

ALIMENTARY TRACT

Inherited Susceptibility to Colorectal Adenomas and Carcinomas: Evidence for a New Predisposition Gene on 15q14-q22

IAN TOMLINSON,^{*,†,§} NAZNEEN RAHMAN,^{*} IAN FRAYLING,^{||} JON MANGION,^{*} RITA BARFOOT,^{*} RIFAT HAMOUDI,^{*} SHEILA SEAL,^{*} JOHN NORTHOVER,^{||} HUW J. W. THOMAS,^{||} KAY NEALE,[¶] SHIRLEY HODGSON,[#] IAN TALBOT,^{**} RICHARD HOULSTON,^{*} and MICHAEL R. STRATTON^{*}

^{*}Section of Cancer Genetics, Haddow Laboratories, Institute of Cancer Research, Sutton, Surrey; [†]Department of Clinical Medicine, Oxford University, Radcliffe Hospitals, Oxford; [§]Molecular and Population Genetics Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London; ^{||}Colorectal Cancer Unit, Imperial Cancer Research Fund, St. Mark's and Northwick Park NHS Trust, Harrow; [¶]Polyposis Registry, St. Mark's Hospital, Harrow; [#]Department of Clinical Genetics, Guy's Hospital, London; and ^{**}Department of Pathology, St. Mark's Hospital, Harrow, England

Background & Aims: The aim of this study was to evaluate the role of known colorectal adenoma and carcinoma susceptibility genes and to locate a novel susceptibility gene in an Ashkenazi family (SM1311) with dominantly inherited predisposition to colorectal adenomas and carcinomas. **Methods:** Clinicopathologic and family history data were collected. Genetic linkage and mutational analyses were used to investigate the genetic basis of the family's disease. **Results:** Affected members of SM1311 develop multiple tubular, villous, tubulovillous, and/or serrated colorectal adenomas throughout the large bowel, and some develop colon carcinoma. There are no extracolonic features clearly associated with disease in SM1311. We have shown that the family's phenotype does not result from *APC* mutations (including the I1307K variant) or from genetic changes in the other known genes that predispose to colon cancer. Using genetic linkage analysis, supplemented by allele loss in tumors, we have provided evidence for a new colorectal cancer susceptibility gene, *CRAC1* (colorectal adenoma and carcinoma), mapping to chromosome 15q14-q22. **Conclusions:** We provide evidence for a novel colorectal adenoma and carcinoma susceptibility gene on chromosome 15q14-q22. Further studies are needed to confirm this localization and to evaluate the contribution of *CRAC1* to this disease.

Two major colorectal cancer predisposition syndromes have been described, familial adenomatous polyposis coli (FAP) and hereditary nonpolyposis colon cancer (HNPCC).¹ FAP usually presents in the second decade and is characterized by large numbers of adenomatous polyps (usually more than 1000) that carpet the large bowel. Malignant change usually takes place in one or more polyps by age 50 years.² Almost all FAP cases result

from truncating (nonsense/frameshift) mutations in the *APC* gene,³ and it has been estimated that 1% of colorectal cancers are caused by these mutations.^{4,5} Patients with 5' or 3' mutations in *APC* tend to present with fewer adenomas (0–100) and are described as having attenuated FAP.⁶ Recently, a missense *APC* polymorphism (I1307K) was reported that indirectly leads to colorectal adenoma and carcinoma predisposition by creating a region of the gene that is prone to somatic mutation. This *APC* variant allele was observed in 6% of Ashkenazi controls, 10% of Ashkenazi patients with colorectal cancer, and 28% of Ashkenazim with a family history of colorectal cancer.⁷

Therefore, it is possible that the contribution of *APC* mutations to colorectal cancer incidence has been underestimated. In contrast to FAP, patients with HNPCC have a normal or only slightly increased tendency to develop adenomas, but the probability and rate of progression to carcinoma are increased.⁸ An increased risk of other carcinomas is also a recognized feature of this syndrome.⁹ Germline defects in the mismatch repair genes, *hMSH2* and *hMLH1*, account for the majority of HNPCC families, and rare mutations in *hPMS1*, *hPMS2*, and *hMSH6* have also been reported.^{10,11} A number of other rare inherited syndromes, including juvenile polyposis¹² and the Peutz-Jeghers syndrome,¹³ are also associated with increased susceptibility to colorectal cancer.

