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Fine-Scale Mapping of the Gene Responsible for Multiple Endocrine Neoplasia Type I (MEN1)

Minoru Fujimori,* Samuel A. Wells, Jr., † and Yusuke Nakamura*

*Department of Biochemistry, Cancer Institute, Tokyo; and †Department of Surgery, Washington University School of Medicine, St. Louis

Summary

We have constructed a high-resolution genetic linkage map in the vicinity of the gene responsible for multiple endocrine neoplasia type 1 (MEN1). The mutation causing this disease, inherited as an autosomal dominant, predisposes carriers to development of neoplastic tumors in the parathyroid, the endocrine pancreas, and the anterior lobe of the pituitary. The 12 markers on the genetic linkage map reported here span nearly 20 cM, and linkage analysis of MEN1 pedigrees has placed the MEN1 locus within the 8-cM region between D11S480 and D11S546. The markers on this map will be useful for prenatal or presymptomatic diagnosis of individuals in families that segregate a mutant allele of the MEN1 gene.

Introduction

Although hormone-producing endocrine tumors are not usually multiple or familial, several types of a familial syndrome called multiple endocrine neoplasia (MEN) are known. One of them, MEN type 1 (MEN1), is characterized by a combination of tumors in the parathyroid, the endocrine pancreas, and the anterior lobe of the pituitary gland. The mutant gene responsible for MEN1 was localized recently to the proximal long arm of chromosome 11 by linkage analysis of several affected families, by using markers defined by RFLPs (Larsson et al. 1988). Subsequent linkage analysis, based on a linkage map of the proximal long arm of chromosome 11, placed the MEN1 locus within a 12-cM region encompassing the PYGM and PGA loci (Nakamura et al. 1989). Loss of heterozygosity (LOH) on chromosome 11q, suggesting the presence of a tumor-suppressor gene in that region, has been observed in MEN1-associated tumors (Bale et al. 1991). By deletion mapping in such tumors, Byström et al. (1990) placed the MEN1 locus telomeric to PYGM.

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Address for correspondence and reprints: Yusuke Nakamura, M.D., Ph.D., Department of Biochemistry, Cancer Institute, 1-37-1 Kami-Ikebukuro, Toshima, Tokyo 170, Japan.

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To map the MEN1 locus more precisely and to obtain reagents for more accurate presymptomatic diagnosis, we have developed new RFLP markers on chromosome 11q13. Here we present a high-resolution linkage map of 12 polymorphic loci around the MEN1 locus on chromosome 11q13, and we describe both the analysis of linkage between each of these markers and the locus harboring a mutation responsible for MEN1.

Subjects and Methods

Family

The MEN1 pedigree used in the present study has been described elsewhere (Nakamura et al. 1989). All individuals at risk were screened by measurement of serum Ca^{2+}, prolactin, and various pancreatic hormones, according to methods described elsewhere (Skogseid et al. 1987; Larsson et al. 1988).

DNA Markers

cCI11-probes have been reported by Tokino et al. (1991), and Tanigami et al. (1992), and pMCMP1 has been reported by Carlson et al. (1988). All markers were genotyped in 40 of the reference families in the panel maintained by the CEPH (Centre d'Etude du Polymorphisme Humain) in Paris. Methods and conditions for enzyme digestion, electrophoresis, blot-

