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Genetic Identification of *Mom-1*, a Major Modifier Locus Affecting *Min*-Induced Intestinal Neoplasia in the Mouse

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Summary

Mutations in the human *APC* gene cause various familial colon cancer syndromes. The Multiple intestinal neoplasia (Min) mouse provides an excellent model for familial colon cancer: it carries a mutant mouse *Apc* gene and develops many intestinal adenomas. Here, we analyze how this tumor phenotype is dramatically modified by genetic background. We report the genetic mapping of a locus that strongly modifies tumor number in *Min*/+ animals. This gene, *Mom-1* (Modifier of Min-1), maps to distal chromosome 4 and controls about 50% of genetic variation in tumor number in two intraspecific backcrosses. The mapping is supported by a LOD score exceeding 14. Interestingly, *Mom-1* lies in a region of synteny conservation with human chromosome 1p35-36, a region of frequent somatic loss of heterozygosity in a variety of human tumors, including colon tumors. These results provide evidence of a major modifier affecting expression of an inherited cancer syndrome.

Introduction

Colon cancer is one of the most prevalent malignancies in the Western world, with an estimated 145,000 new cases and 60,000 deaths per year in the United States alone (Burt and Lipkin, 1992). Although environmental factors such as diet markedly influence the incidence of the disease, genetic factors also play a key role, as evidenced by striking familial clustering of colon cancer (Bishop and Skolnick, 1984; Hansen and Cavenee, 1987; Burt and Lipkin, 1992).

In some human pedigrees, predisposition to colon cancer is inherited in a dominant Mendelian fashion (Lynch et al., 1992a, 1992b). The best known example is familial adenomatous polyposis, in which individuals inheriting a single mutant allele of the *APC* (or adenomatous polyposis coli) gene develop hundreds to thousands of adenomatous polyps in the colon that, unless removed, eventually progress to become carcinomas (Bulow, 1987; Hamilton, 1989). The *APC* gene was shown to map to chromosome 5q21-22 on the basis of genetic linkage analysis (Bodmer et al., 1987; Leppert et al., 1987) and was subsequently cloned on the basis of its chromosomal position (Groden et al., 1991; Kinzler et al., 1991; Nishisho et al., 1991). The *APC* gene has also been implicated in various other familial colon cancer syndromes with clinical features distinct from classical familial adenomatous polyposis. These include attenuated adenomatous polyposis coli, in which some patients may have only a few polyps; hereditary flat adenoma syndrome, in which the polyps have a distinctive morphology; and Gardner's syndrome, in which the polyps are often accompanied by a variety of extracolonic neoplasms (Spirio et al., 1992; Lynch et al., 1992b; Nishisho et al., 1991). In short, individuals inheriting mutations in *APC* can show a striking range of phenotypic variation.

Although some of the phenotypic variation may be due to differences between mutant alleles of *APC* (R. L. White, personal communication), there can be striking variation even among family members who inherit the same *APC* mutation. Some carriers in a family may have only a few colonic polyps, others may show more than 100 colonic polyps, and still others may manifest gastric polyps or a variety of other nongastrointestinal neoplasias, including osteomas, sarcomas, and carcinomas (Leppert et al., 1990; Spirio et al., 1992). Such variation within families might be due to differences in environmental factors (such as diet), genetic background (such as modifier genes), or both. Unfortunately, it is difficult to dissect the effects of environmental and genetic modifiers in human pedigrees.

The mouse offers a powerful system for studying complex genetic interactions in general and colon cancer in particular. Several years ago Moser et al. (1990) isolated a dominantly transmitted, fully penetrant mouse mutation named *Min* (for multiple intestinal neoplasia) that causes a phenotype closely resembling human inherited colonic polyposis syndromes. Heterozygotes for the *Min* mutation develop numerous intestinal and colonic adenomas similar in morphology to the adenomas seen in familial adenomatous polyposis and Gardner's syndrome patients; if left untreated, they can eventually become locally invasive (Moser et al., 1992). In addition, about 10% of *Min*/+ females on a hybrid background develop spontaneous mammary adenoacanthomas or adenocarcinomas (Moser et al., 1993). Finally, homozygotes for *Min* die in utero (unpublished data). Genetic mapping studies showed that the *Min* mutation maps to chromosome 18 and is tightly linked to *Apc*, the mouse homolog of the human *APC* gene (Luongo et al., 1993). Su et al. (1992) subsequently determined that *Min* mice carry a nonsense mutation in exon 15 of the mouse *Apc* gene (a mutation of the sort typically seen in human colon cancer kindreds) that is recombinationally inseparable from *Min*.