It has been estimated that approximately 15% of colorectal cancers are caused by dominantly inherited predisposition to the disease.^{14,15} However, only 2%–6%

Abbreviations used in this paper: FAP, familial adenomatous polyposis; HNPCC, hereditary nonpolyposis colon cancer; LOH, loss of heterozygosity; PCR, polymerase chain reaction.

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have been attributed to FAP and HNPCC.^{4,5,16} The indirect evidence for additional colorectal cancer predisposition genes provided by segregation analyses is supported by the existence of families with colorectal cancer that show evidence against linkage to the known loci.¹⁷ A proportion of this residual risk may be caused by primary predisposition to colorectal adenomas that subsequently progress to carcinoma. Epidemiological studies support this notion; relatives of patients with colorectal cancer have a 2–3-fold elevated risk of developing adenomas,^{18–20} and relatives of probands with adenoma(s) have a 2-fold increased risk of developing colorectal cancer.²¹ Indeed, it has been suggested that predisposition to colorectal adenoma is common in the general population and that colorectal adenomas and carcinomas may occur predominantly in susceptible individuals.¹⁴

To evaluate further the possible existence of additional colorectal cancer susceptibility genes, we investigated an Ashkenazi family with multiple colorectal adenomas and carcinomas by genetic linkage and mutational analyses.

Materials and Methods

Pedigree and Samples

Family SM1311 was ascertained through St. Mark's Hospital, Harrow, England. Interviews and samples were obtained with full informed consent and local Ethical Review Board approval. Details of affection status were derived from histopathology reports and medical records and confirmed, wherever possible, by histological review of colorectal and other tumors. Twenty-micrometer sections were cut from formalin-fixed, paraffin-embedded tumors and microdissected to enrich for neoplastic tissue. DNA was extracted from peripheral blood and archival tumor samples using standard methods.

DNA Analysis

The initial genome-wide linkage search was performed using fluorescence-labeled primers for polymorphic microsatellite markers at approximately 15–20-cM intervals. Dye-labeled polymerase chain reaction (PCR) products were detected on ABI 377 DNA sequencers and analyzed using Genescan and Genotyper software (Applied Biosystems, Foster City, CA). For analysis of loss of heterozygosity (LOH), one primer was end-labeled with γ -[³²P]adenosine triphosphate using T4 polynucleotide kinase. Radiolabeled products were electrophoresed through 6% denaturing polyacrylamide gels, visualized on x-ray film, and quantitated on a Molecular Dynamics PhosphorImager using Imagequant software. Allele loss was scored if the area under an allelic peak was reduced to <25% of its original value (compared with the other allele), thus making a conservative allowance for the presence of contaminating stromal tissue or inflammatory infiltrate. For all tumors with possible LOH, PCR reactions and allele quantitation were performed at least four times at every marker. Only a tumor with unambiguous loss in all reactions was classed as showing allele loss.

Linkage Analysis

The disease was modeled as a dominant trait ($q = 0.001$) with age-specific penetrance. Whereas the penetrance of the disease gene cannot be reliably calculated using data from a single family, age-specific risks were estimated in SM1311 by survival analysis assuming that individuals with colorectal cancers had polyps 5 years before cancer was diagnosed. Under this model, the *CRAC1* gene confers a polyp cumulative risk of 20% by age 30 years, 35% by 40 years, 51% by 50 years, 80% by 60 years, and 96% at greater than 60 years. Although this is an approximation, the evidence for linkage is primarily based on affected individuals and the logarithm of the odds (LOD) scores do not significantly change as the penetrance is altered. Individuals were classified as unaffected at the age at which their most recent colonoscopic examinations showed no adenomas or carcinoma. Cases that had not undergone colonoscopy and had not developed symptomatic colorectal adenomas or carcinoma were scored as of unknown phenotype. Analyses were carried out using all individuals of known phenotype and separately using affected individuals only.

