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A New DNA Marker (D10S94) Very Tightly Linked to the Multiple Endocrine Neoplasia Type 2A (MEN2A) Locus

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Summary

Combined somatic cell hybrid and linkage studies between D10S94 and five pericentromeric loci (FNRB, D10Z1, MEN2A, RBP3, and D10S15) have localized the new DNA sequence pcl1/A1S-6-c23 at D10S94 to 10q11.2. No recombinants were observed between D10S94 and D10Z1 or MEN2A. D10S94 maps in proximal 10q11.2 very near to MEN2A. There are three possible orders for the six loci that we investigated from the centromeric region of chromosome 10. At present the genetic data do not allow us to order MEN2A with respect to D10Z1 and D10S94. The three possible orders are FNRB–D10Z1–D10S94–MEN2A–RBP3–D10S15, FNRB–D10Z1–MEN2A–D10S94–RBP3–D10S15, and FNRB–MEN2A–D10Z1–D10S94–RBP3–D10S15. In view of the fact that no recombinants between D10S94 and MEN2A or between D10S94 and D10Z1 were observed, the combined haplotypes formed from RFLPs and D10Z1 and D10S94 will increase the informativeness and accuracy of genotype prediction for at-risk members of the families having the MEN 2A syndrome, particularly when the affected parent is female. The localization of D10S94 with respect to MEN2A will prove valuable in experiments directed toward cloning the MEN2A locus.

Introduction

Multiple endocrine neoplasia type 2A (MEN2A) is a dominantly inherited cancer syndrome characterized by medullary thyroid cancer (MTC) with and without pheochromocytomas (PHEO) and/or parathyroid adenoma. The MEN2A locus has been assigned to the pericentromeric region of chromosome 10 by DNA marker linkage studies (Mathew et al. 1987; Simpson et al. 1987). The closest single-copy flanking markers described to date are the genes encoding the interstitial retinol binding protein (RBP3), the beta subunit of the fibronectin receptor (FNRB), and the DNA sequence TB14.34 (D10S34) (Nakamura et al. 1989; Wu et al. 1990). RBP3 and FNRB flank the MEN2A locus at a recombination frequency (θ) of .05 and .06, respectively, on the basis of studies of six large families (Wu et al. 1990). D10S34 flanks the MEN2A locus with a θ of .07, on the basis of analyses of 29 families (Nakamura et al. 1989). In the same collection of families, RBP3 was found to flank the MEN2A locus at a θ of .02 (Nakamura et al. 1989).

RBP3 maps in the proximal region of the long arm of chromosome 10, in band 10q11.2 (Liou et al. 1987; Lathrop et al. 1988). FNRB is located on the short arm of the chromosome in band 10p11.2 (Goodfellow et al. 1989; Wu et al. 1989), and D10S34 is localized to the region 10p11-p12 (Mathew et al. 1990). MEN2A is very tightly linked to the alphoid repeat sequences at the centromere (D10Z1) (Wu et al. 1990). No recombinants between MEN2A and D10Z1 have been observed (Wu et al. 1990; C. P. G. Mathew, personal communication). Because of the lack of recombination between MEN2A and D10Z1, it is not known whether the MEN2A locus maps to the long or short arm of chromosome 10.

Recently the probe pcl1/A1S-6-c23 (D10S94) was iso-

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lated by Alu element–mediated polymerase chain reaction amplification of DNA from the somatic cell hybrid H2CL1 (Brooks-Wilson et al. 1990, and in press; P. J. Goodfellow, A. R. Brooks-Wilson, and D. Smailus, unpublished data). Mapping in a large series of somatic cell hybrids enriched for the pericentromeric region of chromosome 10 (Brooks-Wilson et al. 1990, and in press; Goodfellow et al. 1990; P. J. Goodfellow, A. R. Brooks-Wilson, and D. Smailus, unpublished data) allowed the assignment of pcl1/A1S-6-c23 to the most proximal portion of 10q11.2. This localization indicated that pcl1/A1S-6-c23 must map near the MEN2A locus. pcl1/A1S-6-c23 detects RFLPs in *Pvu*II- and *Rsa*I-digested DNAs (Brooks-Wilson et al., in press).

