

Four New Colon Cancer Susceptibility Loci, *Scs6* to *Scs9* in the Mouse¹

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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, *Scs1–Scs5*, have been described previously using the recombinant congenic strains. Two of these loci, *Scs4* and *Scs5*, show a reciprocal, genetic interaction. Here we report the mapping of four new colon tumor susceptibility genes: (a) *Scs6* on chromosome 11; (b) *Scs7* on chromosome 3; (c) *Scs8* on chromosome 8; and (d) *Scs9* on chromosome 10. *Scs7* and *Scs8* show a genetic interaction; *Scs7* is only detected by virtue of its interaction with *Scs8*.

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,⁴ 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, *Scs1*, *Scs2*, *Scs3*, *Scs4*, and *Scs5*, 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, *Scs1*, 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 *Scs1*, *Scs2*, *Scs4*, and *Scs5* 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 *Scs3*, which cannot be the cause of its susceptibility. Therefore, the susceptibility of these strains is caused by loci other than the known loci *Scs1–Scs5*. 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) *Scs6* on chromosome 11; (b) *Scs7* on chromosome 3; (c) *Scs8* on chromosome 8; and (d) *Scs9* on chromosome 10. *Scs7* and *Scs8* show a genetic interaction, a phenomenon we also observed for *Scs4* and *Scs5* (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).⁵ Three strains were tested: (a) CcS-3; (b) CcS-5; and (c) CcS-11. For each strain, a BALB/c × (BALB/c × CcS)_{F1} 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 proteinase 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*, *D4Mit7*, *D4Mit17*, *D6Mit14*, *D6Mit15*, *D6Mit48*, *D6Mit58*, *D6Mit158*, *D7Mit9*, *D7Mit10*, *D7Mit14*, *D7Mit47*, *D7Mit67*, *D7Mit117*, *D8Mit12*, *D8Mit17*, *D10Mit14*, *D10Mit46*, *D10Mit47*, *D11Mit4*, *D11Mit20*, *D11Mit21*, *D11Mit139*, *D11Mit164*, *D11Nds9*, *D11Nds10*, *D16Mit19*, *D16Mit56*, *D16Mit73*, *D16Mit81*, *D16Mit134*, *D16Nds2*, and *D18Mit40*. The CcS-5 backcross was genotyped with *D1Mit129*, *D3Mit46*, *D5Mit27*, *D5Mit112*, *D5Mit164*, *D6Mit10*, *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 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>.

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

The number of mice per genotype is shown in parentheses.

Marker	CC ^a	CS ^b	<i>P</i> ^c	Corrected <i>P</i> ^{c,d}	Locus	Chromosome	Strain
<i>D11Mit2</i>	10.1 ± 0.02 (44)	14.9 ± 0.02 (45)	0.000125	0.0043	<i>Sc6</i>	11	CcS-5
<i>D1Mit129</i>	14.9 ± 0.01 (52)	10.2 ± 0.02 (37)	0.0021	0.074		5 ^e	CcS-5
<i>D3Mit163</i>	12.9 ± 1.1 (17)	11.6 ± 1.1 (12)	Not significant		<i>Sc7</i>	3	CcS-3
<i>D8Mit17</i>	9.0 ± 1.1 (14)	16.7 ± 1.1 (15)	0.000088	0.0057	<i>Sc8</i>	8	CcS-3
<i>D10Mit46</i>	8.65 ± 1.1 (14)	17.4 ± 1.1 (15)	0.000040	0.0056	<i>Sc9</i>	10	CcS-3
<i>D10Mit46</i>	9.9 ± 0.02 (51)	14.8 ± 0.02 (40)	0.0011	0.06		10	CcS-5

^a Homozygous for the BALB/c alleles.

^b Heterozygous for BALB/c and STS alleles.

^c *P* includes some interactions.

^d *P*s are corrected according to Lander and Kruglyak (18).

^e *D1Mit129* is linked to *D5Mit304* on chromosome 5 (H. Havelkova, personal communication).

Table 2 Reciprocal genetic interaction between *D8Mit17* and *D3Mit163*

P = 0.0001, corrected *P* = 0.012. The STS allele at *Sc7* increases the susceptibility in mice heterozygous at *Sc8* but decreases in mice homozygous for the BALB/c allele at *Sc8*. The average number of tumors is given for each of the four genotype combinations. The number of mice/genotype is shown in parentheses. CC, homozygous for BALB/c alleles; CS, heterozygous for BALB/c and STS alleles.