A phenocopy rate was also included in the analysis. With the exception of FAP, multiple adenomatous polyps are almost never seen in individuals before the age of 40 years. The overall incidence of a single polyp in this age group is approximately 0.001.²² There are no published data on age-specific population risks of multiple polyps (detected by colonoscopy) in older age groups, and we therefore based these phenocopy rates on the population rates of colorectal cancer (0.006 before age 65 years).²³ These phenocopy rates are likely to be greater than the population rates of multiple polyps; hence, in terms of producing evidence in favor of linkage, the analysis is conservative.

Two-point linkage analyses were performed using the MLINK program in the FASTLINK package.²⁴ Multipoint analyses were undertaken using the VITESSE program.²⁵ Marker allele frequencies were estimated from the Genome Data Base at <http://gdbwww.gdb.org/> or from the genotyping of 30 unrelated samples. The order of and distances between markers were based on the Genethon map.

Results

SM1311 is an Ashkenazi pedigree with 12 affected members (Figure 1) in which susceptibility to colorectal adenoma and carcinoma is segregating in a dominant fashion. The clinicopathologic features of the family's tumors are shown in Table 1. Most members of the family have been regularly screened by colonoscopy, although some older family members died before surveillance screening was introduced. None has undergone esophagogastroduodenoscopy. Four individuals developed colorectal cancer. One of them (II.5) died at an early age (31 years); the others died at 55 (II.10), 59 (II.3), and 67 (I.1) years. It is not known whether the individuals with colorectal cancer had adenomas. Six individuals (II.1, II.7, II.11, III.2, III.4, III.5) have developed

