Evidence of Linkage to Chromosome 9q22.33 in Colorectal Cancer Kindreds from the United Kingdom


Abstract
About 30% of all colorectal cancers are thought to have a genetic basis and the known predisposing genes can only account for a small fraction of cases. A previous report suggested that a colorectal cancer candidate gene, explaining at least 20% of colorectal cancer cases with family history, was located within a 25 cM region on chromosome 9q22.2-q31.3. We typed 16 polymorphic markers encompassing the region of putative linkage in 57 colorectal tumor families from the United Kingdom. Known Mendelian syndromes had been excluded. We found suggestive evidence of linkage, as positive parametric (HLOD = 1.23) and nonparametric (NPL = 1.21, \( P = 0.11 \)) LOD scores were obtained by analysis of the whole family set. Enrichment for cases with \textit{a priori} genetic etiology by analyzing families with at least one person affected at \(<45\) years of age (\( n = 39 \) families) gave a maximum multipoint NPL score of 2.65 (\( P = 0.007 \)). In this group, significant NPL scores \( >1.67 \) (\( P < 0.05 \)) were found in a 6.5 cM region between D9S1851 and D9S277. With a more stringent threshold (NPL-2.4, \( P < 0.01 \)), the linked region was 1.7 cM between D9S971 and D9S272/D9S173. Exclusion from the analysis of kindreds with a phenotype of multiple polyposis also found evidence of linkage in the same region (NPL = 2.47 at close to D9S277, \( P = 0.009 \)). The type 1 transforming growth factor-\( \beta \) receptor, a prime candidate gene, was excluded as a cause of disease. The results presented here further support the existence of a colorectal cancer susceptibility gene on chromosome 9q and refine its likely location. (Cancer Res 2006; 66(10): 5003-6)

Introduction
Colorectal cancer is the third most common cause of cancer-related death in the Western world and has an annual incidence of 35,000 in the United States. In the United States, it represents the second most common cause of cancer death (1). Mendelian syndromes with mutations in known genes (\textsc{APC}, mismatch repair genes, \textsc{MUTYH/MYH}, \textsc{SMAD4}, \textsc{ALK3}, and \textsc{STK11/LKB1}) account for 2% to 6% of cases (2). However, a number of factors have suggested a greater role for inherited predisposition. Siblings of affected individuals have a 2- to 3-fold increased relative risk for both adenomas and colorectal carcinomas, and the risk is greater if the patient is diagnosed at a young age or if there is more than one affected family member. Segregation analyses suggest dominant inheritance of the uncharacterized susceptibility genes (3, 4), whereas twin studies estimate that 35% of colorectal cancers are attributable to genetic influence (5).

Recently, Wiesner et al. (1) analyzed a set of 74 affected sibling pairs from 53 colorectal cancer kindreds, supplemented by analysis of discordant siblings. They reported that a new colorectal cancer predisposition locus maps to chromosome 9q22.2-q31.2 (\( P = 0.0049 \) for the affected siblings), with a support interval of \(~25\) cM between D9S283 and D9S938, and a minimum \( P \) value at D9S1786. This region includes a number of candidate genes, including \textit{patched} (\textsc{PtcH}) and the DNA repair gene \textsc{Xpa}.

We have tested the reported region of linkage on 9q in 57 colorectal tumor kindreds from the United Kingdom. Our results suggest that a susceptibility locus for colorectal tumors may indeed map to this site, and we provide evidence for refinement of the region within which the putative disease gene lies.

Materials and Methods
Families were ascertained as part of the ColoRectal Tumour Gene Identification (\textsc{CORGI}) study. Each kindred had at least three affected individuals (confirmed by pathology reports). Blood and paraffin-embedded tumor samples were requested from all living family members. Genomic DNA was extracted from whole blood using the Chemagic Magnetic Separation Module 1. A total of 201 affected individuals and 169 other individuals were available for genotyping. In the 57 kindreds, the number of affected individuals available for genotyping per family were as follows: 2 in 11 families, 3 in 20 families, 4 in 14 families, 5 in 7 families, 6 in 2 families, and 7 in 1 family. No patient had clinical features of Peutz-Jeghers syndrome, juvenile polyposis, hereditary mixed polyposis, or inflammatory bowel disease. Germ line mismatch repair gene mutations were excluded by microsatellite instability testing (\textsc{Bat25}, \textsc{Bat26}) in two colorectal cancers from each family; kindreds in which both cancers were unstable were excluded. Where one unstable cancer was found (or if the only available cancer was unstable), direct mutation screening of all coding regions of \textsc{MSH2} and \textsc{MLH1} was undertaken using denaturing high-performance liquid chromatography analysis based on constitutional DNA (details...
available from authors). Additionally, for all individuals with more than five adenomas, germ line APC and MYH mutations were excluded by denaturing high-performance liquid chromatography screening of the full coding region of each gene; and linkage to the APC gene was excluded where family size made this possible (details available from authors in all cases).

