described (13,14). Clinical data and scoring have been published (15,16).

Results and Discussion

Physical mapping

The region of chromosome 11 containing the MEN 1 gene is defined by seven anchor markers for which the relative order and map distance have been determined in reference families: (D11S149-D11S288)-PGA-PYGM-(D11S97-D11S146-INT2)-11qter. A total of 58 DNA markers have been mapped to this region by hybridization to somatic cell hybrids (11). By analyzing the same markers on a panel of radiation-reduced somatic cell hybrids, 53 of the clones were assigned to the D11S149-INT2 region. The radiation-reduced hybrids are designed to carry different parts of chromosomal region 11q13 on a hamster background, and from the hybridization pattern the markers were divided into nine groups (11).

This sublocalization of the markers provides a starting point for more precise mapping with PFGE and linkage analysis (11,13). Comparison of the physical map (Figure) with the present linkage map indicates considerable variability in the recom-

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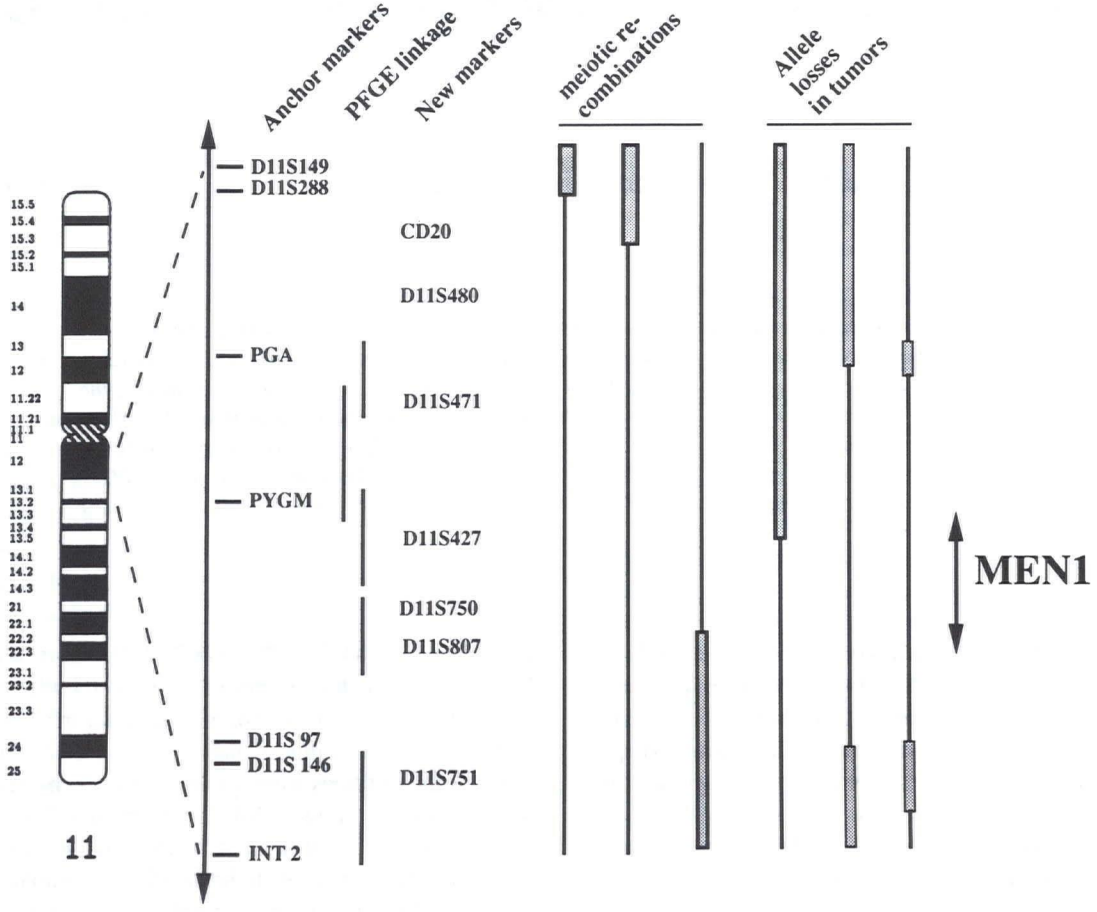

Figure—Mapping of the MEN1 region on chromosome 11. An enlarged map of the 11q11-13 region shows the gene order based on combined physical mapping and linkage analysis. Anchor markers are those previously mapped in reference families. In each case the region "excluded" from analysis of recombinational events or deletion mapping is hatched.

bination frequency over the region. In particular, the recombination rate is higher than expected for markers flanking the MEN1 region. PGA and PYGM are located 3 cM apart (8). They are physically linked through D11S471 and the distance is less than 875 kb (13). A similar situation is found on the telomeric side where D11S97, D11S146, and INT2 (which are within 2% meiotic recombinations) are located on the same 1,200 kb Nrul fragment (13).

Linkage analysis

The anchor markers have been used for linkage analysis of MEN 1 families, to identify meiotic crossovers, and to locate the disease gene between flanking markers. By using lod score calculations, several recombinants have been detected for INT2 and D11S146 on the telomeric side of MEN1 (7,16,17). By the same method, multiple recombinants have been detected for D11S288 (7,16) and one for PGA (16). These findings indicate that the MEN 1 gene is flanked centromerically by D11S288-D11S149 and telomerically by INT2-D11S146. However, sublocalization of MEN1 is most reliable when the crossing-over event has been thoroughly outlined in individual cases. Since such analysis also requires genotyping of the closest relatives, a detailed analysis is not always possible. The two recombinations outlined in the Figure indicate that the gene is located between CD20 and D11S807.

Deletion mapping in tumors

MEN 1-associated tumors may result from the unmasking of a recessive mutation, according to the two-mutation model postulated originally by Knudson (2). Such rearrangements would then be present in all tumor cells, restricted to a specific chromosome (18), and serve to eliminate the wild type allele of MEN1. In MEN 1 families this has been shown for parathyroid and pancreatic tumors (1,3,4) but not for pituitary or adrenocortical tumors. In the sporadic counterparts of these tumors, allele losses for chromosome 11 markers have been found in parathyroid but not in pituitary tumors (3-5). The results from similar analysis of sporadic pancreatic tumors have been conflicting.

In order to define the minimal region of overlap for deletions of chromosome 11, a number of MEN 1-associated tumors have been screened for allele losses (3-6). From these studies, it is evident that mitotic recombinations do occur within the MEN1 region (4-6). Thus it is difficult to establish an accurate and reliable deletion map. As illustrated in the Figure, for markers

within the PGA and D11S146 regions, both alleles may be retained while the rest of one chromosome 11 complement is lost. In one case, combined pedigree and tumor genotype analysis showed that the lost alleles were always derived from the nonaffected father. In two other parathyroid tumors, the allele losses were interpreted as simple deletions (Figure).

Taken together, the linkage and deletion mapping analysis would place MEN1 telomeric of PYGM and centromeric of D11S807.

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