Mice carrying *Min* thus provide a model system for studying human familial adenomatous polyposis and, in particular, for identifying genes that can modify the phenotype caused by an *Apc* mutation. In fact, in the course of genetic mapping experiments, the Wisconsin group noticed that the number of intestinal tumors in *Min*/+ mice was strongly

Table 1. Tumor Multiplicity in *Min*/+ Mice of Different Genetic Backgrounds

Min/+ Mice		Tumor Number (Mean ± SD)	Number of *Min*/+ Animals
Parental strain	B6 Min/+	28.5 ± 7.9	(n = 26)
F1 hybrids	(AKR × B6-Min)F1	5.8 ± 4.3	(n = 40)
	(MA × B6-Min)F1	5.7 ± 4.0	(n = 15)
	(CAST × B6-Min)F1	3.0 ± 1.8	(n = 12)
Backcross to B6	B6 × (AKR × B6-Min)F1	17.7 ± 11.7	(n = 110)
	B6 × (MA × B6-Min)F1	15.9 ± 11.0	(n = 82)
	B6 × (CAST × B6-Min)F1	14.4 ± 11.9	(n = 142)
Backcross to AKR	AKR × (AKR × B6-Min)F1	1.7 ± 1.7	(n = 57)

Crosses were performed and tumors were counted as described in Experimental Procedures. B6 refers to C57BL/6J; AKR to AKR/J; MA to MA/MyJ; and CAST to CAST/EiJ. All backcrosses, except the backcross to AKR, were performed in both parental orientations, and no significant differences in tumor numbers among progeny were seen, owing to the sex of the *Min*/+ parent.

affected by genetic background: whereas the *Min*/+ mice of the parental congenic strain C57BL/6J-*Min*/+ (denoted B6-Min) have an average of 29 tumors, their *Min*/+ F1 progeny with AKR mice showed an average of only 6 tumors (Moser et al., 1992). This finding suggested that the AKR strain carries alleles that can act in a dominant fashion to modify the tumorigenic effect of *Min*. Similar results were found in F1 progeny with two other mouse strains. In addition, a backcross of (AKR × B6-Min)F1 mice to B6 gave a segregation of tumor multiplicities consistent with a small number of modifier loci (Moser et al., 1992). To test whether these presumptive modifier loci could be mapped, we jointly analyzed a number of experimental crosses to identify modifier genes that influence tumor multiplicity in *Min*/+ mice.

Here we report the genetic mapping of a locus that strongly modifies tumor number in *Min*/+ animals. This gene, named *Mom-1* (for Modifier of Min-1), maps to distal chromosome 4. Allelic variation in *Mom-1* appears to control about 50% of the genetic variation in tumor number in two different intraspecific backcrosses. The mapping is supported by a cumulative LOD score exceeding 14. Interestingly, the *Mom-1* locus lies in a region of synteny conservation with the human chromosomal segment 1p35-36, a region that shows frequent somatic loss of heterozygosity in a variety of human tumor types, including colon tumors. These studies provide evidence that a major modifier locus affects the expression of an inherited cancer syndrome.

Results

Tumor Multiplicity in *Min*-Bearing Mice Is under Genetic Control

The *Min* mutation has been maintained by backcrossing to the C57BL/6J (B6) strain for more than 23 generations. On this genetic background, the dominant intestinal tumor phenotype is fully penetrant: *Min*/+ mice (B6-Min) typically have 28.5 ± 7.9 (mean ± 1 SD) tumors, and most die within 4 months of birth (Table 1). When B6-Min mice were crossed to AKR mice, however, the *Min*/+ F1 progeny showed a greatly reduced tumor multiplicity: these mice had an average of 5.8 ± 4.3 tumors, and most survived until sacrifice at 300 days. This suggests that the AKR strain carries genetic modifiers that are at least partially dominant in suppressing the intestinal tumor phenotype. When the *Min*/+ F1 progeny were backcrossed to B6, the backcross (BC1) progeny had a mean tumor number intermediate between the F1 and B6-Min parents, with a large standard deviation (17.7 ± 11.7), as would be expected from the segregation of genetic modifying factors in the backcross progeny. (For details of the cross and counting of tumors, see Experimental Procedures.)