		<i>D3Mit163</i> (<i>Sc7</i>)	
		CC	CS
<i>D8Mit17</i> (<i>Sc8</i>)	CC	13.3 ± 1.11 (8)	6.1 ± 1.12 (6)
	CS	12.6 ± 1.1 (9)	22.1 ± 1.12 (6)

D3Mit162, *D3Mit163*, *D7Mit7*, *D7Mit8*, *D7Mit9*, *D7Mit10*, *D7Mit14*, *D7Mit15*, *D7Mit26*, *D7Mit47*, *D7Mit54*, *D7Mit55*, *D7Mit67*, *D7Nds1*, *D7Nds2*, *D7Nds5*, *D7Nds4*, *D8Mit40*, *D8Mit85*, *D10Mit12*, *D10Mit14*, *D10Mit24*, *D10Mit46*, *D10Mit47*, *D10Mit133*, *D10Mit150*, *D12Mit37*, *D16Mit34*, *D16Mit73*, *D19Mit12*, *D19Mit41*, *D19Mit56*, *D19Mit60*, *D19Mit61*, and *D19Mit62*. These markers cover the known STS-derived segments from strains CcS-3, CcS-5, and CcS-11 with a spacing of approximately 5 cM (16)

Statistical Analysis. To obtain a normal distribution, the numbers of colon tumors in the CcS-3 backcross were log-transformed. In the CcS-5 and CcS-11 backcrosses, the exponent 0.2 normalized the colon tumor numbers. Linkage between the number of colon tumors and the markers in the three different backcrosses was determined by advanced ANOVA (NCSS, Kaysville, UT) using gender and marker(s) as fixed factors. For CcS-5 and CcS-11, the experimental group was used as a random factor.

All single markers and all pairs of nonlinked markers were tested. Markers and interactions with *P* < 0.05 were combined in one model, and, subsequently, markers and interactions with a *P* > 0.05 were eliminated one by one, starting with the one with the highest *P*. Final *P*s were corrected for multiple comparisons using the following formula:

$$\mu(T) = [C + 2\rho GT^2]\alpha(T) \text{ (Ref. 18)}$$

where $\alpha(T)$ is the observed *P*, the F ratio from ANOVA is used for T^2 , the genome length in Morgans $G = 2$ (the length of the segregating part of the donor genome; 12.5% of 16 M), the constant $\rho = 1$ (crossover rate) for a backcross, and *C* (the number of chromosomes segregating in the cross) is 8 for CcS-11 and 9 for CcS-3 and CcS-5 (16, 18).

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 (*Sc7*, *Sc8*, and *Sc9* on chromosomes 3, 8, and 10, respectively; Tables 1 and 2). *Sc8* was found to be linked with *D8Mit17* (*P* = 0.0057). Backcross mice carrying the STS allele at the *Sc8* locus developed almost twice as many tumors as their littermates that were homozygous for the BALB/c allele at this locus. *Sc8* 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, *Sc9*, 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* (*Sc7*) and *Sc8* (*P* = 0.012). *Sc7* has no apparent effect on itself (Table 1) but can only be detected because of a genetic interaction with *Sc8*. When *Sc8* is homozygous for the BALB/c alleles, the STS allele of *Sc7* shows resistance to colon tumors. However, when *Sc8* carries a STS allele, the STS allele of *Sc7* determines susceptibility (Table 2). Consequently, the BALB/c or STS alleles of *Sc7* are not intrinsically susceptible or resistant, but their effect depends on the genotype at the interacting locus, *Sc8*. *Sc7* 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 *Sc6* was detected. Linkage of susceptibility was found to the STS allele of *D11Mit2* (*P* = 0.0043; Table 1). *Sc6* 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

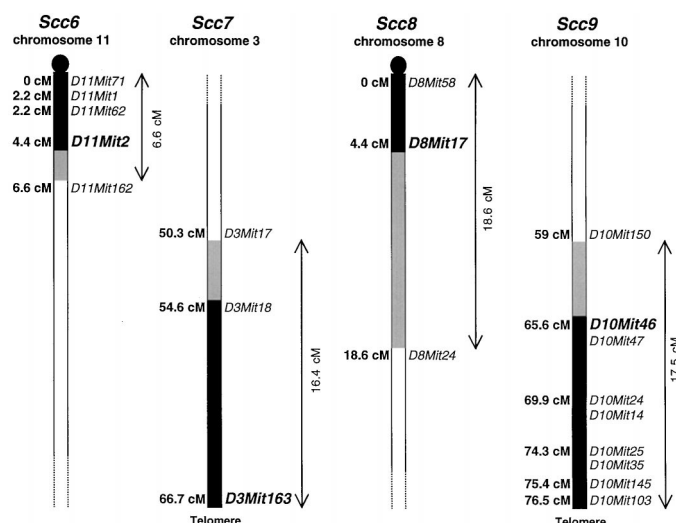


Fig. 1. STS-derived genomic segments containing the four *Sc* loci. *Sc6* is found in the backcross of CcS-5, and *Sc7*, *Sc8*, and *Sc9* are found the CcS-3 backcross. Linkage is found with the markers depicted in bold. The centimorgan position is taken from the Whitehead Institute Genetic and Physical Maps of the Mouse Genome (<http://carbon-wi.mit.edu:8000/cgi-bin/mouse/index>). □, the BALB/c-derived genomic regions; ■, the STS-derived segments; ▨, the region of crossover. The vertical arrows indicate the maximum length of the STS genomic segment (12).

mouse chromosome 5, closely linked to *D5Mit304*.⁶ 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 *Sec7*, *Sec8*, and *Sec9* were mapped are also present in some of the other backcrosses (*Sec7* in CcS-11, *Sec8* in CcS-5, and *Sec9* in both CcS-5 and CcS-11). However, only *Sec9* 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 *Sec9* and *Sec6*, 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 *Sec* loci.

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⁶ H. Havelkova, personal communication.