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 polysomy 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 Kingdom. In the United States, it represents the second most common cause of cancer death (1). Mendelian syndromes with mutations in known genes (\textit{APC}, mismatch repair genes, \textit{MUTYH}/\textit{MYH}, \textit{SMAD4}, \textit{ALK3}, and \textit{STK11}/\textit{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 \( \sim 25 \) cM between D9S283 and D9S938, and a minimum \( P \) value at D9S1786. This region includes a number of candidate genes, including \textit{patched} (\textit{PTCH}) and the DNA repair gene \textit{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 (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 (BAT25, 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 \textit{MSH2} and \textit{MLH1} was undertaken using denaturing high-performance liquid chromatography analysis based on constitutional DNA (details...
Cycling conditions consisted of an initial denaturation of 94 °C for 5 minutes, 35 cycles of 95 °C/55 °C/72 °C for 1 minute each, and a final step of 72 °C for 10 minutes. 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 PedCheck 1.1 (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 = 1.21, P = 0.11) 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

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**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.

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most families were not informative. We also applied the pedigree evidence for linkage (NPL = 0.19, HLOD = 0.20) simply because polymorphism (details available from the authors). We did not find (13). We typed all individuals for the increased risk of colorectal cancer (12), although not in all studies is adenomas in mouse models (9). Another gene of greater interest has been associated with predisposition to mutagen-induced repair gene polymorphism exists in a poly-alanine tract within this gene and previously been implicated in susceptibility to cancer (10, 11). A number that provides most support for linkage in our study contains at least 20 known genes and several hypothetical proteins.7 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 associated predisposition to mutagen-induced adenomas in mouse models (9). Another gene of greater interest is type I transforming growth factor-β receptor (TGFBR1), which has previously been implicated in susceptibility to cancer (10, 11). A polymorphism exists in a poly-alanine tract within this gene and the rarer allele (TGFBR1*6A, q = 0.08) has been associated with increased risk of colorectal cancer (12), although not in all studies (13). We typed all individuals for the TGFBR1 polyadenylic acid polymorphism (details available from the authors). We did not find evidence for linkage (NPL = 0.19, HLOD = 0.20) simply because most families were not informative. We also applied the pedigree disequilibrium test (14) and failed to find significant association between the TGFBR1*6A allele and the disease (pedigree disequilibrium test, P = 0.32). The frequency of the TGFBR1*6A allele was actually lower among our family founders than that reported in the general population (6.2% versus 8.0%; ref. 10). Our results show that TGFBR1*6A is not responsible for the linkage signal observed in the region.

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 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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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</td>
<td>2.23 (D9S930)</td>
<td>1.88 (D9S930, 0.04)</td>
</tr>
<tr>
<td>&lt;45 y (39)</td>
<td>0.37 (D9S1865)</td>
<td>0.30 (D9S2146, 0.37)</td>
</tr>
<tr>
<td>&gt;45 y (18)</td>
<td>1.23 (D9S930)</td>
<td>1.42 (D9S272, 0.08)</td>
</tr>
<tr>
<td>Mean age of onset</td>
<td>0.36 (D9S930)</td>
<td>0.90 (D9S930, 0.18)</td>
</tr>
<tr>
<td>&lt;55 y (39)</td>
<td>2.80 (D9S277)</td>
<td>2.44 (D9S277, 0.03)</td>
</tr>
<tr>
<td>&gt;55 y (18)</td>
<td>0.55 (D9S173)</td>
<td>0.67 (D9S173, 0.24)</td>
</tr>
<tr>
<td>Male sex</td>
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<td></td>
</tr>
<tr>
<td>Female sex</td>
<td></td>
<td></td>
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</tbody>
</table>

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

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

References


http://genome.ucsc.edu/.
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