Considerable information about the genetic control of tumor number can be gleaned by examining the distribution of tumor multiplicity in the B6-Min parent, the F1 progeny, and the BC1 progeny (Figure 1). The phenotypic variance in the parental and F1 generations must be entirely environmental variance because the animals are genetically identical. By contrast, the phenotypic variance in the BC1 progeny is the sum of environmental and genetic variance, because allelic differences are segregating in the backcross progeny (Wright, 1968). We found that the genetic component of the variance in tumor number in this backcross was approximately 69%. In addition, by suitably transforming the data and applying a classical formula of Wright, one can estimate the effective number n of genetic factors controlling tumor number in the *Min*/+ backcross progeny (see Experimental Procedures concerning calculation and interpretation of n). Applying this approach, we found that the effective number of genetic factors controlling the trait was approximately 1.8 (Table 2). This suggested that it should be possible to perform genetic mapping to identify at least one major quantitative trait locus (QTL) controlling the expressivity of *Min* in a cross of modest size (Lander and Botstein, 1989).

Mom-1, a Major Modifier of Tumor Multiplicity, Maps to Chromosome 4

With the aim of genetically mapping QTLs affecting the expression of *Min*, we genotyped 110 *Min*/+ progeny from an (AKR × B6-Min) × B6 backcross with 75 simple sequence length polymorphisms (SSLPs) distributed

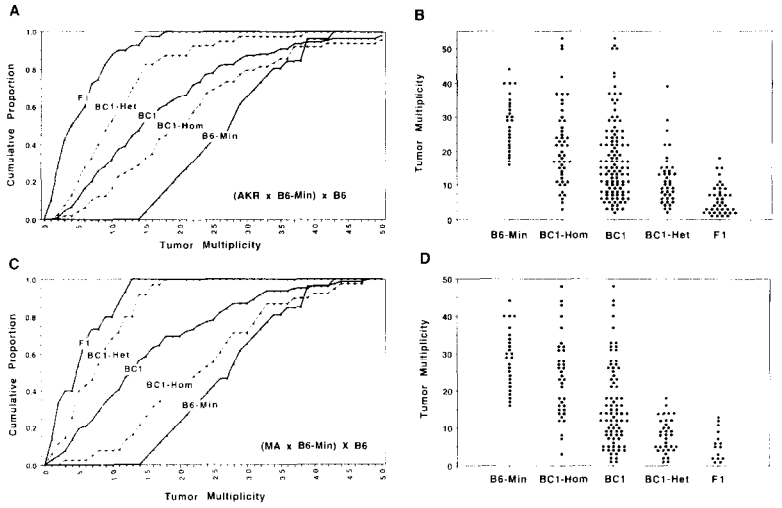

Figure 1. Distribution of Tumor Number in *Min*/+ Animals in Two Backcrosses Involving Min

For each backcross, tumor multiplicity is depicted both by a cumulative probability distribution and a scatterplot for five groups: the B6-Min parental strain (B6-Min), the F1 hybrid between B6-Min and the other parental strain (F1), the total backcross population (BC1), the backcross progeny heterozygous at *Mom-1* (BC1-Het), and those backcross progeny homozygous for the B6 allele around *Mom-1* (BC1-Hom). The backcrosses are AKR backcross to B6 (A and B) and MA backcross to B6 (C and D).

throughout the genome, chosen from a genetic map of more than 1000 SSLPs previously constructed by the Whitehead group (Dietrich et al., 1992, 1993; unpublished data). When we analyzed the quantitative phenotype in relation to the inheritance pattern of genetic markers (Lander and Botstein, 1989), we found strong evidence for a QTL on distal chromosome 4 that has a profound effect on the number of tumors in *Min*/+ animals (Figure 2). The presence of a major QTL between *D4Mit12* and *D4Mit13* was supported by a LOD score of 4.7. We refer to the locus as *Mom-1* (Modifier of Min-1). Based on QTL linkage analysis, *Mom-1* accounts for a reduction of about 10 tumors in animals heterozygous for *Mom-1* compared with animals homozygous for the B6 allele of *Mom-1* and explains 35% of the genetic variance in tumor number in this backcross (Figure 1A and Table 3). No other QTLs with statistically significant LOD scores were detected in the cross.

