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Molecular Genetic Mapping of the Multiple Endocrine Neoplasia Type 1 Locus

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Familial multiple endocrine neoplasia type 1 (MEN 1) is an autosomal dominant disorder characterized by the combined occurrence of tumors of the parathyroid glands, the endocrine pancreas, and the pituitary gland. MEN 1 tumors have previously been shown to be associated with the loss of alleles on chromosome 11, and deletion mapping studies together with family linkage studies have localized the MEN 1 gene to 11q13. A detailed genetic map around the MEN 1 locus is required to facilitate further characterization and cloning of the gene (MEN1). We have characterized a panel of seven rodent-human somatic cell hybrids which contain fragments of human chromosome 11 with breakpoints in the pericentromeric region by using eight DNA sequences (D11S149, PGA, PYGM, D11S97, INT2, D11S37, D11S533, and D11S147) to define the region containing MEN1. This will facilitate the rapid localization of additional DNA sequences in this region. In addition, we have used a highly polymorphic repetitive degenerate hexanucleotide sequence, designated D11S533, for segregation studies in one family with MEN 1. These molecular genetic approaches will help to define a precise 1 to 2 centiMorgan map around MEN1. (Henry Ford Hosp Med J 1992;40:162-6)

Multiple endocrine neoplasia type 1 (MEN 1) is characterized by the combined occurrence of tumors of the parathyroid glands, the pancreatic islet cells, and the anterior pituitary (1). The disease may arise sporadically or be inherited as an autosomal dominant condition. The genetic abnormalities which cause inherited neoplastic disorders may involve two or more recessive mutations (2) involving tumor suppressor genes (3) and these can be investigated by using the techniques of molecular biology (1,4). Thus, cloned DNA sequences, which reveal restriction fragment length polymorphisms (RFLPs), have been used to identify allelic deletions involving chromosome 11 in parathyroid tumors (5-7), insulinomas (8), prolactinomas (7), and somatotrophinomas (9) from patients with MEN 1. Further studies have revealed that such allele loss involving chromosome 11 also occurs in non-MEN 1 parathyroid and anterior pituitary tumors (6,7,9). In addition, linkage studies using chromosome 11 RFLPs in families affected with MEN 1 have localized the mutant gene (MEN1) to the pericentromeric region, band 11q13, of the long arm of chromosome 11 (5,8,10). The genetic map of the region has been defined with polymorphic markers to be 11cen-(D11S288,D11S149)-PGA-PYGM-(D11S97,D11S146)-INT2-11qter (11), and MEN1 has been located by linkage studies to a region distal to D11S288 and proximal to INT2 (12). This region is approximately 10 centiMorgans (cM) which is equivalent in size to 10 million base pairs. Such a large region is difficult to analyze either by physical mapping methods using pulsed field gel electrophoresis (PFGE) and linking libraries (13,14) or by the construction of a series of overlapping clones (CONTIG) using cosmids or yeast artificial chromosomes (YACs) (15). These studies would be facilitated by defining a 1 to 2 cM map around MEN1 (Fig 1) using two parallel and complementary approaches. In the first approach the smallest region of allele loss in tumors is defined and the results from one MEN 1 parathyroid tumor indicate that this tumor suppressor gene is telomeric to the PYGM locus but centromeric to the D11S146 locus (7). In the second approach multipoint crossovers are explored by linkage studies in MEN 1 families, using highly polymorphic DNA sequences from 11q13 as genetic markers. These detailed genetic mapping studies will be facilitated by the use of the polymerase chain reaction (PCR) to detect polymorphisms in Alu sequences (16) and in microsatellite tandem repetitive sequences (17,18), as these are highly informative and enable maximal genetic information to be gained from the limited number of MEN 1 families. We report on the use of one such highly polymorphic repetitive sequence in a family with MEN 1. In addition, in order to enable the rapid localization of DNA sequences to the region containing MEN1, we have characterized a panel of seven rodent-human somatic cell hybrids which contain fragments of human chromosome 11 with breakpoints in the pericentromeric region.