We report here the linkage relationship between D10S94 and MEN2A and between D10S94 and the four DNA markers FNRB, D10Z1, RBP3, and D10S15 from the pericentromeric region of chromosome 10.

Material and Methods

Identification of the Disease Phenotype

The families included in the present study are five kindreds (R, C, S, W, and B) in which linkage has been shown between the disease locus and chromosome 10 markers (Wu et al. 1990). In the present study, the N family (Wu et al. 1990) was not included. Individuals at risk for MEN2A but not presenting with either of the tumors which commonly characterize the syndrome—i.e., MTC or PHEO—were assigned a disease allele genotype on the basis of the results of highly reliable clinical screening tests (Gagel et al. 1988; Easton et al. 1989). Elevated serum calcitonin levels following pentagastrin stimulation on at least two consecutive assays was considered an indication for thyroidectomy, and raised urinary catecholamine levels were considered an indication for adrenalectomy. Whenever possible, there was histopathological confirmation of C-cell hyperplasia or MTC, in the case of a thyroid tumor, or confirmation of PHEO, in the case of the adrenal tumor.

Linkage Analysis

D10S94 *Pvu*II alleles were typed for all members of the five MEN2A families studied, while the rare *Rsa*I polymorphism detected by pcl1/A1S-6-c23 (the frequency of the uncommon allele is .03) (Brooks-Wilson et al., in press) was used in only one family. All family members had been previously typed for the pericentromeric markers FNRB, D10Z1, RBP3, and D10S15 (Wu et al. 1990). Genotypes used for linkage analysis in the present study were based on typings using the following probe/enzyme combinations: FNRB typing was done for *Bgl*II, *Ban*II, and *Hin*fI alleles detected by the pGEM-32 cDNA and for the *Msp*I alleles detected by the pB/R2 genomic sequence (Wu et al. 1989). D10Z1 genotypes were determined using *Pst*I and *Hin*fI variants and the pα10RP8 probe (Devilee et al. 1988; Carson et al. 1990). RBP3 *Bgl*II and *Msp*I variants detected by the H.4IRBP cDNA, as well as the *Taq*I variants detected by the cTBIRBP.9 probe, were determined (Liou et al. 1987; Simpson et al. 1987; Nakamura et al. 1988). D10S15 *Rsa*I alleles were determined using the pMCK2 probe (Lathrop et al. 1988). The lod score (Z) value (Morton 1955) was calculated using the LIPED program (Ott 1974) modified with a straight-line age-at-onset correction (Farrer et al. 1987; Hodge et al. 1979). Z values were calculated for both combined and separate male and female meioses. Confidence intervals were determined using 1-lod-unit estimates.

Results

Pairwise Z values (table 1) indicate linkage between D10S94 and the five loci examined from the pericentromeric region of chromosome 10. Of the loci tested, D10S94 is closest to MEN2A and D10Z1. No recombination between D10S94 and MEN2A and between D10S94 and D10Z1 was observed. Close linkage was observed between D10S94 and RBP3, D10S15, and FNRB. No recombination events separating D10Z1, MEN2A, D10S94, RBP3, and D10S15 were observed in male meioses (table 1). Our linkage data are consistent with the previously published order: FNRB–D10Z1–RBP3–D10S15 (Wu et al. 1990).

Two critical crossover events which define the map position of D10S94 with respect to other pericentromeric markers were observed in a nuclear family within the C kindred (fig. 1 *A*), and one was observed in a nuclear family from the R kindred (fig. 1 *B*). A recombination event in the mother (1–2)—i.e., that between FNRB and D10S94—was also detected in II-2 (fig. 1 *A*). The recombinant (II-2) is noninformative for the centromeric marker (D10Z1), and therefore the genetic data do not distinguish between the two possible map orders: FNRB–D10Z1–D10S94 and FNRB–D10S94–D10Z1. This recombination event which took place between FNRB and D10S94, however, places D10S94 on the centromeric side of FNRB. The second crossover in 1–2 was detected in II-5, who is a recombinant with respect to D10S94 and RBP3. This second recombina-

Table 1

Data on Pairwise Linkage between D10S94 and Five Pericentromeric Loci on Chromosome 10

