Four New Colon Cancer Susceptibility Loci, Scc6 to Scc9 in the Mouse

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Abstract

Germ-line mutations in APC and mismatch repair genes explain only a small percentage of all colorectal cancer cases. We have used the recombinant congenic strain mouse model to find new loci that are involved in the control of susceptibility to colon cancer. Five different colon cancer susceptibility genes, Scc1–Scc5, have been described previously using the recombinant congenic strains. Two of these loci, Scc4 and Scc5, show a reciprocal, genetic interaction. Here we report the mapping of four new colon tumor susceptibility genes: (a) Scc6 on chromosome 11; (b) Scc7 on chromosome 3; (c) Scc8 on chromosome 8; and (d) Scc9 on chromosome 10. Scc7 and Scc8 show a genetic interaction; Scc7 is only detected by virtue of its interaction with Scc8.

Introduction

Colon cancer is one of the leading causes of cancer death in the Western world. Several genes have been identified that are involved in familial colon cancer. Germ-line mutations in the APC gene cause FAP, and mutations in mismatch repair genes, mainly MSH2 and MLH1, lead to HNPCC (1, 2). FAP and HNPCC together account for only a small percentage of all colorectal cancer cases. The sporadic type of colon cancer, without obvious genetic linkage, represents the majority of cases. Evidence for inherited susceptibility to colon cancer distinct from FAP and HNPCC comes from studies that show familial clustering for apparent sporadic colorectal cancer cases. Relatives of colorectal cancer patients have an increased risk of cancer of the same type (3, 4). In addition, even colon cancer without familial clustering may preferentially affect genetically predisposed individuals (5, 6). Therefore, the analysis of genes that might affect the susceptibility to sporadic colon cancer can have considerable impact.

Mouse models are powerful tools for identifying susceptibility genes because inbred strains differ widely in their susceptibility to DMH-induced colon adenomas (7). We used the CcS series of the recombinant congenic strains (8) to study colon cancer susceptibility (9). The CcS strains are derived from the mouse strains BALB/c and STS which are resistant and susceptible, respectively, to chemically induced colon adenomas. When treated with DMH, STS mice develop a large number of tumors, and BALB/c mice develop only a few tumors. This difference is caused by multiple genes (7). Each individual CcS strain has obtained a random subset of 12.5% genes from strain STS on the genetic background of mouse strain BALB/c. In this way, the STS alleles of different loci involved in the susceptibility to colon cancer are divided between the 20 CcS strains, thus converting a multigenic difference into oligogenic or monogenic differences (10).

Previously, five colon cancer susceptibility loci, Scc1, Scc2, Scc3, Scc4, and Scc5, were identified using the RC strains CcS-16, CcS-17, and CcS-19, which are highly susceptible to colon cancer (11, 12). One of these loci, Scc1, has been mapped to a small region on chromosome 2 (13).

The CcS strains CcS-3, CcS-5, and CcS-11 are also more susceptible to colon cancer than the BALB/c strain (9). However, loci Scc1, Scc2, Scc4, and Scc5 cannot be responsible for this difference because these strains carry the BALB/c allele for these loci.

The CcS-11 strain has the resistant STS allele of Scc3, which cannot be the cause of its susceptibility. Therefore, the susceptibility of these strains is caused by loci other than the known loci Scc1–Scc5. In the present study, we used backcrosses of CcS-3, CcS-5, and CcS-11 to the resistant strain BALB/c to search for additional susceptibility loci. This approach led to the detection of four new colon cancer susceptibility loci: (a) Scc6 on chromosome 11; (b) Scc7 on chromosome 3; (c) Scc8 on chromosome 8; and (d) Scc9 on chromosome 10. Scc7 and Scc8 show a genetic interaction, a phenomenon we also observed for Scc4 and Scc5 (12) and for several lung cancer susceptibility loci (14, 15).

Materials and Methods

Animals and Tumor Induction. The mice received a standard laboratory diet (Hope Farms, Woerden, the Netherlands) and acidified drinking water ad libitum (pH 2.5 to pH 3.0). The genetic composition of the RC strains used has been described previously (16). Three strains were tested: (a) CcS-3; (b) CcS-5; and (c) CcS-11. For each strain, a BALB/c × (BALB/c × CcSF) backcross was produced. The CcS-3 cross consists of 29 mice. The backcrosses for CcS-5 and CcS-11 each consist of two experiments performed at different points in time; 38 and 55 animals were used in the two CcS-5 experiments, and 36 and 38 animals were used in the CcS-11 experiments. Mice (11–15 weeks old) received 26 weekly s.c. injections of DMH [15 mg/kg body weight, freshly dissolved in 1 mM EDTA (pH 6.8)]. Males were sacrificed at 32 weeks after the start of treatment, and females were sacrificed at 36 weeks after the start of treatment, or earlier if the animals became visibly ill (9). At autopsy, the colon was removed, and the number of tumors was counted using a dissection microscope.

