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Genetic Linkage Map of Six Polymorphic DNA Markers around the Gene for Familial Adenomatous Polyposis on Chromosome 5

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Summary

A genetic linkage map of six polymorphic DNA markers close to the gene (*APC*) for familial adenomatous polyposis (FAP) on chromosome 5q is reported. One hundred fifty-five typed members of nine FAP kindred provided more than 90 meioses for linkage analysis. A number of crucial recombination events have been identified which are informative at three or more loci, allowing confident ordering of parts of the map. There was no evidence of genetic heterogeneity, with all families showing linkage of at least one chromosome 5 marker to the gene. Recombination data and two-point linkage analysis support a locus order of centromere–π227–C11P11–ECB27–L5.62–*APC*–EF5.44–YN5.48–telomere, although EF5.44 could lie in the interval L5.62–*APC* or ECB27–L5.62. No recombinants were identified between *APC* and either EF5.44 or YN5.48, but published deletion mapping in colorectal carcinomas and linkage analysis in FAP suggest that YN5.48 is 1–3 cM from *APC*. The present study suggests that YN5.48 and L5.62 delineate a small region of chromosome 5 within which the EF5.44 locus lies very close to the *APC* gene. These data not only allow use of flanking markers for presymptomatic diagnosis of FAP but also provide a high-density map of the region for isolation of the *APC* gene itself and for further assessment of the role of chromosome 5 deletions in the biology of sporadic colorectal cancer.

Introduction

The genetic aberration resulting in familial adenomatous polyposis (FAP) has an incidence of approximately 1:10,000 (Reed and Neel 1955) but is of great clinical importance as a gene defect causing an autosomal dominant heritable cancer syndrome in which large-bowel cancer is preventable by prophylactic colectomy (Bulow 1987). The gene for FAP has been shown to be linked to markers which map to chromosome 5q (Bodmer et al. 1987; Leppert et al. 1987; Meera-Khan et al. 1988; Nakamura et al. 1988; Dunlop et al. 1989; Murday et al. 1989; Varesco et al. 1989) and is known as *APC*. *APC* has also been implicated as a tumor suppressor gene involved in the biology, if not in the genesis, of sporadic colorectal cancer. Loss of genetic material from chromosome 5 has been demonstrated in FAP and in sporadic colorectal adenomas and carcinomas (Solomon et al. 1987; Vogelstein et al. 1988; Ashton-Rickardt et al. 1989; Sasaki et al. 1989). The mechanism of such loss is usually by interstitial deletion (Ashton-Rickardt et al. 1989). The published data broadly support the hypothesis that *APC* mutations are recessive at the cellular level and, in common with other tumor suppressor genes, require inactivation of both alleles if malignancy is to supervene (Knudson 1971).

In addition to causing the full FAP syndrome, *APC* mutations may also be involved in the inheritance of susceptibility to non-FAB colorectal cancer. Familial clustering of colorectal cancer has been well described (Woolf 1958; Macklin 1960; Lovett 1976; Duncan and Kyle 1982; Lynch et al. 1985; Bonelli et al. 1988). Dominant inheritance of colorectal adenomas and of carcinomas may even account for the majority of cases of these neoplasms with a gene frequency in the general population as high as 19% (Cannon-Albright et al.

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1988). Clinically, certain extracolonic features of FAP occur with excess frequency in apparently sporadic colorectal cancer (Sondergaard et al. 1985*b*) and in hereditary nonpolyposis colorectal cancer (Sondergaard et al. 1985*a*), suggesting a common genetic etiology. In addition, genetic linkage has recently been established using chromosome 5q DNA probes in a family in which affected individuals have variable numbers of polyps which do not all meet the criteria for the diagnosis of FAP (Leppert et al. 1990). It seems likely that a number of different mutant genes are responsible for colorectal cancer susceptibility, and so *APC* may be considered a candidate member of such a group of genes.

A high density of polymorphic DNA markers in the region around *APC* will greatly facilitate efforts toward identification of *APC*. Even after full characterization of *APC*, it is likely that closely linked markers will still be valuable for confirmation of mutation analysis and for presymptomatic diagnosis of FAP in families with rare mutations.

The present study reports a genetic linkage map of six polymorphic DNA markers. Two new probes have been mapped, and one of these appears to be very close to *APC* itself.

Material and Methods

Nine Scottish kindreds with FAP were studied, comprising 155 genotyped and phenotyped individuals. This provided in excess of 90 meioses for linkage analysis if all families were fully informative for any marker. Clinical, pathological, and genealogical data were complete, and affection status and penetrance were assigned in the manner which we have described elsewhere (Dunlop et al. 1989).

