

A new polymorphic marker (D10S97) tightly linked to the multiple endocrine neoplasia type 2A (MEN2A) locus

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Abstract. Familial multiple endocrine neoplasia type 2A (MEN 2A) is a cancer syndrome that is inherited as an autosomal dominant with high penetrance. Its clinical features are medullary carcinoma of the thyroid, pheochromocytomas, and hyperparathyroidism. A new polymorphic locus D10S97 (probe: KW6Δ*Sac*I) detects a codominant *Eco*RI polymorphism that is tightly linked to the MEN2A locus. The peak lod score for linkage between D10S97 with MEN2A is 13.03 at $\theta = 0.00$. The polymorphic locus D10S97 maps, by linkage analysis, into the previously defined interval between FNRB and RBP3 to which MEN2A has been assigned. We present physical mapping data showing that the probe pKW6 originates from 10p13 and that the polymorphic locus D10S97 in 10q11.2 is detected by cross-hybridization.

Introduction

The locus responsible for familial multiple endocrine neoplasia type 2A (MEN 2A) has been mapped to the pericentromeric region of chromosome 10 (Mathew et al. 1987; Simpson et al. 1987; Kidd and Simpson 1990). The primary clinical feature of MEN2A is medullary carcinoma of the thyroid (Gagel et al. 1988). The MEN2A locus is flanked by FNRB and D10S34 on the proximal short arm and RBP3 and D10S15 on the proximal long arm of chromosome 10 (Wu et al. 1990; Mathew et al. 1991; Lichter et al. 1992b). The α satellite repeat polymorphism at D10Z1 show tight linkage to MEN2A (Wu and Kidd 1990a; Carson et al. 1990). These five loci are sufficient to achieve a DNA-based diagnosis for carrier status in most cases (Lichter et al. 1992a). Tight linkage between two marker loci from proximal 10q11.2, D10S94 (Goodfellow et al. 1990) and D10S102 (Mathew et al. 1991), and MEN2A have been reported. These newer

loci add additional markers for DNA-based testing for MEN2A. Here we describe another locus, D10S97 (Wu and Kidd 1990b), that is tightly linked to MEN2A and has potential value for DNA based presymptomatic tests for MEN2A. The D10S97 polymorphism has been studied in a global survey and is polymorphic in almost all of our population samples. This locus, however, is not simple; the probe pKW6 recognizes sequences in at least two places. We have physical evidence supporting the linkage data placing the polymorphic sequence at 10q11.2. We also demonstrate that the pKW6 probe is derived from a monomorphic locus at 10p13 and have assembled a yeast artificial chromosome (YAC) contig for that locus called D10F38S2.

Materials and methods

Patient material

All subjects in MEN2A kindreds who participated in this study have been previously described by Wu et al. (1990). Population samples have been previously described (Bowcock et al. 1987; Kidd et al. 1991; Kidd et al. 1992).

DNA and probes

The probe pKW6Δ*Sac*I is available from ATCC (Rockville, Md.). Preparation of plasmid and genomic DNA, nick translations, DNA digestions and hybridizations were carried out as previously described in Wu et al. (1990). Sequencing was accomplished using the Sequenase (USB) kit. Somatic cell hybrids used in regionally localizing sequences recognized by pKW6 have been described previously (Brooks-Wilson et al. 1990; Mathew et al. 1990; Astrin et al. 1991; Carson and Simpson 1991). The fluorescence in situ hybridization (FISH) was performed according to the procedure of Lichter et al. (1990) using Cot1 DNA (BRL) for suppression of highly repetitive sequences. The chromosomes were counterstained with DAPI to give a G-like banding pattern for easy chromosome identification. Each of the YACs were imaged at least three times, 18SE2 was imaged five times.