To localize the *Mom-1* gene relative to known mouse genes, we typed the flanking genetic markers *D4Mit16* and *D4Mit13* in a (B6 × SPRET) × B6 interspecific backcross in which nearly 1000 mouse genes have been mapped (Copeland and Jenkins, 1991). (Because *D4Mit12* was not polymorphic in the B6 × SPRET cross, the tightly linked locus *D4Mit16* was used in its place.) On the basis of linkage analysis of this cross, *D4Mit16* lies less than 1 cM distal to *Lck* (the gene encoding a lymphocyte-specific protein-tyrosine kinase), and *D4Mit13* is recombinationally inseparable from the more distal locus *Pnd* (the gene encoding pronatriodilatin [Yang-Feng et al., 1985]). *Mom-1* thus lies in the interval between *Lck* and *Pnd*. The *Lck–Pnd* interval on distal mouse chromosome 4 shows strong mouse–human synteny conservation (i.e., conservation of gene order) with the *LCK–PND* interval on human chromosome 1p35-36 (Nadeau et al., 1991; Marth et al. 1986; Yang-Feng et al., 1985), which suggests that a human homolog of *Mom-1* would lie in the region 1p35-36.

Mom-1 Is Confirmed in a Second Backcross

We next studied a backcross involving a different inbred laboratory strain, MA/MyJ (MA). An (MA × B6-Min) F1 × B6 backcross was produced, and tumors were counted in the F1 and BC1 progeny. At the level of phenotype, the results were remarkably similar to the backcross with AKR: the *Min*/+ F1 progeny had 5.7 ± 4.0 tumors; the *Min*/+ BC1 progeny had 15.9 ± 11.0 tumors; 71% of the total variance was attributable to genetic variance; and Wright's formula gave an estimate of 1.7 apparent factors segregating (Tables 1 and 2). We then scored a total of 82 *Min*/+ backcross progeny with 53 SSLP markers distributed over the genome. We again found strong evidence for a major modifier in the same region of distal chromosome 4, with a peak LOD score of 10.9 in the interval between *D4Mit54* and *D4Mit13* (Figure 2). It seems likely that the effect is due

Table 2. Genetic Analysis of *Min*/+ Mice in Different Crosses

Cross	Genetic Contribution to Phenotypic Variance (%)[a]	Number of Genetic Factors Controlling Tumor Number[b]
B6 × (AKR × B6-Min)F1	59	1.8
B6 × (MA × B6-Min)F1	61	1.7
B6 × (CAST × B6-Min)F1	87	2.1

All analyses were performed on transformed data, as explained in Experimental Procedures.
[a] Defined as σ_g^2/σ^2, as explained in Experimental Procedures.
[b] Calculated according to Wright's formula, as explained in Experimental Procedures.

Table 3. Tumor Number of Different *Mom-1* Classes

Cross	*Mom-1* Genotype[a]		
	B6/B6	B6/X	X/X
Backcrosses to B6			
B6 × (AKR × B6-Min)F1	22.0 ± 1.7 (n = 49)	11.7 ± 1.2 (n = 40)	NA
B6 × (MA × B6-Min)F1	23.6 ± 1.7 (n = 38)	8.2 ± 0.7 (n = 41)	NA
B6 × (CAST × B6-Min)F1	16.7 ± 12.5 (n = 64)	11.3 ± 8.1 (n = 54)	NA
Backcross to AKR			
AKR × (AKR × B6-Min)F1	NA	1.8 ± 1.6 (n = 20)	1.16 ± 1.5 (n = 20)

The *Mom-1* genotype is written in terms of two alleles B6 and X, with X denoting the non-B6 allele in each cross (i.e., AKR, MA, or CAST). The *Mom-1* genotype was inferred for each animal on the basis of its genotype at the closest flanking genetic markers; animals showing recombination between the markers were excluded. The flanking markers used were *D4Mit16* and *D4Mit13* in the AKR backcross, *D4Mit54* and *D4Mit13* in the MA backcross, and *D4Mit16* and *D4Mit54* in the CAST backcross. NA, not applicable.