Genotyping was carried out as we have reported elsewhere (Dunlop et al. 1989), and all techniques were carried out by established protocols. In brief, DNA was purified from fresh peripheral blood leukocytes, digested with the appropriate restriction endonuclease, agarose-gel fractionated, and transferred onto nylon membranes, and hybridization of ^{32}P-labeled DNA probe was revealed by autoradiography.

Genotypes were obtained for six DNA probes which recognize RFLPs on chromosome 5. Probe DNA fragments used were as follows: π227 (D5S37) (Stewart et al. 1987) as a 900-bp, *Hin*dIII/*Eco*RI fragment in πAN7; C11P11 (D5S71) (Bodmer et al. 1987) as a 3.6-kb, *Eco*RI fragment in pUC8; ECB27 (D5S98) (Murday et al. 1989; Varesco et al. 1989) as a 2.8-kb, *Sal*I fragment in phage lambda; pL5.62 as a 10.5-kb, *Bgl*II fragment cloned into the *Bam*HI site of pUC18; pEF5.44 as a 2.3-kb, *Msp*I fragment cloned into the *Acc*I site of pUC18; and YN5.48 (D5S81) (Nakamura et al. 1988) as a 2.4-kb, *Taq*I fragment cloned into the *Acc*I site of pUC18. Table 1 shows polymorphisms recognized by each of these probes and the appropriate restriction enzyme used in the present study. Allele sizes and frequencies from a local control population are also tabulated in table 1. Probes pL5.62 and pEF5.44 are DNA fragments recognizing polymorphisms as noted above and are subclones from cosmids selected from a genomic cosmid library derived from part of chromosome 5q (Nakamura et al. 1988).

Linkage analysis was carried out using the *LINKAGE* group of programs. The 1-lod unit of support was

Table 1

Allele Size and Frequency Recognized at Six Polymorphic Chromosome 5q DNA Marker Loci in a Control Scottish Population

Probe (enzyme recognizing polymoprhism)	Frequency/Size (kb)			No. of Chromosomes Examined
	A1	A2	A3	
EF5.44 (*Msp*I)	.18/2.9	.82/2.1		84
L5.62 (*Bgl*II)	.93/9.0	.07/5.5		76
YN5.48 (*Msp*I)	.45/9.0	.55/8.0		60
ECB27 (*Bgl*II)	.39/11.9	.61/10.5		74
C11P11 (*Taq*I)	.14/4.4	.86/3.9		100
π227 (*Pst*I)	.25/3.0	.75/4.3		66
π227 (*Bcl*I)	.17/3.0	.46/1.8	.37/1.2	54
π227 (*Bst*XI)	.29/2.7	.71/2.3		28
π227 (*Mbo*I)	.25/.55	.75/.45		20

taken as representative of 95% probability limits. Multipoint linkage analysis was not felt to be valid for the present study, since only affected families were analyzed, without Centre Etude de Polymorphisme Humaine (CEPH) data. Instead, the data are presented as a series of pairwise analyses between marker and disease loci and between all pairs of marker loci. Crucial rare recombinant events informative at three or more loci were identified and have allowed confident locus ordering for some parts of the map. The lod scores for linkage between markers are relatively low because the families were not specifically selected for a high level of informativity such as is the case with CEPH families.

Validation of paternity and of typing for chromosome 5 markers was inherent in the multiple two-point analyses which were carried out. In addition, paternity testing was carried out on all *APC*-marker obligate recombinants by using the probe 29C1 (DXYS14), which is derived from the sex chromosomes and recognizes a pseudoautosomal hypervariable polymorphism (Cooke et al. 1985). This probe is extremely useful for paternity testing since typing can be carried out simply by Southern blot analysis. In addition, Cooke et al. (1985) found that, of 83 individuals studied, no two had the same genotype.

Results

The results of the analysis for linkage of *APC* to each of the six polymorphic DNA marker loci are presented in table 2. The peak lod score (Z_{max}) and recombination fraction (θ) indicating the maximum likelihood of marker location relative to *APC*, along with 95% probability limits, are tabulated together with lod-score tables. No recombinants have been identified between *APC* and YN5.48 or between *APC* and EF5.44. The Z_{max} for APC–YN5.48 was 7.00 at θ = .00, with 95% probability limits .00–.07. EF5.44 was not very informative in these families, and so the Z_{max} for *APC*–EF5.44 was only 3.5 at θ = .00, with resultant wide 95% probability limits.

