Four New Colon Cancer Susceptibility Loci, \textit{Scc6} to \textit{Scc9} in the Mouse

Tom van Wezel, Claudia A. L. Ruivenkamp, Alphons P. M. Stassen, Corina J. A. Moen, and Peter Demant

Division of Molecular Genetics, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands

Abstract

Germ-line mutations in \textit{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, \textit{Scc1}–\textit{Scc5}, have been described previously using the recombinant congenic strains. Two of these loci, \textit{Scc4} and \textit{Scc5}, show a reciprocal, genetic interaction. Here we report the mapping of four new colon tumor susceptibility genes: (a) \textit{Scc6} on chromosome 11; (b) \textit{Scc7} on chromosome 3; (c) \textit{Scc8} on chromosome 8; and (d) \textit{Scc9} on chromosome 10. \textit{Scc7} and \textit{Scc8} show a genetic interaction; \textit{Scc7} is only detected by virtue of its interaction with \textit{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 \textit{APC} gene cause FAP\(^3\), and mutations in mismatch repair genes, mainly \textit{MSH2} and \textit{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 \textit{CcS} series of the recombinant congenic strains \(8\) to study colon cancer susceptibility \(9\). The \textit{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 \textit{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 \textit{CcS} alleles of different loci involved in the susceptibility to colon cancer are divided between the 20 \textit{CcS} strains, thus converting a multigenic difference into oligogenic or monogenic differences \(10\).

Materials and Methods

\textbf{Animals and Tumor Induction.} The mice received a standard laboratory diet (Hope Farms, Woerden, the Netherlands) and acidified drinking water \textit{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) \textit{CcS}-3; (b) \textit{CcS}-5; and (c) \textit{CcS}-11. For each strain, a BALB/c \(\times\) (BALB/c \(\times\) C57F1) backcross was produced. The \textit{CcS}-3 cross consists of 29 mice. The backcrosses for \textit{CcS}-5 and \textit{CcS}-11 each consist of two experiments performed at different points in time: 38 and 55 animals were used in the two \textit{CcS}-5 experiments, and 36 and 38 animals were used in the \textit{CcS}-11 experiments.

\textbf{Materials and Methods.} The mice received a standard laboratory diet (Hope Farms, Woerden, the Netherlands) and acidified drinking water \textit{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) \textit{CcS}-3; (b) \textit{CcS}-5; and (c) \textit{CcS}-11. For each strain, a BALB/c \(\times\) (BALB/c \(\times\) C57F1) backcross was produced. The \textit{CcS}-3 cross consists of 29 mice. The backcrosses for \textit{CcS}-5 and \textit{CcS}-11 each consist of two experiments performed at different points in time: 38 and 55 animals were used in the two \textit{CcS}-5 experiments, and 36 and 38 animals were used in the \textit{CcS}-11 experiments. Mice (11–15 weeks old) received 26 weekly s. i. injections of DMH \(15 \text{ mg/kg body weight, freshly dissolved in } 1 \text{ mm EDTA (pH 6.8)}\). 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.

\textbf{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 MapPars TM; Research Genetics, Huntsville AL). The \textit{CcS}-3 backcross used microsatellite markers \(D3Mit18, D3Mit162, D3Mit163, D4Mit17, D4Mit14, D6Mit15, D6Mit153, D6Mit158, D7Mit10, D7Mit37, D8Mit17, D8Mit58, D8Mit155, D10Mit12, D10Mit14, D10Mit46, D10Mit47, D11Mit4, D11Mit20, D11Mit21, D11Mit139, D11Mit164, D11Nds9, D11Nds10, D16Mit19, D16Mit56, D16Mit73, D16Mit81, D16Mit134, D16Nds2, and D18Mit40). The \textit{CcS}-5 backcross was genotyped with \(D1Mit129, D3Mit46, D5Mit127, D5Mit112, D5Mit164, D6Mit23, D6Mit35, D6Mit173, D7Mit137, D8Mit17, D8Mit58, D8Mit155, D10Mit12, D10Mit14, D10Mit24, D10Mit25, D10Mit46, D10Mit47, D10Mit103, D10Mit133, D11Mit1, D11Mit2, D11Mit62, D11Mit71, D17Mit10, D17Mit13, D17Mit18, D17Mit19, D17Mit22, D17Mit35, D17Mit46, D17Nds3, D17Nds4, and D18Mit19). The \textit{CcS}-11 backcross was genotyped with \(D1Mit15, D1Mit36, D1Mit205, D1Mit208, D3Mit18\).