Individuals were classed as affected if they fulfilled one or more of the following criteria: (a) colorectal cancer at age \( \leq 75 \) years; (b) “significant” adenoma (three or more synchronous or metachronous, and/or villous morphology, and/or severe dysplasia, and/or diameter \( > 1 \) cm) at age \( \leq 75 \) years; (c) any adenoma at age \( \leq 45 \) years; (d) \( > 10 \) hyperplastic polyps at age \( \leq 75 \) years; and (e) any hyperplastic polyp at age \( \leq 35 \) years. Of those affected, 76 met the criteria by a diagnosis of carcinoma (with or without adenoma), 116 had adenomas, and 9 had hyperplastic polyps. Of the carcinomas, 27 were right sided, 45 were left sided, 1 is in the transverse colon, and 3 are of unknown location. Dukes classification showed 12 to be stage A, 37 stage B, 23 stage C, and 4 unknown. The mean ages for meeting the CORGI criteria were as follows: for diagnosis of carcinoma, 58; for diagnosis of adenoma(s), 47; and for diagnosis of hyperplastic polyps, 36. All other individuals were scored as of unknown status, except for 13 who were scored unaffected because they had developed no colorectal tumor of any type and were \( > 75 \) years of age.

Sixteen polymorphic microsatellite markers (Fig. 1) were chosen so as to encompass the area of reported linkage (1) with an average spacing of 2.7 cM/2.15 Mb. Marker positions, physical and genetic distances, and primer sequences were obtained from the University of California-Santa Cruz genome browser [May 2004 assembly]. PCR amplification of these markers was done using standard 50 µl reactions containing 50 ng genomic DNA, 1 × standard PCR buffer, 2 mmol/L MgCl2, 200 µmol/L deoxynucleotide triphosphates, 1 unit Taq polymerase, and 200 µmol/L of each oligonucleotide primer. Each forward primer was labeled with FAM, HEX, or TET.

Genotyping was done on an ABI3100 semi-automated sequencer and results were analyzed using the Genotyper software (Perkin-Elmer Applied Biosystems, United Kingdom). Two investigators (Z.K. and L.G.C-C.), blinded to family structure and phenotype, reviewed allele calls. Consensus genotype tables were created for the linkage analyses. Mendelian inheritance was verified with PedCheck1.1 (6).

Two-point and multipoint parametric and nonparametric (NPL) LOD scores were calculated using Genehunter 2.1 (7). Marker allele frequencies were estimated from the genotypes of pedigree founder using Recode (8) and the frequency of the mutant allele was set to 0.01. Nonparametric analyses were evidently based on allele sharing between affected individuals only. For parametric analyses, we estimated LOD scores under heterogeneity (HLOD) and assumed an autosomal dominant mode of inheritance with incomplete, age-dependent penetrance. Four penetrance groups were defined for the analyses (group 1: \( < 40 \) years; group 2: 41-50 years; group 3: 51-60 years; and group 4: \( > 61 \) years). Penetrances for carriers were 0.25, 0.50, 0.70, and 0.80 for groups 1 to 4, respectively, and were obtained using information on ages of onset from the full CORGI data set of 900 colorectal cancer families from the United Kingdom. Phenocopy rates were set at 0.0001, 0.005, 0.01, and 0.04, respectively, to provide conservative estimates of the population rates (Cancer Research UK Cancer Statistics). Our analyses were conducted in the whole data set (57 families) and, on a strictly pre hoc basis in three different family groups, subdivided by (a) the age of the youngest affected individual in the pedigree (under versus over 45 years of age); (b) the mean age of presentation of the affected pedigree members (under versus over 55 years of age); and (c) the disease phenotype observed in the family [presence or absence of multiple (more than five) polyps in one or more family member(s)]. Groups (a) and (b) were used to enrich for cases with a genetic etiology. Group (c) was used because Mendelian syndromes suggest that individuals may be primarily predisposed either to colorectal cancer or colorectal polyps.

Results and Discussion

In the whole data set, we found suggestive evidence for linkage to chromosome 9q because positive multipoint LOD scores were observed in the region using both parametric (HLOD = 1.23) and nonparametric (NPL = 1.11) analyses. Approximately 20% of the families showed linkage to this chromosomal segment (data not shown). Marker D9S277 showed the highest two-point HLOD (1.36) and NPL scores (1.44, \( P = 0.07 \)). This marker was the most polymorphic and informative microsatellite in the study (17 alleles versus a mean of 9 alleles/locus, polymorphic information content = 0.62 versus a mean of 0.43).