Table 1

Characteristics of RFLP Markers

Probe (locus)	Restriction Enzyme	Allele Size (kb)	Allele Frequency[a]
cCI11-282 (D11S467)...	*Taq*I	7.0	.83
		5.5	.17
cCI11-231 (D11S453)...	*Taq*I	3.6	.58
		3.5	.42
cCI11-8 (D11S429).....	*Pst*I	4.8	.42
		3.1 + 1.7	.58
cCI11-319 (D11S480)...	*Taq*I	4.5	.50
		3.3	.50
cCI11-410 (D11S559)...	*Pvu*II	4.4	.67
		3.1	.33
cCI11-288 (D11S469)...	*Taq*I	3.6	.58
		3.4	.42
pMCMP1 (PYGM)	*Taq*I	1.7	.11
		1.6	.26
		1.4	.63
cCI11-4 (D11S427).....	*Pvu*II	2.3–2.9	4 Alleles
		70% Heterozygosity	
cCI11-363 (D11S546)...	*Taq*I	5.6	.42
		4.9	.58
cCI11-254 (D11S460)...	*Pst*I	5.5	.33
		4.1	.67
cCI11-44 (D11S443)	*Pst*I	3.5	.58
		2.5	.42
cCI11-283 (D11S468)...	*Pst*I	3.2	.17
		3.0	.83

[a] Calculated from data on 80 unrelated Caucasians.

ting, hybridization, and autoradiography have been described elsewhere (Nakamura et al. 1988).

Linkage Analysis

Linkage analysis was performed with the LINKAGE program package (Lathrop et al. 1985). The genetic map was constructed from the reference families by the Gene Mapping System algorithm (Lathrop et al. 1988). Sex-specific differences in recombination estimates were investigated by comparing the likelihoods under two different models: (1) a model assuming a constant ratio of female:male genetic distance throughout the chromosomal region spanned by the marker loci and (2) a model assuming no between-sex difference in frequency of crossing-over in this region. Odds against inversions of adjacent loci were calculated with at least five flanking markers on each side of the inverted pair.

Results

The characteristics of the 12 polymorphic markers used in the present study are shown in table 1. For

Table 2

Pairwise Lod Scores (below diagonal) and Recombination Estimate (above diagonal) for 12 Loci in MEN1 Region of Chromosome 11, Typed in 40 CEPH Families

Locus	Locus											
	cCI11-282	cCI11-231	cCI11-8	cCI11-319	cCI11-410	cCI11-288	MCMP1	cCI11-4	cCI11-363	cCI11-254	cCI11-44	cCI11-283
cCI11-282...		.04	.04	.03	.05	.04	.03	.04	.08	.10	.11	.14
cCI11-231...	8.8		.01	.10	.00	.04	.09	.04	.11	.06	.08	.14
cCI11-8.....	5.9	17.0		.04	.02	.09	.08	.06	.16	.09	.04	.06
cCI11-319...	11.7	4.9	13.3		.00	.01	.04	.01	.06	.07	.04	.03
cCI11-410...	11.4	10.5	11.9	20.2		.00	.02	.01	.02	.05	.04	.03
cCI11-288...	9.9	14.1	7.3	24.1	15.3		.02	.01	.04	.05	.06	.04
MCMP2.....	7.5	9.3	8.7	11.9	12.4	13.8		.01	.02	.07	.02	.00
cCI11-4.....	12.3	14.1	10.6	22.1	15.9	13.0	25.9		.01	.03	.03	.06
cCI11-363...	11.8	9.7	2.6	12.5	18.2	21.0	25.7	27.7		.01	.03	.02
cCI11-254...	7.4	13.1	7.8	9.0	7.8	13.2	6.4	13.1	21.5		.03	.03
cCI11-44.....	8.6	7.5	11.0	13.8	9.7	8.1	11.3	23.3	15.5	16.4		.03
cCI11-283...	2.5	2.8	7.2	16.3	14.5	8.7	7.0	11.4	8.4	7.4	7.3	