Tests for linkage between each pair of marker loci revealed no discrepancies to suggest nonpaternity or mistyping. There were no cases of nonpaternity when all marker-*APC* recombinants were tested with the probe 29C1.

Lod-score calculations were carried out for linkage between each pair of markers, and these data are shown in table 3. The θ is given above the diagonal and the Z_{max} is given below the diagonal, for each marker-marker combination. No recombination events were detected between the following pairs of markers: L5.62–ECB27, ECB27–C11P11, C11P11–π227, and EF5.44–L5.62. However, the lod scores for EF5.44–L5.62 and for L5.62–ECB27 were low because of poor informativity of these markers in the local population (see table 1).

Table 4 shows all recombination events which were fully informative for three or more loci. The vertical line represents the recombination event in each case, and the informative markers are ranged either side of the breakpoint, depending on the haplotypes resulting from the recombination. Figure 1 shows this information in graphic form. The shaded area represents all possible *APC* locations, relative to the markers studied. Thus, *APC* could overlap EF5.44 or YN5.48 but cannot overlap L5.62, ECB27, C11P11, or π227, because of the presence of recombinants. These data suggest that YN5.48 and L5.62 delineate a small region which spans *APC*. The interval L5.62–YN5.48 could be as much as 11 cM (see table 3) or 7 cM (since L5.62–EF5.44 is 0 cM and since EF5.44–YN5.48 is 7 cM) or as little as 2 cM (since L5.62–*APC* is 2 cM and since *APC*–YN5.48 is 0 cM). The latter interval

Table 2

Linkage Analysis in Nine Scottish FAP Kindreds (KMD1–6, 8, 14, and 16)

Linkage vs. *APC*	Z_{max}	θ	95% Probability Limits	θ					
				.00	.05	.10	.15	.20	.25
EF5.44	3.50	.00	.000–.160	3.50	3.22	2.91	2.58	2.23	1.86
YN5.48	7.00	.00	.000–.070	7.00	6.31	5.56	4.79	4.01	3.24
L5.62	13.31	.02	.005–.095	...	12.99	11.94	10.65	9.21	7.65
ECB27	1.89	.06	.002–.260	...	1.88	1.76	1.50	1.20	.90
C11P11	5.45	.09	.025–.210	...	5.23	5.43	5.13	4.59	3.90
π227	4.40	.11	.035–.235	...	4.00	4.40	4.26	3.88	3.35

Table 3

Two-Point Z_{max} Values (below diagonal) and θ Values (above diagonal) for Linkage between Marker Loci in Nine FAP Kindreds

	EF5.44	YN5.48	L5.62	ECB27	C11P11	π227
EF5.44		.07	.00	.15	.16	.25
YN5.48	2.11		.11	.19	.11	.25
L5.62	1.23	.98		.00	.04	.04
ECB27	.69	.52	1.03		.00	.12
C11P11	.64	1.60	4.11	1.86		.00
π227	.00	1.05	1.48	1.72	3.05	

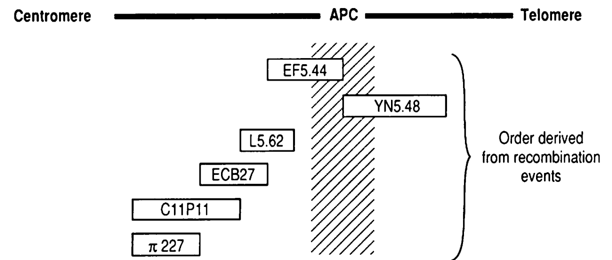

Figure 1 Markers ordered from recombination events as detailed in table 5. *APC* is represented by the shaded area, and the site of marker locus possible at any point in the box indicated.

(2 cM) seems most likely, since lod scores are highest for linkage of each of the markers to *APC*. When two-point linkage analysis is added to the order derived from recombination events (table 4), and given that C11P11 is centromeric to *APC* (Varesco et al. 1989) and that YN5.48 is telomeric to *APC* (Nakamura et al. 1988), these data support the locus order centromere–π227–C11P11–ECB27–L5.62–*APC*–EF5.44–YN5.48–telomere, although EF5.44 could lie in the interval L5.62–*APC* or, least likely, in the interval ECB27–L5.62.