We found significant NPL scores \( (P < 0.05) \) in our subsequent analyses based on disease subtype and age of onset. Significant linkage was observed in those pedigrees without multiple polyps (maximum NPL score of 2.47 at close to D9S277, \( P = 0.009 \); Table 1), whereas in those families having members with multiple adenomas or hyperplastic polyps, disease was not linked to the region (NPL score of –0.10, \( P = 0.52 \); HLOD = 0). We also obtained, at marker D9S277, the highest two-point LOD (2.80) and NPL (2.44) scores in the subgroup of families without multiple polyp cases. A significant NPL score was additionally found in those families with a mean age of onset \(< 55 \) years (HLOD = 1.3, NPL score 2.08, \( P = 0.02 \); Table 1), whereas those with older patients showed a weaker linkage signal (HLOD = 0.42, NPL = 1.32, \( P = 0.10 \)). Our most significant finding (Table 1) was observed in those families with at least one young affected

![Figure 1. Multipoint nonparametric LOD score in the set of families with at least one affected member \(< 45 \) years of age. X axis, approximate location of the markers; Y axis, NPL value.](image-url)
patient (<45 years of age), with the highest NPL score observed, 2.65 (P = 0.007). In this group, which comprised about two thirds of all families, support for linkage (P < 0.05) was found in a 6.5 cm region between D9S1858 and D9S277. The strongest support for linkage (P < 0.01) was found in a region of 1.7 cm between D9S971 and D9S272/D9S173 (Fig. 1). This latter region overlaps with that reported by Wiesner et al. (1), although it is much smaller and slightly shifted toward the telomere.

The region of ~1.7 cm, between D9S971 and D9S272/D9S173, that provides most support for linkage in our study contains at least 20 known genes and several hypothetical proteins. A number of these genes have been previously implicated in tumorogenesis and are good colorectal cancer candidates. One of them, the DNA repair gene XPA, lies in the middle of the linkage signal peak and has been associated with predisposition to mutagen-induced adenomas in mouse models (9). Another gene of greater interest has been associated with predisposition to mutagen-induced repair gene (10, 11). A number of these genes have been previously implicated in tumorigenesis and are good colorectal cancer candidates. One of them, the DNA repair gene XPA, lies in the middle of the linkage signal peak and has been associated with predisposition to mutagen-induced adenomas in mouse models (9). Another gene of greater interest has been associated with predisposition to mutagen-induced repair gene (10, 11).

In conclusion, we have shown linkage to a refined region on chromosome 9q22.32-q31.1 in a set of United Kingdom Caucasian colorectal cancer families, with best evidence for location of a colorectal cancer susceptibility gene to a region of ~1.7 cm. Stratification of the sample based on disease severity replicated the original linkage report of Wiesner et al. (1) and greatly narrowed down the region likely to contain the susceptibility gene. Although the original finding (1) formally failed to achieve a significant LOD score (≥3), our result in the families with the younger cases met the significant criteria (P < 0.01) for a replication linkage study (7, 15). Both studies found that the putative disease locus accounts for around 20% of all colorectal cancer patients with family history and both noted that such a causal gene may be involved in a severe form of the disease.

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References


Table 1. Maximum two-point and multipoint parametric and nonparametric scores estimated in the whole sample and in the different phenotypic categories

<table>
<thead>
<tr>
<th>Group (no. of families)</th>
<th>Maximum two-point scores</th>
<th>Maximum multipoint scores</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HLOD (marker)</td>
<td>NPL (marker, P*)</td>
</tr>
<tr>
<td>All families (57)</td>
<td>1.36 (D9S277)</td>
<td>1.44 (D9S272, 0.07)</td>
</tr>
<tr>
<td>Youngest case (&lt;45 y)</td>
<td>2.23 (D9S930)</td>
<td>1.88 (D9S930, 0.04)</td>
</tr>
<tr>
<td>&gt;45 y (18)</td>
<td>0.37 (D9S1865)</td>
<td>0.30 (D9S2146, 0.37)</td>
</tr>
<tr>
<td>Mean age of onset</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;55 y (39)</td>
<td>1.23 (D9S930)</td>
<td>1.42 (D9S272, 0.08)</td>
</tr>
<tr>
<td>&gt;55 y (18)</td>
<td>0.36 (D9S930)</td>
<td>0.90 (D9S930, 0.18)</td>
</tr>
<tr>
<td>Disease type</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No multiple polyps (41)</td>
<td>2.80 (D9S277)</td>
<td>2.44 (D9S277, 0.03)</td>
</tr>
<tr>
<td>≥1 case with multiple polyps (16)</td>
<td>0.55 (D9S173)</td>
<td>0.67 (D9S173, 0.24)</td>
</tr>
</tbody>
</table>

*Significant P values (<0.05) are highlighted in bold.

1Genetic distance from the most centromeric marker D9S922.

2HLOD across the whole region was 0.

3http://genome.ucsc.edu/.

4Significant level was set at P < 0.05.

5 Odds ratios were calculated using a log-linear model and a case-control design with 18 U.S.C. Section 1734 solely to indicate this fact.

6We thank the Cancer Research UK LBJ Equipment Park for technical help and the anonymous reviewers for their valuable comments.

7 http://genome.ucsc.edu/.

8 The number of families with at least 20 known genes and several hypothetical proteins in the region of interest.

9 The frequency of the TGFBR1*6A allele and the disease (pedigree disequilibrium test) was not significantly different from the general population (P = 0.32). The frequency of the TGFBR1*6A allele was actually lower among our family founders than that reported in the literature (10). Our results show that TGFBR1*6A is not responsible for the linkage signal observed in the region.

10 We thank the Cancer Research UK LBJ Equipment Park for technical help and the anonymous reviewers for their valuable comments.

11 The number of families with at least 20 known genes and several hypothetical proteins in the region of interest.


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