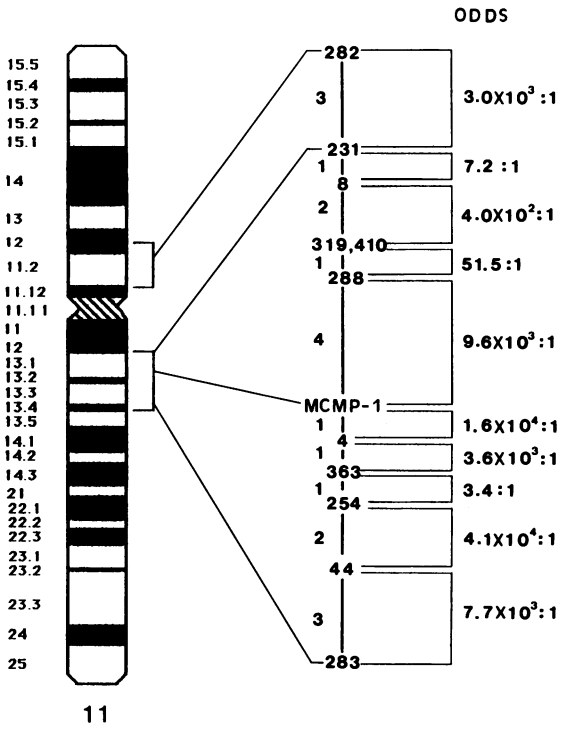

Figure 1 Sex-average map of 12 loci in MEN1 region of chromosome 11. Odds against inversion of adjacent loci are indicated on the right. Genetic distances (in cM) between loci are indicated on the left.

cCI11-4, a VNTR marker, 70% heterozygosity has been observed among 80 unrelated Caucasians. Table 2 summarizes the results of pairwise analyses of genotypic data from the CEPH families, in which every marker was tested against each of the other 11. Except for four pairs, recombination frequencies between markers were less than 10%, and almost all pairs gave lod scores higher than 3. On the basis of these data, we constructed a genetic linkage map (fig. 1) by using the LINKAGE and GMS programs (Lathrop et al. 1988). The map spans nearly 20 cM, and the best-supported order of loci was cCI11-282(D11S467)–cCI11-231(D11S453)–cCI11-8(D11S429)–cCI11-319 (D11S480), cCI11-410(D11S559)–cCI11-288(D11S-469)–MCMP1(PYGM)–cCI11-4(D11S427)–cCI11-363(D11S546)–cCI11-254–(D11S460)–cCI11-44 (D11S443)–cCI11-283(D11S468). Although the odds against alternative orders for the pairs cCI11-231–8, cCI11-410–288, and cCI11-363–254 were small (7.2:1, 51.5:1, and 3.4:1, respectively), all other orders could be rejected with odds of at least 10^2 against them. Figure 2 shows sex-specific recombination

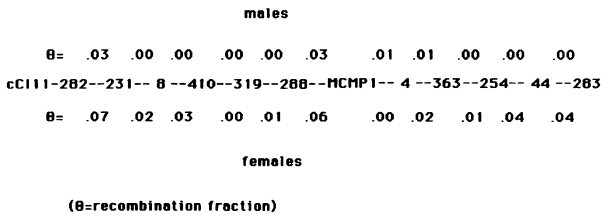

Figure 2 Sex-specific recombination fractions (θ) in 11 intervals, under model assuming no between-sex difference in frequency of crossing-over in this region.

rates. In females, the regions between cCI11-231 and cCI11-288 and between cCI11-363 and cCI11-283 were approximately 6 and 9 cM, respectively, but we detected no male recombinants in either of these regions. Overall, observed recombination was more frequent in females than in males, in the part of chromosome 11 covered by the map.

Table 3 shows the results of linkage analysis of each marker in the MEN1 family. Four probes (cCI11-4, cCI11-288, cCI11-363, and MCMP1) showed no recombination with the MEN1 locus; on the other hand, cCI11-319 and cCI11-254, nearly 8 cM apart in the linkage map, showed 7% and 6% recombination and lod scores of 1.92 and 3.38, respectively. A location score (Lathrop et al. 1984) was calculated for the MEN1 locus within the map of closely linked markers. As figure 3 shows, the most likely position for MEN1 was near the marker cCI11-4 (D11S427), and almost certainly the gene lies within the 8-cM interval between cCI11-410, cCI11-319 and cCI11-254.