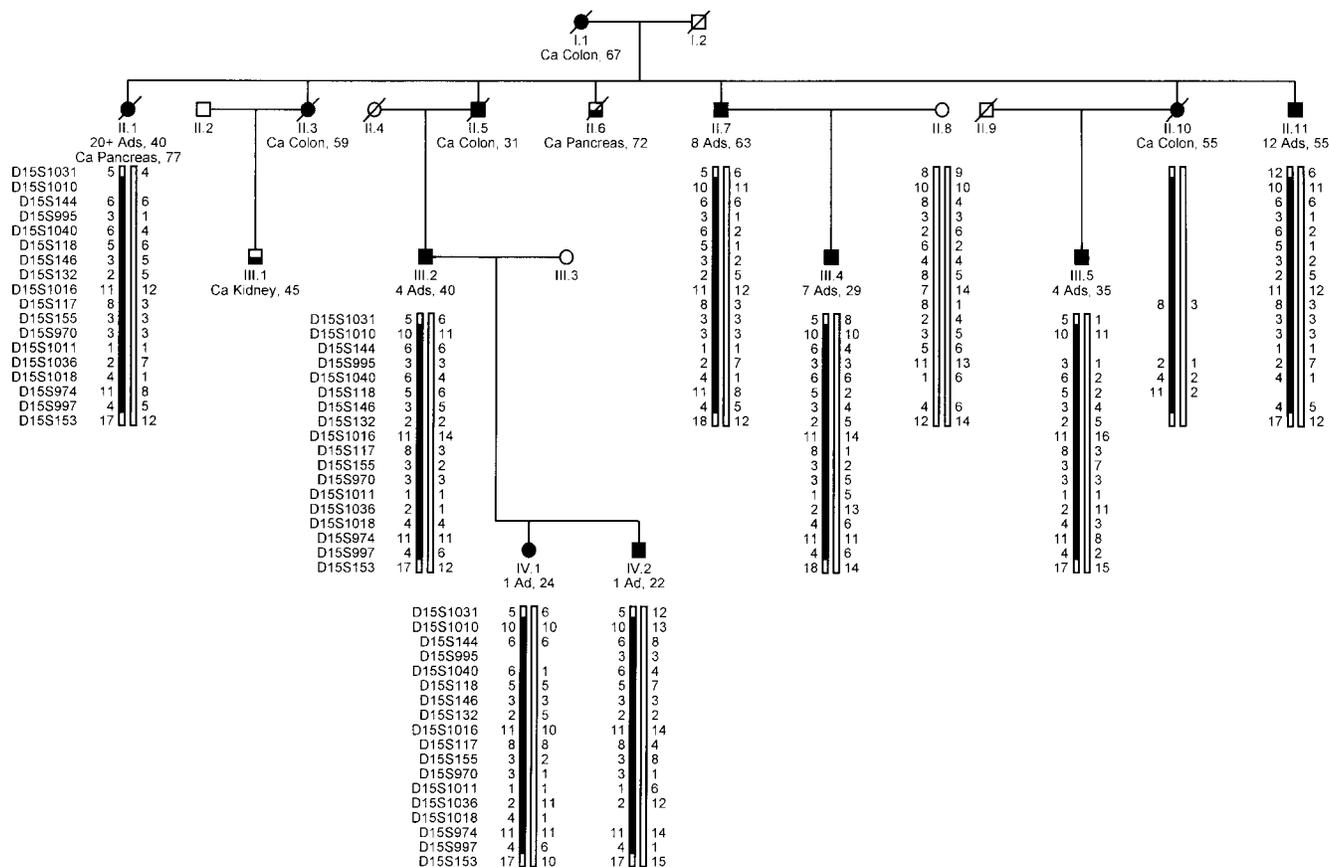


Figure 1. Pedigree of family SM1311. Closed symbols indicate affected individuals. Half-closed symbols indicate individuals affected with a cancer other than colon cancer. Numbers after cancer designation indicate age at death. Numbers after adenoma designations (Ads) indicate age at presentation. The black bar indicates the 15q14-q22 haplotype shared by all affected individuals. Data on unaffected individuals who are potential gene carriers are omitted to preserve confidentiality.

multiple adenomatous polyps but no colorectal cancer, possibly because of regular colonoscopic surveillance and elective polypectomy. The adenomas occur throughout the large bowel and include lesions of tubular, villous, tubulovillous, and serrated histological types. Two patients (IV.1 and IV.2) have developed single adenomatous polyps. These polyps were large (more than 2 cm) and were detected by colonoscopy at a very young age (24 years and 22 years, respectively). Therefore, in the context of this family, they are likely to have resulted from genetic predisposition. One of the individuals (II.1) with multiple adenomas died of pancreatic cancer at age 77 years, and another (II.6) also developed pancreatic cancer (age 72 years), although it is not known whether he had adenomas. III.1 had renal adenocarcinoma diagnosed at age 45 years. He is undergoing regular endoscopy, and no colorectal adenomas have been observed.

SM1311 was first analyzed for evidence of linkage to known colorectal cancer susceptibility loci. Analysis of six markers flanking *APC* shows that the disease phenotype in this family is unlinked to this gene (LOD score of -3.21 at $\theta = 0.01$ using *D5S2084*, which is 1 cM from *APC*). Moreover, germline *APC* mutations have not been

detected in this family in a mutation screen of the full *APC* coding sequence and intron/exon junctions by a combination of the protein truncation test and single-stranded conformational polymorphism/heteroduplex analysis.²⁶ To exclude the T-to-A I1307K missense mutation predicted to account for 28% of Ashkenazi families with colorectal cancer, we sequenced this region of the *APC* gene in all 17 individuals from SM1311 from whom DNA was available (8 affected individuals, 6 unaffected individuals, and 3 spouses). The variant *APC* allele was not observed, and all individuals were homozygous for the wild-type allele (data not shown).