Materials and Methods

Rodent-human somatic cell hybrids

Seven rodent-human somatic cell hybrids (J1, EJNAC, J1-11, J1-44, CF37, CF52, and R28-4D) containing all or part of hu-

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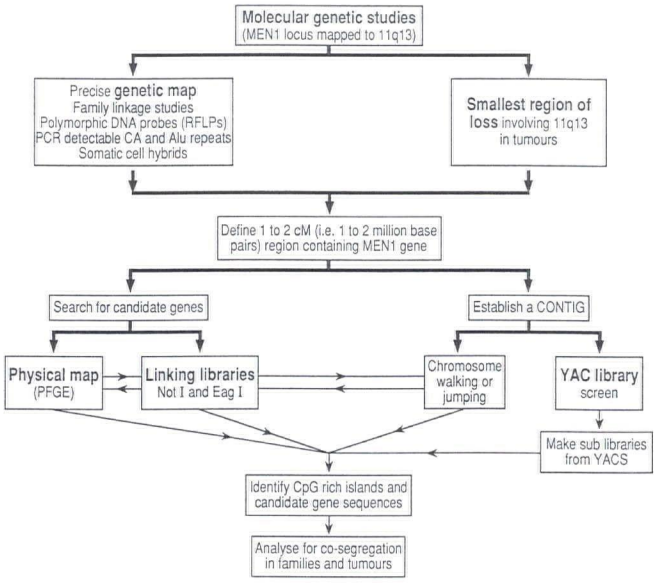

Fig 1—Molecular genetic approaches to the gene for MEN 1. MEN1 has been localized to 11q13, and a 1 to 2 centiMorgan (cM) region around the disease locus is being defined by deletion mapping studies in tumors and by family linkage studies using highly polymorphic DNA sequences. This will facilitate the construction of a series of overlapping clones (CONTIG) using cosmids or yeast artificial chromosomes (YACs) and the identification of candidate gene sequences by using pulsed field gel electrophoresis (PFGE) and linking libraries to reveal CpG-rich islands which occur at the 5′ region of many vertebrate genes. Such candidate gene sequences can in turn be used for further deletion mapping and family linkage studies.