The STSs for pKW6 (D10F38S2) are: (1) A1–A2, GTA TGG AGC TTT AAG GTA GGA, GCC ATG GAT TGG GTG TTG G (5' to 3') 206 base pairs (bp); (2) S1–A2, CTG ATT CCT TAA CAT TTT TCT CTG CA, GCC ATG GAT TGG GTG TTG G

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(5' to 3') 850 bp; (3) S2-H1, AGC TTT GGC CTC CGT GCG, GTC AAA ACT ACC TCC ACG AC (5' to 3') 263 bp. All PCR was done as follows: 2.25 mM MgCl₂, 30 cycles with 30 min at 94°C, 30 min at 60°C, and 30 min at 72°C, in the Perkin-Elmer 9600 thermocycler. YACs were isolated from the Centre d'Etude du Polymorphisme Humain (CEPH) YAC library (Albertsen et al. 1990) using PCR-based screening procedures similar to those described by Green and Olsen (1990).

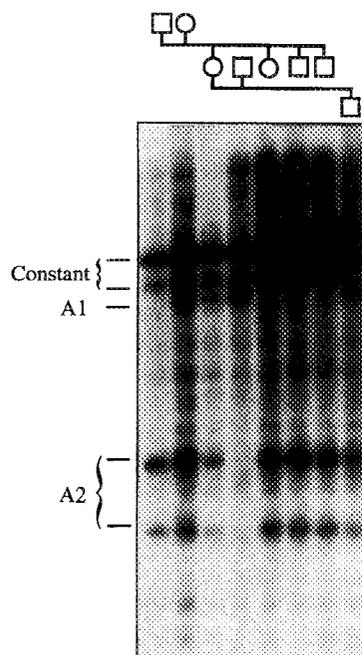


Fig. 1. Inheritance pattern of D10S97 restriction fragment length polymorphism (RFLP) in the MEN2IS family (Wu et al. 1990). DNA was digested with *EcoRI* and probed with pKW6Δ*SacI* as described in the methods. Lanes 1–8, contain DNA samples from individuals JK2368, JK2376, JK2369, JK2366, JK2339, JK2380, JK2378, and JK2365, respectively. Alleles A1 (6.0 kb) and A2 (3.5 + 2.5 kb) are indicated. Several constant bands are detected by this probe; two particularly prominent ones, 7.0 kb and 6.5 kb, are labeled *constant*. The genotypes for these individuals are: lane 1 A2/A2; lane 2 A1/A2; lane 3 A1/A2; lane 4 A1/A1; lane 5 A2/A2; lane 6 A2/A2; lane 7 A2/A2; and lane 8 A1/A2

Analytical and statistical methods

Linkage analysis was carried out using LIPED (Ott 1985) with local revisions. Lod scores were summed across all of our family material. Allele frequencies, heterozygosities, and polymorphism information content (PIC) values were calculated empirically using formulas from Botstein et al. (1980).

Results

Polymorphic system

Figure 1 shows the inheritance pattern of the codominant two-allele system for D10S97 using the probe pKW6Δ*SacI*. The A1 allele produces an approximately 6-kb *EcoRI* fragment, while the A2 allele produces both the 2.5- and 3.5-kb fragments. This indicates that the polymorphic *EcoRI* site probably resides near the middle of the 6.0-kb *EcoRI* fragment and that the probe spans this site. Several constant bands are detected with this probe. The three most prominent ones are: 7 kb, 6.5 kb, and 1.0 kb. The parent clone, pKW6, only allows the A2 bands to be scored because it detects an additional constant band that comigrates with the A1 allele. Additionally, the parent clone also detects a prominent 2.0-kb *EcoRI* constant fragment.

Molecular details

Figure 2 summarizes the molecular analysis of the probe pKW6 and indicates the polymorphic components of D10S97 detected by various subfragments. The 2.7-kb insert identifies several bands (about 35 kb) on a genomic Southern blot. In addition to some level of genomic duplication needed to yield this much hybridization with such a short genomic clone, we believe that this clone has some low level repetitive element that results in many faint "background" bands.