Table 4

Recombination Events in Which Three or More Loci Were Informative

C11P11 π227	*APC* EF5.44 L5.62	C11P11	*APC* EF5.44 YN5.48
L5.62 π227	*APC*	π227	*APC* EF5.44 L5.62
ECB27 π227	*APC*	L5.62 C11P11 π227	YN5.48
ECB27 C11P11	YN5.48	ECB27	EF5.44 YN5.48
ECB27 π227	YN5.48	ECB27 C11P11 π227	EF5.44 YN5.48
EF5.44 ECB27 C11P11 π227	YN5.48	π227	EF5.44 YN5.48 ECB27

NOTE.—The vertical line represents where the recombination has occurred in relation to each of the informative loci.

Discussion

The present study has provided a genetic linkage map of six polymorphic DNA markers which lie very close to *APC* on chromosome 5q. The large number of meioses studied and the detection of a number of rare recombination events which were informative at multiple loci have allowed confident ordering of parts of the map. The possibility of double crossovers for these events is remote, since the genetic area under scrutiny is very small indeed. The localization of YN5.48 distal to *APC* (Nakamura et al. 1988) now seems certain, following deletional analysis in colorectal carcinomas (Ashton-Rickardt et al. 1989) and the recent identification of an *APC*–YN5.48 recombinant which was nonrecombinant for APC–π227 (Tops et al. 1989). No *APC*–YN5.48 recombinants were detected in the present study, but, when the relevant two-point linkage analysis data are added into the map, the localization of YN5.48 distal to *APC* and on the other side from π227, C11P11, ECB27, and L5.62 is highly likely. The present study has not provided firm data on the exact location of EF5.44, but the latter certainly lies centromeric to YN5.48 and telomeric to ECB27. Because of this and the absence of *APC*–EF5.44 recombinants with a lod score of 3.50, we believe EF5.44 to be very close indeed to *APC*. In the search for relatively small deletions, use of EF5.44 to screen pulsed-field DNA fragments for rearrangements in patients with FAP could provide the key to isolation and characterization of *APC*.

The data presented here, combined with those already published for linkage of several of these markers to the *APC* gene, give lod scores sufficiently high (and hence 95% probability limits sufficiently narrow) to allow their use in presymptomatic diagnosis of FAP. Estimates of genetic distance from *APC* for the markers published as lod-score tables when data from this anal-

ysis are combined are as follows: YN5.48–*APC*, approximately 18.5 at $\theta = .025$ (Nakamura et al. 1988; Tops et al. 1989); π227–*APC*, 18.95 at $\theta = .075$ (Meera-Khan et al. 1988; Murday et al. 1989); and C11P11–*APC*, 11.97 at $\theta = .025$ (Bodmer et al. 1987; Leppert et al. 1987).

The establishment of a high-density map of the region around *APC* is of great importance, since, until isolation of the gene itself, multiple markers will be required for presymptomatic diagnosis of FAP. Even after *APC* is cloned and sequenced, it is likely that there will be a need for linked markers in some families. We have detected no evidence of genetic heterogeneity in nine Scottish FAP kindreds, with linkage being apparent for at least one of the six markers studied in all families; and, to date, there are no families in the world literature which do not exhibit linkage to 5q21–22. This is of paramount importance to clinicians who will use genetic linkage data to influence management of families and at-risk individuals under their care.

In the biology of colorectal carcinomas, the use of this high-resolution map will also be of great benefit for further examination of the role of loss of heterozygosity at loci close to *APC*. Examination of DNA from both FAP and non-FAP colorectal adenomas and carcinomas at the EF5.44 locus will also be of great interest, since the present study has demonstrated the close proximity of EP5.44 to *APC*. Preliminary data on such analysis have suggested that the frequency of EF5.44 loss is very high in colorectal cancer tissue (Wyllie et al. 1989). This again supports the linkage data presented here, suggesting that EF5.44 is very close to *APC*.

The present study has delineated between YN5.48 and L5.62 a small chromosome 5q region, which may be as little as 2 megabases. This region includes the gene for FAP, and we have identified a marker which we believe lies between the two flanking markers and appears to be very close to *APC* itself. It is now possible to proceed to isolate clones from this region and to screen these for the presence of expressed sequences. Any such sequences must be strong candidates for *APC* itself, given both the relatively small distance between these markers and that *APC* is probably a fairly large gene, since the rate of new mutations giving rise to sporadic cases of FAP is high (Reed and Neel 1955). Isolation of *APC* will only be the opening chapter of a fascinating book providing new understanding of a heritable cancer syndrome and also of the biology of colorectal cancer.

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