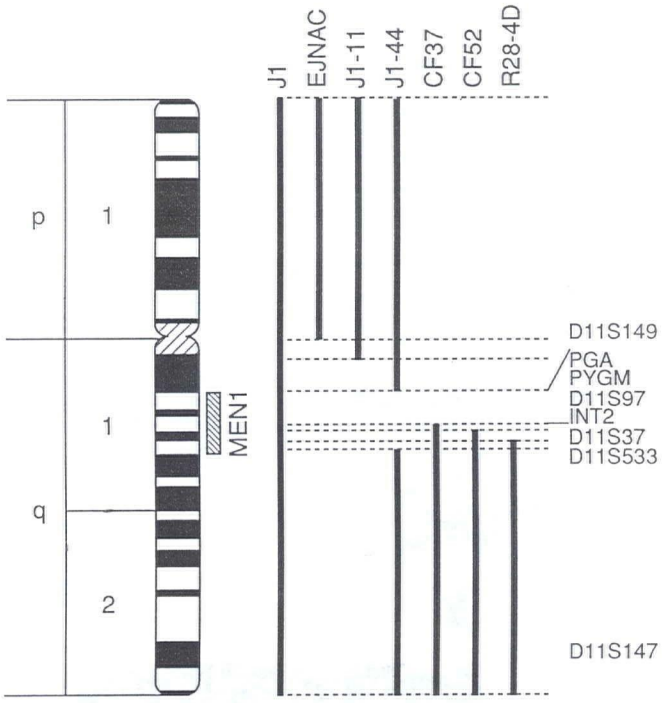

Fig 2—Schematic diagram of a panel of seven rodent-like human somatic cell hybrids containing fragments of human chromosome 11 represented with Giemsa bands. The eight DNA sequences (D11S149, PGA, PYGM, D11S97, INT2, D11S37, D11S533, and D11S147) are shown juxtaposed to their region of origin on the short (p) and long (q) arms of chromosome 11. The chromosomal region 11q13 to which the MEN 1 gene (MEN1) has been localized is shown by the hatched-line box. The fragment of chromosome 11 contained in each hybrid cell line, established by previous studies (19-22) and our study, is shown by the solid vertical line and the designated name of each hybrid cell line is shown above. This panel enables the localization of DNA sequences to the region containing MEN1.

man chromosome 11 were investigated (Fig 2). The cell line J1 contained the whole of human chromosome 11 (19) whilst the remaining six cell lines contained fragments of human chromosome 11 with breakpoints involving the pericentromeric region. Thus, the cell line EJNAC contained 11p only (20); J1-11 contained 11pter to 11q11 (21); J1-44 contained 11pter to 11q12 and also 11q21 to 11qter (21); CF37 and CF52 contained 11q13 to 11qter (22); and R28-4D contained 11q13 to 11qter (21).

DNA hybridization analysis

Genomic DNA was extracted from the rodent-human somatic cell hybrid cell lines, human leukocytes, and mouse thymus and spleen by standard methods (23). From each cell line, 10 μg of DNA was digested with an eightfold excess of the restriction endonuclease PstI (Boehringer Mannheim). Human leukocyte and mouse spleen and thymus DNA was similarly digested with PstI. The resulting DNA fragments were separated by electrophoresis through a 0.8% agarose gel and transferred onto Hybond-N membrane (Amersham) by Southern's (24) method. Prehybridization was performed in 4 × SSC (1 × SSC is 0.15 M sodium chloride and 15 mM sodium citrate), 10 × Denhardt's solution, 0.1% sodium tetrapyrophosphate, 50 μg/mL sonicated salmon sperm DNA, and 5% dextran sulphate at 65 °C four hours prior to the addition of a radiolabeled DNA probe in which α [^{32}P]-dCTP had been incorporated by oligonucleotide primed synthesis (25). Sonicated human placental DNA (130 μg/mL) was used for competitive hybridization when the probes PYGM, D11S97, and D11S37 were used. Overnight hybridization at 65 °C was performed and the filters were washed to a stringency of 1 × SSC and 0.1% SDS at 65 °C. Autoradiography was performed with dual intensifying screens and preflashed Fuji medical x-ray film at −70 °C for one to five days.

Detection of the polymorphic repetitive element (D11S533) by the polymerase chain reaction

The polymorphic repetitive element, designated locus D11S533, was analyzed using the previously reported oligonucleotide primers: L-5′GCCTAGTCCCTGGGTGTGGTC3′ and R-5′GGGGGTCTGGGAACATGTCCCC3′ (26). These oligonucleotides, which are complementary to the unique sequences flanking the repetitive element, were synthesized using a 7,500 DNA synthesizer (MilliGen). The technique of PCR (27) was used to detect the polymorphic repetitive element us-