DNA Preparation and Genotyping. DNA was prepared from mouse tails using a standard protease K procedure. The backcross mice were genotyped as described previously (17) using microsatellite markers (Mouse MapPairs TM; Research Genetics, Huntsville AL). The CcS-3 backcross used microsatellite markers D3Mit18, D3Mit162, D3Mit163, D4Mit17, D4Mit19, D6Mit14, D6Mit15, D6Mit48, D6Mit58, D6Mit158, D7Mit9, D7Mit10, D7Mit44, D7Mit47, D7Mit57, D7Mit117, D8Mit12, D8Mit14, D10Mit14, D10Mit46, D10Mit47, D11Mit4, D11Mit20, D11Mit21, D11Mit139, D11Mit164, D11Mit9, D11Mit10, D16Mit19, D16Mit56, D16Mit73, D16Mit81, D16Mit134, D16Mit52, and D18Mit40. The CcS-5 backcross was genotyped with D1Mit129, D1Mit46, D5Mit27, D5Mit112, D5Mit164, D6Mit23, D6Mit55, D6Mit173, D7Mit137, D8Mit17, D8Mit58, D8Mit155, D10Mit12, D10Mit14, D10Mit24, D10Mit25, D10Mit46, D10Mit47, D10Mit103, D10Mit133, D11Mit1, D11Mit2, D11Mit62, D11Mit71, D17Mit10, D17Mit13, D17Mit18, D17Mit19, D17Mit22, D17Mit35, D17Mit46, D17Nd3, D17Nd4, and D18Mit19. The CcS-11 backcross was genotyped with D1Mit15, D1Mit36, D1Mit205, D1Mit208, D3Mit18.

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4 The abbreviations used are: FAP, familial adenomatous polyposis; DMH, 1,2-dimethylhydrazine; CcS, CcS/Dem; BALB/c, BALB/cHeA; STS, STS/A; HNPCC, hereditary nonpolyposis colorectal cancer.

5 In addition to Ref. 16, see also http://www.informatics.jax.org/rcset.html.

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Table 1  Mean tumor number and standard error per genotype and Ps for linkage per genotype at the markers on chromosomes 3, 5, 8, 10, and 11 as determined by analysis of variance

<table>
<thead>
<tr>
<th>Marker</th>
<th>CC(^a)</th>
<th>CS(^b)</th>
<th>(P)</th>
<th>Corrected (F_{\text{adj}})</th>
<th>Locus</th>
<th>Chromosome</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1Mit12</td>
<td>10.1 ± 0.2 (44)</td>
<td>14.9 ± 0.2 (45)</td>
<td>0.000125</td>
<td>0.0043</td>
<td>Scc6</td>
<td>11</td>
<td>CsC-5</td>
</tr>
<tr>
<td>D1Mit19</td>
<td>14.9 ± 0.1 (52)</td>
<td>10.2 ± 0.2 (37)</td>
<td>0.0021</td>
<td>0.074</td>
<td>Not significant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3Mit19</td>
<td>12.9 ± 1.1 (17)</td>
<td>11.6 ± 1.1 (12)</td>
<td>0.000088</td>
<td>0.0057</td>
<td>Scc8</td>
<td>8</td>
<td>CsC-3</td>
</tr>
<tr>
<td>D8Mit17</td>
<td>9.0 ± 1.1 (14)</td>
<td>16.7 ± 1.1 (15)</td>
<td>0.000292</td>
<td>0.0086</td>
<td>Scc9</td>
<td>10</td>
<td>CsC-5</td>
</tr>
<tr>
<td>D10Mit67</td>
<td>8.65 ± 1.1 (14)</td>
<td>17.4 ± 1.1 (15)</td>
<td>0.000240</td>
<td>0.0056</td>
<td>Scc9</td>
<td>10</td>
<td>CsC-5</td>
</tr>
<tr>
<td>D10Mit64</td>
<td>9.9 ± 0.2 (51)</td>
<td>14.8 ± 0.2 (40)</td>
<td>0.0011</td>
<td>0.06</td>
<td>Not significant</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(\text{a}\) Homozygous for the BALB/c alleles.
\(\text{b}\) Heterozygous for BALB/c and STS alleles.
\(\text{P}\) includes some interactions.
\(\text{P}\) values are corrected according to Lander and Kruglyak (18).

Table 2  Reciprocal genetic interaction between D8Mit17 and D3Mit163

<table>
<thead>
<tr>
<th>Marker</th>
<th>CC</th>
<th>CS</th>
<th>(P)</th>
<th>(R)</th>
<th>(R^2)</th>
<th>Scc8</th>
<th>Chromosome</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>D3Mit162</td>
<td>13.3 ± 1.1 (8)</td>
<td>6.1 ± 1.12 (6)</td>
<td>0.0011</td>
<td>0.06</td>
<td>Not significant</td>
<td>Not significant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D3Mit163</td>
<td>12.6 ± 1.1 (9)</td>
<td>22.1 ± 1.12 (6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
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</table>

\(\text{P} = 0.0001, \text{corrected} P = 0.0012\). The STS allele at Scc7 increases the susceptibility in mice heterozygous at Scc8 but decreases in mice homozygous for the BALB/c allele at Scc8. The average number of tumors is given for each of the four genotype combinations. The number of mice per genotype is shown in parentheses. CC, homozygous for BALB/c alleles; CS, heterozygous for BALB/c and STS alleles.