Discussion

We have described here a genetic linkage map of 12 markers in the vicinity of the MEN1 locus. Linkage analysis of MEN1 pedigrees by the markers on this map placed the MEN1 locus in the 8-cM interval between D11S469 and D11S460. Byström et al. (1990) reported loss of alleles on distal chromosome 11q in a parathyroid tumor which retained heterozygosity at the PYGM locus; they concluded that the MEN1 locus was telomeric to PYGM. When their results are combined with ours presented here, the most likely position for the MEN1 gene would be within the 3-cM region between PYGM and D11S460. Both linkage analysis of larger MEN1 pedigrees and examination of allelic losses in a large number of MEN1-associated tumors will be required before this conclusion can be verified.

Table 3

Linkage Data on 12 Loci for MEN1 Families

Locus	Lod Score at Recombination Fraction of						Peak Lod Score (recombination fraction)
	.00[a]	.05	.10	.20	.30	.40	
CI11-282....	. . .[a]	1.42	2.08	2.13	1.56	.70	2.22 (.15)
CI11-231....	. . .[a]	2.34	2.29	1.85	1.22	.51	2.35 (.06)
CI11-410....	. . .[a]	.43	.79	.86	.63	.30	.89 (.16)
CI11-319....	. . .[a]	1.91	1.87	1.49	.96	.38	1.92 (.07)
CI11-8.....	. . .[a]	3.65	3.68	3.06	2.08	.90	3.72 (.08)
CI11-288....	4.89	4.51	4.10	3.21	2.19	1.03	4.89 (.00)
MCMP1.....	5.03	4.62	4.18	3.22	2.13	.92	5.03 (.00)
CI11-4.....	5.13	4.67	4.19	3.15	2.01	.79	5.13 (.00)
CI11-363....	1.07	1.00	.91	.73	.51	.27	1.07 (.00)
CI11-254....	. . .[a]	3.37	3.31	2.74	1.87	.80	3.38 (.06)
CI11-44	. . .[a]	.49	1.07	1.23	.90	.34	1.26 (.17)
CI11-283....	. . .[a]	−.75	−.39	−.06	.07	.09	.09 (.37)

[a] An ellipsis indicates that lod score was significantly negative.

Julier et al. (1990) observed a significant excess of female:male recombination in a genetic linkage map of the long arm of chromosome 11; for example, in their map the region between PGA and INT2 was approximately 14 cM in females and 6 cM in males. As figure 2 indicates, we also found a higher frequency of recombination in female meioses. A similar tendency characterizes the pericentromeric region of chromosome 10, where the MEN2 gene has been localized (Mathew et al. 1991).

Members of families segregating a gene for MEN1 carry a 50% risk of hypercalcemia or other endocrine dysfunctions, and they often develop carcinomas in endocrine tissues. Early detection of silent tumors would allow more prompt and effective surgical management. Hence, presymptomatic diagnosis is of particular importance for saving the lives of MEN1 patients. The markers reported here will provide useful resources for accurate presymptomatic diagnosis of risk status among members of MEN1 families.

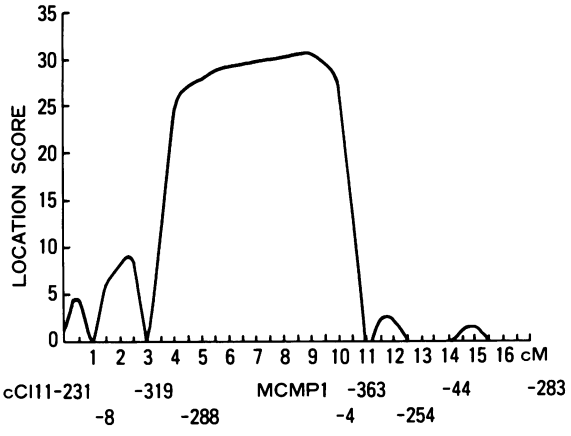

Figure 3 Location score for MEN1 locus on map in fig. 1. The most likely location of MEN1 is close to the cCI11-4 locus.

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