We have made a series of deletions using unique restriction enzyme sites within the clone (Fig. 2). These deletion clones have been used as hybridization probes on

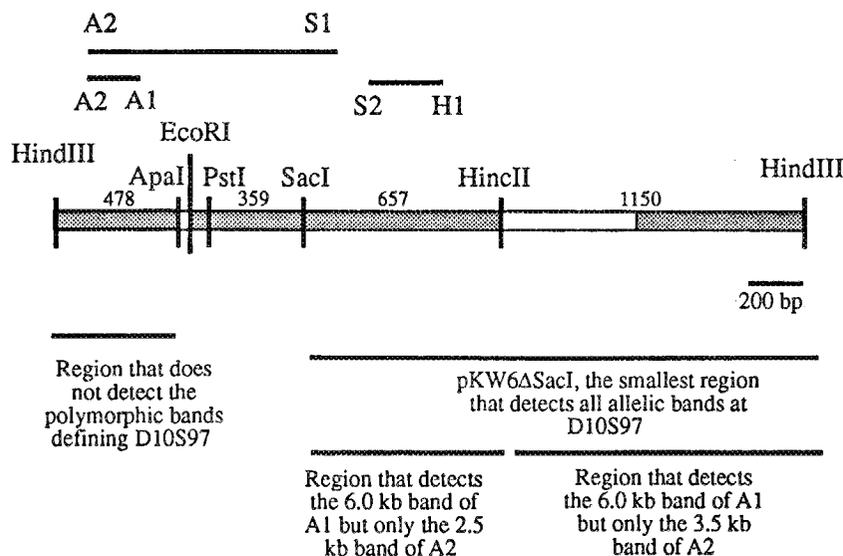


Fig. 2. Molecular map of pKW6, the clone recognizing D10S97. Shaded regions have been sequenced. Unique restriction sites are shown, as are cloning sites. The bars above the map show the PCR amplifiable regions using the primers listed in Materials and methods. The numbers between the restriction sites are approximate sizes of those fragments in base pairs. The bars below the clone summarize the hybridization results of some pKW6 deletion clones.

Table 1. Allele frequencies of D10S97 in populations from around the world, grouped by geographical location

Population sample	Number of chromosomes	A1 frequency	A2 frequency	Observed heterozygosity
<i>Africa</i>				
Ethiopian Jews	32	0.750	0.250	0.500
Pygmy (C.A.R.) Biaka	68	0.544	0.456	0.559
Pygmy (Zaire) M'buti	53	0.679	0.321	0.269
<i>Asia</i>				
Assamese	30	0.733	0.267	0.533
Cambodian	50	0.600	0.400	0.640
Chinese	43	0.767	0.233	0.476
Japanese	88	0.682	0.318	0.500
Thoti	21	0.619	0.381	0.800
<i>Middle East</i>				
Druze	41	0.463	0.537	0.231
Yemenite Jews	42	0.690	0.310	0.368
<i>Americas</i>				
Colombian	26	0.231	0.769	0.462
Karitiana	75	0.213	0.787	0.378
Mayan	101	0.139	0.861	0.280
Muskoke	22	0.318	0.682	0.455
Quechua	44	0.409	0.591	0.409
Surui	94	0.489	0.511	0.554
<i>Other</i>				
Mixed European	144	0.444	0.556	0.400
Roman Jews	48	0.458	0.542	0.583
Global	1022	0.485	0.515	0.453

EcoRI-digested genomic DNA. The portion of the molecule to the "left" of the *EcoRI* site does not detect any polymorphic bands. It only detects the 6.0-, 2.0-, and 1.0-kb constant bands (data not shown). The probe pKW6 Δ *SacI*, a subclone that only possesses DNA from the "right" of the *SacI* site, detects the two-allele, three-band (A1A2) system, as well as the constant 7.0-, 6.5-, and 1.0-kb bands. Further deletion analysis of this clone around the *HincII* site indicates that the polymorphic genomic *EcoRI* site is very close to this *HincII* site; the clone itself does not contain an *EcoRI* site in this region.

Allele frequencies in population samples from around the world

Table 1 summarizes the allele frequencies in several population samples from around the world. The global allele frequencies are 0.485 and 0.515 for the A1 and A2 alleles, respectively, with an observed heterozygosity of 0.453 and a PIC value of 0.328. Allele frequencies vary considerably among populations with A1 frequencies ranging from a low of 0.139 in Mayans to a high of 0.767 in Chinese.