Results and Discussion

We conducted a search for linkage of susceptibility to colon cancer in backcrosses of the strains CcS-3, CcS-5, and CcS-11. Tables 1 and 2 show the detected linkages.

In strain CcS-3, three loci were found (Scc7, Scc8, and Scc9) on chromosomes 3, 8, and 10, respectively; Tables 1 and 2). Scc8 was found to be linked with D8Mit17 (\(P = 0.0057\)). Backcross mice carrying the STS allele at the Scc8 locus developed almost twice as many tumors as their littermates that were homozygous for the BALB/c allele at this locus. Scc8 is located on an 18.6-cM segment between D8Mit58 and D8Mit24, near the centromere on chromosome 8 (Fig. 1). Another linkage was found to the STS allele of D10Mit46 (\(P = 0.0056\)). This locus, Scc9, maps to the telomeric part of chromosome 10 in a 17.5-cM region between D10Mit150 and D10Mit103 (Fig. 1). In the CcS-5 cross, susceptibility was also associated with the STS allele of D10Mit46 (Table 1; \(P = 0.06\)). However, its significance only satisfies the criteria for suggestive linkage (18).

When we looked for two-way interactions between all nonlinked pairs of markers, an interaction was found between D3Mit163 (Scc7) and Scc8 (\(P = 0.012\)). Scc7 has no apparent effect on itself (Table 1) but can only be detected because of a genetic interaction with Scc8. When Scc8 is homozygous for the BALB/c alleles, the STS allele of Scc7 shows resistance to colon tumors. However, when Scc8 carries a STS allele, the STS allele of Scc7 determines susceptibility (Table 2). Consequently, the BALB/c or STS alleles of Scc7 are not intrinsically susceptible or resistant, but their effect depends on the genotype at the interacting locus, Scc8. Scc7 is located on a 16.5-cM segment, between D3Mit17 and D3Mit163 on the telomeric part of chromosome 3 (Fig. 1).

In the CcS-5 cross, the susceptibility locus Scc6 was detected. Linkage of susceptibility was found to the STS allele of D11Mit2 (\(P = 0.0043\); Table 1). Scc6 maps to a 6.6-cM region near the centromere on chromosome 11 between markers D11Mit71 and D11Mit162 (Fig. 1). A suggestive linkage (\(P = 0.074\)) in this cross was found to D1Mit129. The marker D1Mit129 is in fact located on
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mouse chromosome 5, closely linked to D5Mit304. Additional independent experiments are needed to confirm linkage at this locus. No significant or suggestive linkages have been found in the CcS-11 cross.

STS alleles of the markers to which Scc7, Scc8, and Scc9 were mapped are also present in some of the other backcrosses (Scc7 in CcS-11, Scc8 in CcS-5, and Scc9 in both CcS-5 and CcS-11). However, only Scc9 was detected in another cross (CcS-5; Table 1). The failure to detect these loci in all crosses does not disprove them. As pointed out by Lander and Kruglyak (18), the initial, significant linkages can be overestimates of the effect of the loci; due to random fluctuations, the detected linkage will be above the threshold of significance. In other experiments, random fluctuations can push the effect of these genes below the threshold of detection. In addition, some of the loci are detected by virtue of their interactions with other loci (12, 14, 15). Therefore they can probably only be detected in a particular genetic background and are not observed in mice with a different genetic make-up. Paradoxically, in the present experiments, most loci were detected in the cross with the smallest number of animals. However, the detection of four loci in a total of 196 mice is comparable to the number of loci detected in similar experiments (12, 14, 15, 19). The distribution of the linkages between the crosses has a stochastic component and is influenced by interlocus interactions. These interactions, which are presently poorly defined, probably play a much larger role in the genetics of quantitative traits than is generally recognized (15).

In conclusion, we have found four novel loci involved in the complex genetic trait of colon cancer susceptibility. This extends the number of colon tumor susceptibility loci we have detected using the recombinant congenic strain system to nine. Recombinants for the regions on chromosomes 10 and 11 containing Scc9 and Scc6, respectively, have already been produced and will be tested for colon tumor susceptibility to confirm the linkage and map these loci more precisely. Subsequent cloning of these genes could elucidate the underlying mechanisms of cancer susceptibility and of the genetic interactions. The small initial segment to which the loci are mapped, together with the physical maps and the increasing density of the available Expressed Sequence Tag maps, will speed up the identification of candidate genes for these Scc loci.

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