Locus D10S94 vs.	Z at θ of[a]									1-Lod-Unit Confidence	Peak Z Value Parameters		
	0	.001	.01	.05	.1	.2	.3	.4	θ_m:θ_f	Interval	$\hat{Z}_{max}$	θ_m	θ_f
FNRB	-∞	2.45	5.26	6.54	6.30	4.78	2.83	1.02	.06	.02–.15	6.54	.03	.08
D10Z1	7.95	7.93	7.81	7.28	6.56	4.87	2.95	1.07	.0	.0–.07	7.95	.0	.0
MEN2A ...	8.57	8.55	8.43	7.80	7.00	5.25	3.36	1.46	.0	.0–.06	8.57	.0	.0
RBP3.....	-∞	9.72	11.45	11.59	10.60	7.88	4.86	1.97	.03	.01–.09	11.79	.0	.04
D10S15....	-∞	7.02	7.85	7.79	7.09	5.27	3.21	1.22	.02	.0–.10	7.96	.0	.03

[a] $\theta_m = \theta_f$.

[b] Calculated using the program LIPED (Ott 1974) modified by the age at onset correction (Hodge et al. 1979; Farrer et al. 1987). The disease allele frequency used was .0005. For families in the study, see text.

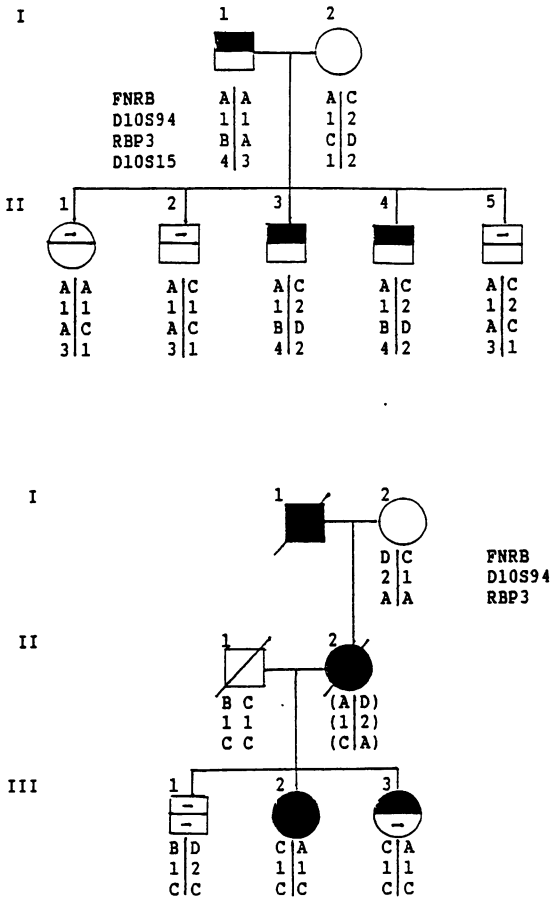

Figure 1 Haplotypes for pericentromeric loci in two nuclear families in which critical crossovers define map position of D10S94. Haplotypes were constructed using the probe/enzyme combinations described in Material and Methods. *A*, Nuclear family from the C kindred. *B*, Nuclear family from R kindred. Parentheses indicate that haplotypes were deduced and were consistent with those in the extended kindred. ■ and ● = MTC; ▣ and ◎ = negative pentagastrin test within past 4 years; ■ and ● = PHEO; ▣ and ◎ = negative catecholamine test.

tion event places D10S94 proximal to RBP3. The third critical crossover (fig. 1 *B*), also between D10S94 and RBP3, maps D10S94 proximal to RBP3. The recombination event which took place in the disease allele carrier, II-2 in the second family, was detected in III-1. III-1 is unaffected at the age of 40 years, and, on the basis of both his age and the results of clinical tests, has a low probability of having inherited the MEN2A allele. Although III-1 is most likely a recombinant with respect to MEN2A and RBP3, this recombination event provides no information on map order for D10S94 and MEN2A. Together, the three recombination events map D10S94 between FNRB and RBP3, in the interval to which MEN2A has been assigned.