Analysis of additional polymorphic markers did not support linkage to the known HNPCC loci. For *hMSH2*, we obtained an LOD score of -1.68 at $\theta = 0.01$ using *D2S391*, which is 1 cM from *hMSH2* (and which also provides information for *hMSH6*, which is within 1 MB of *hMSH2*). For *hMLH1*, the LOD score was -1.39 at $\theta = 0.001$ using *D3S1611*, which is within an intron of *hMLH1*. For *hPMS2*, the LOD score was -0.43 at $\theta = 0.05$ using *D7S517*, which is within 5 cM of *hPMS2*. For *hPMS1*, the LOD score was -0.60 at $\theta = 0.05$ using *D2S2192*, which is within 5 cM of *hPMS1*. Linkage

Table 1. Clinicopathologic Features of Colorectal Tumors of Family SM1311

Patient	Age ^a (yr)	Current age or age at death (yr)	Colorectal carcinomas (site, Dukes stage) or adenomas (site, morphology, degree of dysplasia) to date ^b
I.1	67	67 (died)	Ca (? , ?)
II.1	40	77 (died)	Ad (? , tv, mo), multiple ^c Ads (? , ? , ?)
II.3	59	59 (died)	Ca (s, ?)
II.5	31	31 (died)	Ca (? , ?)
II.7	63 (S)	75	Ad (a, tv, mi), Ad (s, tv, mi), Ad (r, t, mo), Ad (a, se, mi), Ad (a, v, mo), Ad (a, se, mi), 2 Ads (a, t, mi)
II.10	55	55 (died)	Ca (a, C)
II.11	55 (S)	68	Ad (r, tv, mo), Ad (s, t, mo), Ad (r, t, ?), 4Ads (d, t, ?), 2Ads (d, t, mi), Ad (a, t, mi), 2Ads (r, t, mi)
III.2	40 (S)	50	Ad (t, t, mi), Ad (a, t, mi), Ad (a, t, mi), Ad (a, se, mi)
III.4	29 (S)	41	Ad (r, t, mi), Ad (c, t, mi), Ad (r, se), Ad (r, t, mi), Ad (d, t, mi), Ad (t, tv, mi), Ad (t, tv, mo)
III.5	35 (S)	48	Ad (c, t, mo), Ad (a, t, mo), Ad (t, se, mi), Ad (r, se, mi)
IV.1	24 (S)	24	Ad (r, tv, mo)
IV.2	22 (S)	26	Ad (s, tv, mo)

NOTE. These data necessarily represent the minimum number of tumors developed by each patient because of (1) destruction of some adenomas at colonoscopy; (2) prophylactic surgery; (3) loss of material and/or records from some hospital archives (e.g., hospitals closed or merged); (4) inaccurate recollection by patients of the dates and places of colonoscopy or surgery; and (5) failure to examine the whole colon in some patients (e.g., with inoperable carcinoma at presentation).

^aAge at presentation or at screening (S).

^bCarcinoma (Ca) and adenoma (Ad) sites: a, ascending colon; c, cecum; d, descending colon; r, rectum; s, sigmoid; t, transverse colon; ?, unknown. Carcinoma stage: Dukes stage A, B, and C; ?, unknown. Adenoma morphology: s, serrated; t, tubular; tv, tubulovillous; v, villous; ?, unknown. Adenoma degree of dysplasia: mi, mild; mo, moderate; se, severe; ?, unknown.

^cFurther details not available.

evidence in favor of certain other candidates for a colorectal adenoma and carcinoma susceptibility gene was also not observed. For *TP53*, the LOD score was -1.2 at $\theta = 0.05$ using *D17S786*, which is within 1cM of *TP53*. For *PTEN*, the LOD score was -0.46 at $\theta = 0.1$ using *D10S219*, which is 10 cM from *PTEN*. For *SMAD4*, the LOD score was -0.84 at $\theta = 0.05$ using *D18S57*, which is 5 cM from *SMAD4*. (Position of genes obtained from GDB at <http://gdbwww.gdb.org/>, LDB at <http://cedar.genetics.soton.ac.uk/public.html/>.^{17,27}) Previous mutation analysis failed to detect any germline variants in this family at *hMSH2* or *hMLH1*.²²

A genome-wide linkage search using 210 microsatellite markers at a density of 15–20 cM was performed. Eleven markers generated two-point LOD scores of >0.5 . Analysis of additional markers that closely flank 10 of these markers indicated that linkage of the disease to these loci is highly unlikely. The remaining marker,