Pairwise lod scores and recombination distances

We have analyzed this *EcoRI* polymorphism in our reference and MEN2A families (Wu et al. 1990). The pair-

wise lod scores and estimates of genetic distances for D10S97 with several pericentromeric chromosome-10 loci are presented in Table 2. The data show that D10S97 is tightly linked to MEN2A. Previous linkage analyses clearly place D10S97 between FNRB/D10S34 and RBP3/D10S15; 19 identified crossovers placing D10S97 proximal to FNRB/D10S34 and 10 identified crossovers placing D10S97 proximal to RBP3/D10S15 (Lichter et al. 1992b). Of these 29 crossovers, 25 were simultaneously informative at all three locations: proximal short arm, D10S97, and proximal long arm. Two additional markers from the proximal long arm of chromosome 10 (between the centromere and RBP3/D10S15) were informative in the meiotic mapping panel; D10S97 maps between them, distal to D10S94 and proximal to D10S102 (Lichter et al. 1992b).

In the data in Table 2 several of the pairwise distances between D10S97 and other markers in the small pericentromeric region of chromosome 10 are artificially large, higher than estimated distances across the entire region. This is caused by a bias in our data set against non-recombinants. We have identified most of the sections of our extended families in which any crossover between FNRB and D10S15 occurs; only those parts of the families have been typed for many of the new markers that fall into this region. This lack of nonrecombinant individuals artificially increases the genetic distances calculated in these comparisons. This is especially evident for

Table 2. Sex average genetic distances and the corresponding lod scores of D10S97 with other chromosome-10 loci

D10S97 versus	Recombination fraction ($\theta_m = \theta_t$)									Z_{\max}	$\theta_{m=r}$	Confidence interval
	0.000	0.001	0.01	0.05	0.10	0.20	0.30	0.40				
MEN2A	13.03	13.01	12.78	11.73	10.37	7.43	4.30	1.44	13.03	0.000	0-0.028	
FNRB	$-\infty$	-0.30	6.69	10.62	10.85	8.50	5.06	1.79	10.88	0.087	0.037 -0.124	
D10S34	$-\infty$	-8.91	-2.91	0.86	1.80	1.58	0.76	0.15	2.04	0.142	0.077 -0.279	
D10Z1	$-\infty$	6.97	7.93	8.21	7.66	5.66	3.22	1.08	8.21	0.048	0.0004-0.115	
D10S94	$-\infty$	-2.87	0.79	3.48	4.20	3.77	2.40	0.83	4.34	0.133	0.039 -0.227	
D10S102	$-\infty$	0.91	1.89	2.44	2.44	1.92	1.17	0.45	2.46	0.076	0.0008-0.249	
RBP3	$-\infty$	10.82	17.47	20.25	19.20	14.46	8.78	3.41	20.46	0.065	0.044 -0.087	
D10S15	$-\infty$	21.65	23.30	22.89	20.89	15.62	9.57	3.66	24.68	0.029	0.024 -0.042	

Z_{\max} , Maximum lod scores; $\theta_{m=r}$, estimated genetic distance; Confidence interval, 1 lod unit

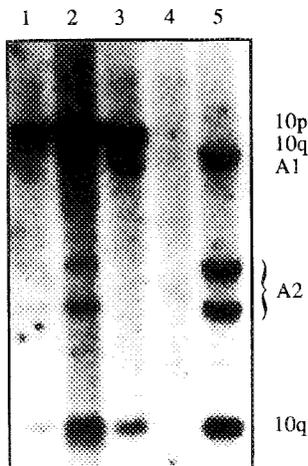


Fig. 3. Somatic cell hybrid mapping of pKW6. Lane 1 762-8A (10+Y only hamster human hybrid, hemizygous for A2); lane 2 WT49 (human female lymphoblastoid cell line, hemizygous for A1); lane 3 R 342-A4 (10 only hamster/human hybrid, hemizygous for A2); lane 4 is W3GH (hamster); and lane 5 64034p61C10 (10cen-qter + Y only, hemizygous A2). No crosshybridization to rodent sequences was detected (lane 4). All samples were digested with *EcoRI*. The 10p, 10q, and allelic bands are shown

loci for which heterozygosity is low. Furthermore, the 1 lod unit confidence intervals for these intervals are quite large, making precise estimates of the recombination distances impossible. These data are not presented as accurate distance estimates but rather as documentation of the close linkage. The meiotic mapping panel (Lichter et al. 1992b) is the basis for ordering the loci.