Discussion

The pairwise linkage analyses of genetic mapping place D10S94 closest to the MEN2A and D10Z1 loci. Critical crossovers (Figs. 1 *A* and 1 *B*) map the new DNA sequence at D10S94 between the FNRB and RBP3; the latter two loci had, until now, been the two closest known single-copy markers flanking MEN2A. The genetic data do not provide a map order for D10Z1, MEN2A, and D10S94. D10S94 was, however, assigned to proximal 10q11.2 through the analysis of somatic cell hybrid DNAs. Of particular importance in regionally localizing D10S94 are the pair of hybrids TRAXK2 and TRAX10TG3. They retain reciprocal translocation chromosomes, which include as their only chromosome 10 material the regions 10q11.2-qter and 10pter-q11.2, respectively (Mathew et al. 1990; Brooks-Wilson et al. 1990). FNRB, localized to 10p11.2 by in situ hybridization, is, as expected, present in the TRAX10TG3 hybrid. RBP3, which was assigned to 10q11.2 through dosage studies, is present in the TRAXK2 hybrid.

D10S94 is present in the TRAXK2 hybrid and absent in TRAX10TG3. Additional hybrids suggest a localization in the most proximal portion of 10q11.2 (P. J. Goodfellow, A. R. Brooks-Wilson, and D. Smailus, unpublished data). Such a localization is compatible with the genetic map proposed in the present study. When the physical and genetic mapping data are combined, the order of the marker loci is unequivocally as follows: FNRB–D10Z1–D10S94–RBP3–D10S15, with the MEN2A locus mapping to one of three intervals. MEN2A must map either between RBP3 and D10S94 (on the long arm of the chromosome) or between D10S94 and D10Z1 (on the long arm of the chromosome) or between D10Z1 and FNRB (on the short arm of the chromosome).

The identification of D10S94 as a marker tightly linked to MEN2A is of considerable importance in DNA-based diagnostics in MEN 2A families. The frequencies of the two alleles recognized in *Pvu*II digests are .67 and .33, giving the RFLP a PIC value of .34, which will mean that the marker will be informative in a considerable number of families. The second pair of alleles will be less useful, with frequencies of .97 and .03 (PIC = .06). D10S94 is the first single-copy marker locus to be linked to the disease locus with no recombination in either sex. The codominant alleles identified at D10S94 result in more recognizable matings of the heterozygous × homozygous (or heterozygous) type that are necessary for informativeness in family studies than the dominant morphs detected at D10Z1, the only other marker with no reported recombination in either sex. The absence, in either sex, of recombination between D10S94 and D10Z1 makes it possible to generate haplotypes in order to further improve the informativeness in DNA marker studies for use in disease allele prediction in MEN2A families. Previously, if the D10Z1 marker was noninformative we had to rely on the use of the closest flanking markers, FNRB and RBP3. Although no recombination between either RBP3 or FNRB and MEN2A has been observed in male meioses, the recombination between the markers in females is considerable; RBP3 and MEN2A recombine at a θ of .10, and FNRB and MEN2A recombine at a frequency of .09 (Wu et al. 1990). In those instances in which an individual is at risk for inheriting the MEN2A allele from the mother, the accuracy of DNA disease genotype prediction is limited. The usefulness of D10Z1 and D10S94 in generating haplotypes for MEN2A studies is greatly enhanced by the fact that neither D10S94 nor D10Z1 has been observed to recombine with MEN2A.

The identification of D10S94 as a single-copy marker which shows no recombination with MEN2A in either sex will greatly facilitate experiments directed toward cloning the disease gene on the basis of its chromosomal localization. We are currently creating a long-range restriction map for the chromosome 10 pericentromeric region to which the disease gene maps.

Testing the linkage relationships between D10S94 and two other dominantly inherited cancers mapped to the pericentromeric region of chromosome 10—i.e., MEN 2B and MTC without PHEO—will allow better definition of the chromosomal region to which they map. The similarities, at the clinical level, between MEN 2A, MEN 2B, and MTC without PHEO, combined with the localization of all three mutations (if they are different) to the same region of the chromosome (at present known as the MEN2A locus and provisionally MEN2B) (Narod et al. 1989; Smith and Simpson 1989; Carson et al. 1990; Wu et al. 1990), makes it appealing to suggest that they may be allelic. By examining the linkage relationships between D10S94 and the three disease mutations, we will gain a better understanding of their relationship to one another at the level of resolution possible through meiotic mapping. Through cloning of the disease loci it will be possible to directly examine the relatedness of these three dominantly inherited cancers involving the medullary thyroid.

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