D15S132 on chromosome 15q, yielded a maximum two-point LOD score of 1.04. Analysis of 10 additional markers that flank *D15S132* supported linkage and provided evidence of a haplotype of marker alleles between *D15S1031* and *D15S153* (a distance of 40 cM) that clearly segregates with the 9 affected individuals (Figure 1). A maximum two-point LOD score of 2.16 was generated at *D15S118* at $\theta = 0$. LOD scores at $\theta = 0$ using other markers in the region were *D15S1031*, $z = 0.40$; *D15S144*, $z = 0.16$; *D15S1040*, $z = 1.35$; *D15S117*, $z = 1.20$; *D15S1016*, $z = 1.92$; *D15S1036*, $z = 1.68$; *D15S997*, $z = 1.91$; *D15S974*, $z = 1.20$; and *D15S153*, $z = 1.11$. Multipoint analysis (using *D15S118*, *D15S1016*, *D15S117*, *D15S1036*, and *D15S997*) generated a maximum LOD score of 2.77 at *D15S118* (and a score of 2.35 using analysis of affected individuals only). Five individuals analyzed (not shown to preserve confidentiality) who carry the linked haplotype have been colonoscopically screened and have not developed polyps. All of these individuals are under age 45 years, and all but 1 is under age 35 years.

Neoplastic tissues dissected from 23 colorectal adenomas and 1 carcinoma from 8 individuals in SM1311 were examined for allele loss at 15q14-q22 using microsatellite markers *D15S117*, *D15S1036*, *D15S132*, and *D15S1018*. Every tumor was informative at a minimum of two of these markers. No tumor had evidence of microsatellite instability at eight other loci tested specifically for this purpose (*D2S123*, *D5S346*, *D6S273*, *D11S871*, *D15S642*, *D15S657*, *D17S806*, and *D19S886*) or at several other loci tested subsequently (see below). Only one tumor (a 2-cm adenoma from individual II.7) showed consistent LOH with repeated amplifications (Figure 2). This allele loss was observed at all four markers and was of the putative wild-type allele at all loci. None of the other tumors showed reproducible evidence of allele loss at any chromosome 15q locus examined (although scrutiny of histological sections from the single carcinoma showed that this lesion was diffusely infiltrative and heavily contaminated with nonneoplastic cells, even after dissection). Because the wild-type allele is lost in cancers arising in susceptibility gene carriers, information from LOH studies can be incorporated into formal genetic linkage analyses of cancer susceptibility traits.^{28–30} This allows the allele loss observed in the single adenoma from individual II.7 to be considered as a surrogate informative meiosis and included in the two-point and multipoint LOD scores. Using this additional information, a maximum multipoint LOD score of 3.06 at *D15S118* was obtained using all individuals of known phenotype (and 2.63 using affected individuals only).