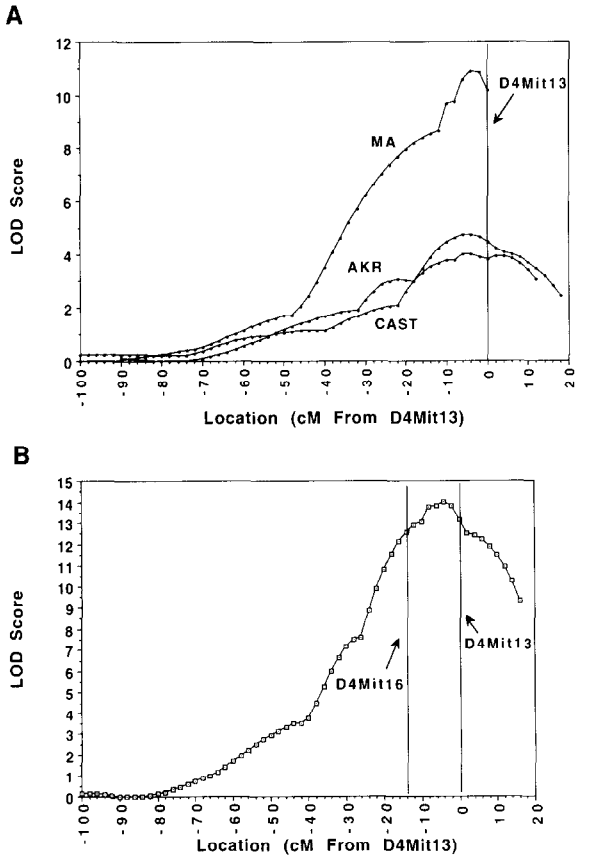

Figure 2. LOD Score Plots for *Mom-1*

(A) The LOD score in support of a locus affecting tumor number is plotted along chromosome 4 in the three crosses involving AKR, MA, and CAST strains backcrossed to B6. Genetic position is indicated relative to *D4Mit13*, which lies in the distal region of chromosome 4. Each plot extends between the furthest polymorphic markers genotyped in the cross.

(B) A composite LOD score plot for the AKR and MA backcrosses to B6 was computed under the assumption that *Mom-1* has an equal effect in both crosses. The genetic map was based on the position of the markers in a previous (B6 × CAST)F2 intercross, in which all the markers are polymorphic (Dietrich et al., 1992). Genetic position is indicated relative to the position of *D4Mit13*.

to the same locus as in the backcross with AKR, *Mom-1*. In this cross, *Mom-1* appears to cause a reduction of about 15 tumors in heterozygotes compared with homozygotes and explains 68% of the genetic variance (Figure 1B and Table 3). When examining the remainder of the genome, we found no other statistically significant QTLs in this backcross.

Mom-1 thus appears to be the major dominant modifier of *Min*-induced tumors in both backcrosses and appears to have virtually identical effects. The difference in the estimated reduction in tumor number (10 for AKR versus 15 for MA) is not statistically significant ($p > 0.10$, likelihood ratio test). Accordingly, the data were pooled. Based on a joint analysis, *Mom-1* appears to reduce tumor number by about 12 and to map about 6 cM proximal to the marker *D4Mit13* with a peak LOD score of 14.0 (Figure 2). The most likely position for *Mom-1* is in the interval between *D4Mit13* and *D4Mit16*, since the LOD score drops by more than 1 unit outside this interval (see, e.g., Conneally et al., 1985; Ott, 1991). The probability of obtaining a LOD score of 14 by chance is less than 10^{-12} (Lander and Botstein, 1989). In short, the genetic evidence for the identification of *Mom-1* is overwhelming.

Evidence for *Mom-1* in a More Distant Backcross

We next studied a backcross involving a different mouse subspecies, Mus musculus castaneus (CAST), which is separated by about 2 million years of evolution. We were interested to determine whether an effect of *Mom-1* could be detected in a cross between such widely divergent strains. A (CAST × B6-Min) × B6 backcross was produced, and progeny were scored for tumors (Table 1). The phenotypic results were slightly different from the backcrosses with AKR and MA: the *Min*/+ F1 progeny had 3.0 ± 1.9 tumors (a greater reduction than in F1 hybrids with either AKR or MA); the *Min*/+ BC1 progeny had 14.4 ± 11.9 tumors; 87% of the total variance was attributable to genetic variance; and Wright's formula gave an estimate of 2.1 apparent factors segregating (Tables 1 and 2). To test for an effect in the region of *Mom-1*, we genotyped 142 progeny with the flanking markers *D4Mit16* and *D4Mit54*. Quantitative linkage analysis indicated the

presence of a QTL causing a reduction of about five tumors in heterozygotes and explaining 25% of the total phenotypic variance. The LOD score for the locus was 2.5 (Figure 2), which is below the recommended statistical threshold for whole genomic search but is highly significant when testing an a priori hypothesis about an individual locus ($p < .005$). This strongly suggests that the CAST strain also carries a dominantly acting modifier in the region, presumably an allele of *Mom-1*. In addition, we genotyped 46 backcross progeny for 70 SSLPs across the genome, but no additional modifier loci were identified.