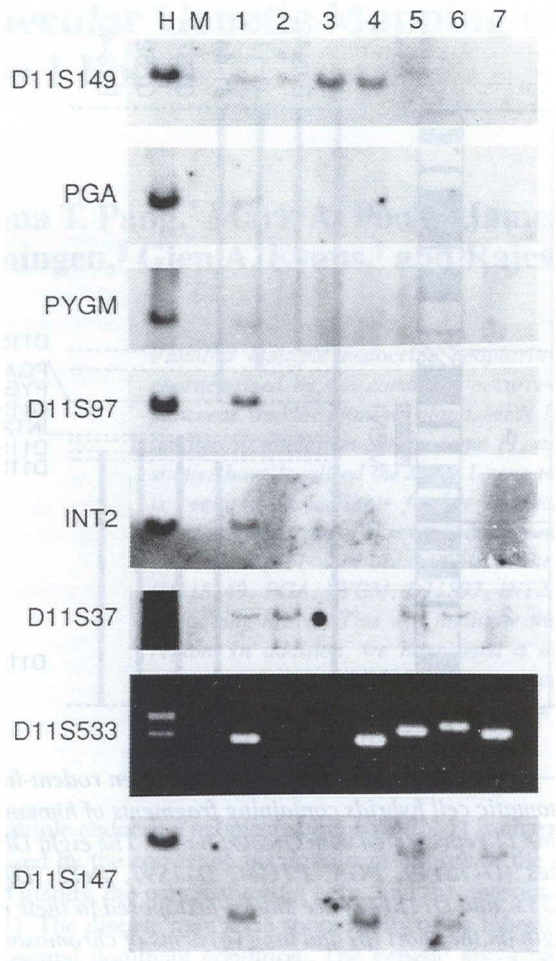

Fig 3—Localization of eight DNA sequences (D11S149, PGA, PYGM, D11S97, INT2, D11S37, D11S533, and D11S147) to regions of human chromosome 11. Normal human (H) and mouse (M) genomic DNA and genomic DNA from each rodent-human somatic hybrid cell line (lanes 1 to 7) was utilized for DNA hybridization analysis or for polymerase chain reaction detection of the repetitive sequence D11S533. The hybrid cell lines J1, EJNAC, J1-11, J1-44, CF37, CF52, and R28-4D (Fig 2) are in lanes 1 to 7, respectively. All eight DNA sequences were present in human DNA and DNA from the cell line J1 and were absent in mouse DNA, thereby indicating their specific origin from human chromosome 11. D11S149 yielded additional hybridization signals from the cell lines EJNAC, J1-11, and J1-44 but not from CF37, CF52, and R28-4D. However, PGA, PYGM, D11S97, and INT2 did not yield hybridization signals with any of these additional cell lines. The probe D11S37 yielded hybridization signals from EJNAC, CF37, and CF52 but not from J1-11, J1-44, or R28-4D. The DNA sequences D11S533 and D11S147 yielded signals from J1-44, CF37, CF52, and R28-4D but not from EJNAC or J1-11. These results indicate that the DNA sequences closest to MEN1 (PGA, PYGM, D11S97, and INT2) are located in a region bounded proximally by the centromeric breakpoint in the cell line J1-44 and distally by the breakpoints in the cell lines CF37 and CF52 (Fig 2). The characterization of this somatic cell hybrid panel will facilitate localization of other DNA probes to the region containing MEN1.

ing a Hybaid TR2 Thermal Reactor as follows: 250 ng genomic DNA was added to a total volume of 25 μl containing 10 mM Tris/HCl pH 8.4, 50 mM KCl, 2.5 mM $MgCl_2$, 0.2 mM each of dATP, dCTP, dTTP, and dGTP, 0.05% W1 detergent (BRL, Gaithersburg, MD), 10 pmol of each primer, and 1 unit of heat-stable DNA polymerase from Thermus aquaticus (Taq polymerase, BRL, Gaithersburg, MD). Thirty-five cycles of PCR amplification were performed. Each cycle consisted of 10 sec at 92 °C to denature double-stranded DNA, 15 sec at 64 °C for the primers to anneal to their complementary sequences, and 1 minute at 72 °C for the extension of the DNA strands. On completion, the PCR amplification products were analyzed by electrophoresis through a composite 4% agarose (3% NuSieve agarose + 1% regular agarose) gel stained with ethidium bromide to reveal the polymorphic repetitive element under ultraviolet light.