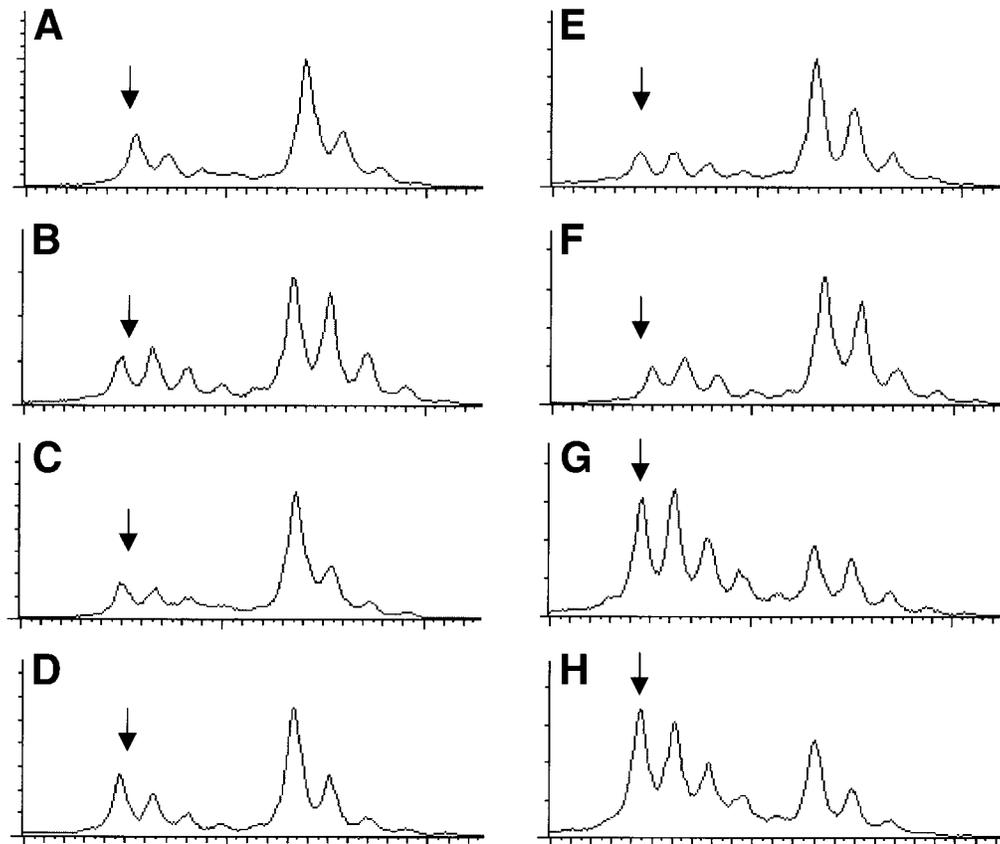


Figure 2. LOH in an adenomatous polyp from individual II.7 in family SM1311. Phosphorimager traces of ^{32}P -radiolabeled, PCR-amplified DNAs from multiple adenomatous polyps from individual II.7 using primers flanking the polymorphic microsatellite repeat *D15S117*. Arrows indicate the allele that is linked to colorectal adenomas and carcinomas in family SM1311. (A) Normal lymphocytes; (B–F) five individual adenomatous polyps showing no evidence of LOH; and (G and H) two different samples from a single adenomatous polyp showing an allelic imbalance ratio of less than 25%, consistent with LOH. Each experiment was repeated at least four times at this marker and confirmed using other markers near the putative location of *CRAC1*.

Discussion

After a genome-wide search on a family (SM1311) with multiple colorectal cancers and adenomas, we obtained a multipoint LOD score of 2.77 (3.06 with inclusion of data from LOH analyses) on chromosome 15q and have demonstrated a haplotype of marker alleles in a 40-cM interval defined by *D15S1031* and *D15S153* that is shared by all affected individuals. The results suggest that there is a previously undescribed susceptibility gene on chromosome 15q, which we have designated *CRAC1* (colorectal adenoma and carcinoma). In addition to colorectal adenomas and carcinomas, the presence of two cases of pancreatic carcinoma in the family suggests that this may also be part of the phenotype conferred by mutations in the gene.

In support of this new locus, there is also strong evidence against the involvement of known colorectal cancer predisposition genes in SM1311. The family clearly does not exhibit the classical features of FAP. However, it does share some features of the attenuated form of FAP in which cases have fewer polyps (1–100) and a later average age at onset of cancer (approximately

55 years).⁶ Most of these families have germline mutations of *APC*, although there are reports of families that are not linked to this locus.³¹ The disease in SM1311 shows strong evidence against linkage to *APC*. Moreover, a complete mutational screen of the coding sequence and flanking introns revealed no truncating mutations in *APC*, and the I1307K variant that is prevalent in Ashkenazi Jews and appears to confer a modest elevated risk of colorectal cancer is not present. The family does fulfill the Amsterdam criteria for HNPCC.³² However, it is not typical of HNPCC because several patients have more polyps than expected. Mutations in known or presently unknown mismatch repair genes are unlikely to account for the clustering of disease in this family because no microsatellite instability has been detected at 12 dinucleotide repeat markers in any of the tumors examined. Moreover, genetic linkage analysis has provided evidence against germline mutations in *hMSH2*, *hMLH1*, *hPMS1*, *hPMS2*, and *hMSH6*, and analysis of genomic DNA showed no mutations in the full coding sequence and flanking introns of *hMSH2* and *hMLH1*.²²