\footnotesize
\(^{1}\) In addition to Ref. 16, see also http://www.informatics.jax.org/rcset.html.

\[^{3}\] Supported by Dutch Cancer Society Grants NKI-97-1463 and NKI-98-1832 (to P. D.).

\[^{2}\] Present address: Department of Human Genetics, Leiden University Medical Center, Wassenarweg 72, 2333 AL Leiden, the Netherlands.

\[^{3}\] To whom requests for reprints should be addressed.

\[^{4}\] The abbreviations used are: FAP, familial adenomatous polyposis; DMH, 1,2-dimethylhydrazine; \textit{CcS}, \textit{CcS}/\textit{F1} BALB/c; \textit{BALB/c}HeA; STS, STS/A; HNPCC, hereditary nonpolyposis colorectal cancer.

Received 3/25/99; accepted 7/19/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
The number of mice per genotype is shown in parentheses. CC, homozygous for BALB/c alleles; CS, heterozygous for BALB/c and STS alleles.

<table>
<thead>
<tr>
<th>Marker</th>
<th>CC</th>
<th>CS</th>
<th>$P^e$</th>
<th>Corrected $P^d$</th>
<th>Locus</th>
<th>Chromosome</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1Mit12</td>
<td>10.1 ± 0.02 (44)</td>
<td>14.9 ± 0.02 (45)</td>
<td>0.000125</td>
<td>0.0043</td>
<td>Scc6</td>
<td>11</td>
<td>CcS-5</td>
</tr>
<tr>
<td>D1Mit129</td>
<td>14.9 ± 0.01 (52)</td>
<td>10.2 ± 0.02 (37)</td>
<td>0.0021</td>
<td>0.074</td>
<td>Scc7</td>
<td>5</td>
<td>CcS-5</td>
</tr>
<tr>
<td>D3Mit163</td>
<td>12.9 ± 0.11 (17)</td>
<td>11.6 ± 0.11 (12)</td>
<td>Not significant</td>
<td>0.0057</td>
<td>Scc8</td>
<td>8</td>
<td>CcS-3</td>
</tr>
<tr>
<td>D3Mit167</td>
<td>9.0 ± 0.11 (14)</td>
<td>16.7 ± 0.11 (15)</td>
<td>0.00088</td>
<td>0.00564</td>
<td>Scc7</td>
<td>3</td>
<td>CcS-3</td>
</tr>
<tr>
<td>D10Mit46</td>
<td>8.65 ± 0.11 (14)</td>
<td>17.4 ± 1.1 (15)</td>
<td>Not significant</td>
<td>0.00040</td>
<td>Scc8</td>
<td>10</td>
<td>CcS-5</td>
</tr>
<tr>
<td>D10Mit46</td>
<td>9.9 ± 0.02 (51)</td>
<td>14.8 ± 0.02 (40)</td>
<td>0.0011</td>
<td>0.06</td>
<td>Scc7</td>
<td>10</td>
<td>CcS-5</td>
</tr>
</tbody>
</table>

$^a$ Homozygous for the BALB/c alleles.
$^b$ Heterozygous for BALB/c and STS alleles.
$^c$ $P$ includes some interactions.
$^d$ $Ps$ are corrected according to Lander and Kruglyak (18).
$^e$ D1Mit129 is linked to D3Mit304 on chromosome 5 (H. Havelkova, personal communication).

**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 teleric 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 teleric 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 D1Mit29. The marker D1Mit29 is in fact located on...
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.

Acknowledgments

We thank Marcelle Treur-Mulder, Joost de Moes, Elly Delzenne-Goette, and Marius Timpico for excellent technical assistance. We also thank Helena Havelkova for sharing information on the chromosomal position of D1Mit129 with us.

References


* H. Havelkova, personal communication.
Four New Colon Cancer Susceptibility Loci, Scc6 to Scc9 in the Mouse


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/59/17/4216

Cited articles
This article cites 13 articles, 2 of which you can access for free at:
http://cancerres.aacrjournals.org/content/59/17/4216.full#ref-list-1

Citing articles
This article has been cited by 13 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/59/17/4216.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, use this link
http://cancerres.aacrjournals.org/content/59/17/4216.
Click on "Request Permissions" which will take you to the Copyright Clearance Center's (CCC) Rightslink site.