Results

Characterization of the panel of somatic cell hybrids

The DNA sequences of D11S149, PGA, PYGM, D11S97, INT2, D11S37, D11S533, and D11S147 were localized to regions of human chromosome 11 using the panel of seven rodent-human somatic cell hybrids shown in Fig 2. The specific human chromosome 11 origin of all of the eight DNA sequences was demonstrated by obtaining hybridization signals or PCR products from human DNA and from the J1 cell line DNA and by observing an absence of signals from mouse DNA (Fig 3). In addition, our further subchromosomal localizations of these DNA sequences was in agreement with previously reported locations (28). For example, the DNA probes PGA, PYGM, D11S97, and INT2 did not yield hybridization signals from the cell lines EJNAC, J1-11, J1-44, CF37, CF52, and R28-4D all of which lack the proximal region of 11q13. Thus, these four markers which are close to MEN1 are localized to 11q13. Further characterization of the somatic cell hybrid panel indicated that the chromosomal segment containing the MEN 1 gene was bounded proximally by the centromeric breakpoint of the J1-44 cell line and distally by the breakpoints in cell lines CF37 and CF52. The telomeric breakpoint in the J1-44 cell line has previously been reported (21) to be 11q21, but our results which obtained a PCR product with D11S533 from this cell line (Fig 3) indicate that the telomeric breakpoint in the J1-44 cell line may be proximal to the region 11q13.3-13.4 where D11S533 has been previously localized by in situ hybridization (26). Our results with D11S37, which revealed hybridization signals from the CF37 and CF52 cell lines but an absence of signals from the J1-44 and R28-4D cell lines, indicate that the breakpoints involved in the CF37 and CF52 lines are centromeric to those occurring in the R28-4D cell line and the telomeric breakpoint in the J1-44 cell line. The DNA sequences D11S37, INT2, and D11S533 have previously been localized to 11q13-11q14 (28), 11q13.1-11q13.2 (29), and 11q13.3-11q13.4 (26), respectively, and our results therefore indicate that the breakpoints in CF37 and CF52 are in the 11q13.2-11q13.4 region. Previous studies have also demonstrated that the cell line EJNAC contains 11p as the sole human chromosome 11 component (20). Our results yielded a hybridization signal from the EJNAC cell line using

D11S37 which has been mapped to 11q13-11q14 (28) indicating that the EJNAC cell line may contain an additional fragment from this region. Thus, our results have further characterized this panel of rodent-human somatic cell hybrids, and the definition of the breakpoints in the cell lines J1-44, CF37, and CF52 will enable rapid localization of DNA probes to the region containing MEN1.

Segregation of D11S533 in an MEN 1 family

PCR detection of the polymorphic repetitive element D11S533 yielded five alleles in one three-generation family suffering from MEN 1 (Fig 4). No recombinants were observed between D11S533 and MEN1. D11S533 has been located by in situ hybridization to 11q13.3-11q13.4 (26) and this would place it distal to the MEN1 locus. However, D11S533 represents an important flanking marker for MEN1 as it is a highly polymorphic, readily detectable sequence by PCR and agarose gel electrophoresis. Thus, D11S533 will help in establishing a more precise genetic map around MEN1 as it will yield genetic linkage data from previously uninformative families.