Most cancer susceptibility genes conform to the model

of a tumor-suppressor gene/recessive oncogene in which both alleles need to be inactivated to contribute to oncogenesis.³³ In tumors arising in susceptible individuals, loss of the wild-type allele (inherited from the non-mutation-carrying parent) is frequently observed at the appropriate locus. Of the 23 adenomas from SM1311 examined, only 1 showed consistent LOH on chromosome 15q (Figure 2). The low level of allele loss observed at *CRAC1* in colorectal adenomas may indicate that inactivation of the second allele is accomplished by small somatic mutations rather than LOH or that contamination by stromal cells is obscuring LOH despite dissection. It also remains possible that inactivation of a single allele is sufficient to contribute to oncogenesis or that the susceptibility gene is activated rather than inactivated during oncogenesis. Whatever the explanation, the pattern is reminiscent of that observed in FAP on chromosome 5q, where frequency of loss of the wild-type allele is variable but is usually seen in fewer than 20% of adenomas.³⁴⁻³⁹ The rate of allele loss on chromosome 5q in carcinomas from individuals with FAP (25%–50%) is higher than in adenomas.³⁴⁻³⁶ However, the single carcinoma available from SM1311 was diffusely infiltrative, with heavy contamination by nonneoplastic cells, and the absence of allele loss on chromosome 15q in this tumor is difficult to interpret.

A previous study of primary sporadic colorectal cancer using a marker at least 30 cM telomeric to *CRAC1* reported LOH at a frequency of 16% on chromosome 15q, which is not substantially greater than background.⁴⁰ However, LOH on 15q has been observed in 37 of 67 (54%) metastatic carcinomas of various types (which included 6 of 9 [67%] metastatic colorectal cancers). In the same series of cancers, LOH was detected at lower levels on chromosomes 2q (3%), 4p (18%), and 4q (20%), suggesting that the allele loss on 15q was greater than background. LOH at similar levels to chromosome 15q was observed on chromosomes 3p (50%), 5q (44%), 17p (62%), and 18q (47%), which are known to harbor tumor-suppressor genes. These data are consistent with the presence of a tumor-suppressor gene on chromosome 15q, and analysis of the subset of tumors showing interstitial LOH maps this gene to a region consistent with our location of *CRAC1*.⁴¹ However, it is possible that this is not a tumor-suppressor gene and that the colocalization with a region of elevated LOH in sporadic cancers is coincidental.

Our study provides evidence for a colorectal adenoma and carcinoma susceptibility gene on chromosome 15q14-q22. Among the genes located in this region (<http://cedar.genetics.soton.ac.uk/public.html/>) that may be considered candidates are the human homologue of *RæA*

(*RAD51*); putative receptor tyrosine kinases *TYRO3* and *Itk*; *BCL8*, a gene rearranged in large cell lymphomas; *FGF 7*; *neogenin*, which was isolated on the basis of sequence similarity to *DCC*; *BUBR1*, the human homologue of a yeast gene that is involved in mitotic checkpoint control and chromosome segregation⁴²; and *SMAD3*, a mediator of transforming growth factor β -induced intracellular signals.⁴³ Further studies are needed in similar families to confirm the localization and to evaluate the contribution of *CRAC1* to colorectal adenoma and carcinoma susceptibility.

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Address requests for reprints to: Michael R. Stratton, M.D., Ph.D., Section of Cancer Genetics, Haddow Laboratories, Institute of Cancer Research, Sutton, Surrey SM2 5NG, England. e-mail: mikes@icr.ac.uk; fax: (181) 643-0549.

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Drs. Tomlinson, Rahman, Frayling, and Mangion contributed equally to this work.

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