Somatic cell hybrid mapping of sequences recognized by probe pKW6 Δ SacI

Hybridization of the probe pKW6 Δ SacI to *EcoRI*-digested DNAs gives rise to a complex pattern of bands, which are derived from both the long and short arms of chromosome 10. Southern blot analysis of DNAs from somatic cell hybrids that retain defined subchromosomal regions (Fig. 3) allowed assignment of the most strongly hybridizing fragment of approximately 7.0 kb to 10p. This 7.0-kb constant band is absent in hybrid 64034p61C10, which retains a derivative chromosome 10 including the

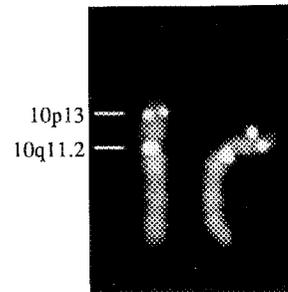


Fig. 4. Fluorescence in situ hybridization of pKW6 shown in micrograph of metaphase spread. Cytogenetic localizations are shown for both signals

region 10cen-qter, but lacks short arm sequences (Fig. 3, lane 5). The more weakly hybridizing polymorphic *EcoRI* restriction fragments (A1, 6.0 kb and A2, 3.5 + 2.5 kb) as well as the constants (6.5-kb and 1.0-kb fragments recognized by pKW6 Δ SacI, however, map to either 10q or 10cen.

Fluorescence in situ hybridization (FISH)

We have mapped the original pKW6 plasmid by FISH to refine the localization of the 10p sequences detected by the probe. The results are shown in Fig. 4. The probe pKW6 maps to two locations on chromosome 10, one in 10q11.2 (D10S97) and one in 10p13. The 10q sequences recognized by pKW6 were detected in only 3 of 11 metaphase spreads examined, while the 10p locus was detected in 9 of the 11. These two loci now have a family symbol from the nomenclature committee at Human Genome Mapping 11 (Simpson and Cann 1991), D10S97 is now also known as D10F38S1 and the 10p13 locus is D10F38S2.

YACs

We have used our DNA sequence information to design PCR primers for pKW6 in the hope of amplifying DNA from D10F38S1. We have used these primers to isolate three YACs for pKW6 from the CEPH YAC library (Albertsen et al. 1990). The presence of sequences recognized by pKW6 has been verified for all three YACs

by PCR and Southern blotting (data not shown). FISH analysis shows that the YACs 27B11, 185E2, and 261B8 all are from the D10F38S2 locus (data not shown; see Material and methods). The localization of these YACs to 10p13 is further evidence that the DNA sequence is from 10p13 and not 10q11.2.

Discussion

One of the goals of genetic studies is to devise new, more reliable techniques to assist in the clinical management of disease. We have shown that the probe pKW6 Δ SacI detects a polymorphism that is tightly linked to MEN2A. The heterozygosity value for this polymorphism makes it an additional useful marker for clinical diagnoses. Although the allele frequencies vary in many of the populations that we have studied, D10S97 is an additional marker that can be used for presymptomatic testing for MEN2A. D10S97 is not a simple, single-copy locus; careful interpretation of the banding pattern is essential to determine correctly the inheritance pattern of the alleles.

While pKW6 Δ SacI detects a useful polymorphism in the MEN2A region, its uses for physical mapping of the MEN2A region are limited. Our data show that the probe originates from 10p13 at the newly defined locus D10F38S2, and that the polymorphic locus D10S97 (D10F38S1) at 10q11.2 is detected by crosshybridization with the 10p13 sequences. Three lines of evidence support this conclusion: (1) FISH with the original pKW6 clone consistently detects the 10p13 locus more readily than the 10q11.2 locus; (2) somatic cell hybrid mapping studies reveal that the most strongly hybridizing fragments recognized by the probe map to 10p, while the polymorphic *Eco*RI fragments map to 10q11.2; (3) YACs isolated using PCR primers made from sequences of pKW6 map to 10p13 and further confirm that the DNA sequence originates from 10p13.

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Note added in proof. Subsequent analyses suggest that the probe pKW6 Δ SacI recognizes additional loci in 10q11.2.