Discussion

Current techniques for mapping the human genome exploit DNA sequence variations affecting recognition sites of restriction endonucleases, as well as DNA-length polymorphisms due to allelic differences in the number of tandemly repeated simple sequence motifs. The resulting RFLP and variable numbers of tandemly repeated (VNTR) markers are routinely detected by the method of Southern (24). PCR provides an alternative and more direct approach to detecting DNA polymorphisms in one of two ways. First, PCR may be used to detect length variations in microsatellite tandem repeats (17,18), for example, $(CA)_n$, where n = 10 to 60. In addition to tandem repeats in the sequence (CA), microsatellite tandem repeats consisting of $(AT)_n$, $(GA)_n$, $(CTTT)_n$, $(ATTT)_n$, $(ATT)_n$, and the hexanucleotide $[T(Pu)T(Pu)T(Pu)]_n$ have also been reported (17,18,26). These tandem repeats, which are highly polymorphic and are inherited in a Mendelian manner, are estimated to occur once in every 50 to 100 Kbp. Thus, they will prove to be a valuable technique in obtaining a detailed genetic map around a disease locus such as MEN1. In this technique oligonucleotide primers are synthesized on either side of the repeat, and PCR is used to amplify the repeat sequence. The smaller and larger fragment length polymorphisms in these repetitive sequences are detected by separation either on a polyacrylamide sequencing gel or on an agarose gel, respectively. In the second method PCR is used to detect either base pair changes or length variations associated with the Alu sequence family (16). These Alu sequence polymorphisms, which are ubiquitous in the human genome and appear to occur once every 6 Kbp, are detected in a manner similar to that for the microsatellite tandem repeats. Thus, the detection by PCR of these highly polymorphic microsatellite tandem repetitive elements and Alu sequences will help to construct a precise genetic map around MEN1 and we have illustrated the use of one such polymorphic repetitive element designated D11S533 in a family with MEN 1 (Fig 4).

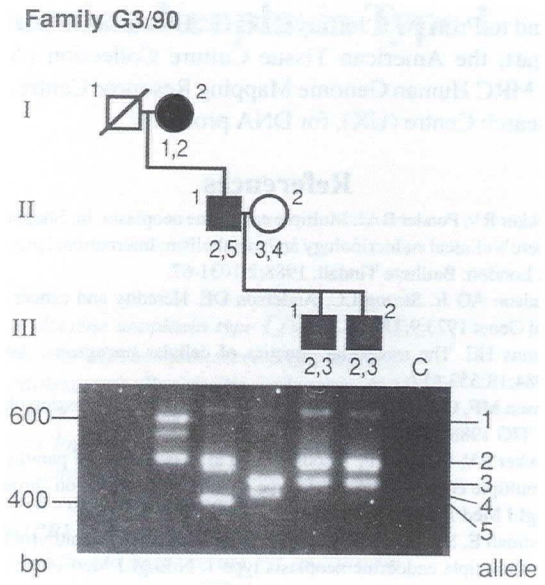

Fig 4—Segregation of D11S533 and MEN1 in family G3/90. Genomic DNA from the family members (upper panel) was used for polymerase chain reaction (PCR) amplification of the polymorphic repetitive element, designated D11S533. The PCR amplification products were detected on an ethidium bromide-stained agarose gel and are shown in the lower panel. In the control lane (C), PCR products were not detected when human genomic DNA was absent from the reaction. Two PCR products were detected from the DNA of each individual, and these ranged in size from 400 to 600 base pairs (bp). Alleles were designated for each PCR product and are indicated on the right. Individual I-2 is affected and heterozygous (alleles 1,2). Her affected son, II-1, is heterozygous (alleles 2,5) and has inherited allele 2 with the disease. The grandchildren III-1 and III-2, who are affected, are heterozygous (alleles 2,3) and have inherited the disease with allele 2. Thus, in this family MEN1 and D11S533 are segregating without recombination.

The rapid localization of such polymorphic repetitive sequences and of additional DNA probes revealing RFLPs or VNTR markers to the chromosomal segment containing MEN1 can be accomplished by the panel of rodent-human somatic cell hybrids which we have characterized. Our results demonstrate that human and mouse polymorphic tandem repetitive sequences can be distinguished thereby enabling the use of this panel of somatic cell hybrids for the localization of such repeat sequences by PCR. Thus, the combined use of a panel of rodent-human somatic cell hybrids and the detection of highly polymorphic repetitive DNA sequences will help to define a precise 1 to 2 cM genetic map around MEN1 and thereby facilitate the construction of a CONTIG, which in turn will permit the cloning of the gene for MEN 1.

Acknowledgments

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