Although the *Mom-1* region shows evidence for a QTL affecting tumor number, the quantitative effect is significantly different from the effects seen in the AKR and MA backcrosses ($p < .01$). Given the considerable evolutionary distance between CAST and laboratory mice, it is perhaps not surprising that the quantitative effect attributable to *Mom-1* is different. In principle, it is possible that the *Mom-1*^{CAST} allele is functionally distinct from the AKR or MA alleles (i.e., is a weaker suppressor of *Min*-induced tumors); the *Mom-1*^{CAST} allele is functionally equivalent but its effect is modified by other factors in the CAST background; or the CAST effect is due to a different locus in the *Mom-1* region.

Tumor Phenotype Can Be Fully Suppressed by Genetic Background

After examining the backcross to the more susceptible B6 strain, we were interested in the reciprocal backcross to the more resistant strain, AKR. Would such backcross animals show an even lower number of tumors than seen in the F1 progeny? Would homozygosity for the resistant allele of *Mom-1* have a stronger protective effect than heterozygosity? To address these questions, we studied 57 *Min*/+ progeny from the reciprocal backcross (AKR × B6-Min) × AKR. The *Min*/+ progeny were identified by DNA typing (see Experimental Procedures) and subsequently scored for tumor number. There was a striking reduction in average tumor number in these *Min*/+ backcross progeny (1.7 ± 1.7) compared with the F1 mice (5.8 ± 4.3) (Table 1; $p < 0.0001$ with either tumor number or transformed tumor number). Remarkably, 19 of 57 *Min*/+ BC1 progeny were completely free of intestinal tumors in the regions scored. Genetic background thus can completely eliminate the tumorigenic effect of *Min*, at least in a proportion of the animals and in the intestinal regions examined. (As described in Experimental Procedures, our standard scoring is based on examining about half of the length of the small intestine and the entire length of the colon. Further investigation of strongly suppressing genetic backgrounds will require scoring of the entire intestine.)

To assess the effect of homozygosity for the AKR allele of *Mom-1* on tumor multiplicity in this backcross, we genotyped the 57 *Min*/+ backcross progeny for genetic markers *D4Mit12* and *D4Mit13* flanking *Mom-1* and inferred the *Mom-1* genotype in the 40 progeny that were nonrecombinant. The number of tumors was somewhat lower in *Mom-1* homozygotes (1.16 ± 1.5, $n = 20$) than in *Mom-1* heterozygotes (1.80 ± 1.6, $n = 20$), but the difference falls just short of statistical significance (one-sided t test, $p = 0.08$; Wilcoxon rank-sum test, $p = 0.05$). These data hint that two copies of *Mom-1*^{AKR} may confer additional reduction of tumor multiplicity; this warrants further study.

In any case, it is clear that the *Mom-1* genotype cannot fully explain the suppression seen in this backcross. In particular, some *Mom-1* heterozygotes (*Mom-1*^{AKR}/*Mom-1*^{B6}) in the backcross are tumor free, yet no tumor-free F1 animals (also *Mom-1*^{AKR}/*Mom-1*^{B6}) have been observed. To search for additional modifier genes, we genotyped the backcross progeny for 40 additional SSLPs distributed across the genome but found no significant evidence for additional QTLs. This is not particularly surprising: in view of the extremely low mean and standard deviation of tumor number in the cross, there is relatively little statistical power to detect QTLs.

Discussion

A powerful approach to studying the function of a gene is to identify other loci that can modify its mutant phenotype. Such modifier loci often shed light on novel interactions, ranging from direct protein–protein contact, to joint participation in a pathway, to indirect signaling between distant organs. In bacteria, yeast, nematodes, and flies, the isolation of modifier alleles, including second-site suppressors and enhancers, is a powerful aspect of any genetic analysis. However, analysis of modifier loci has rarely been employed in mammals, because large-scale mutagenesis to isolate modifiers is not practical and genetic analysis of naturally occuring modifier systems has been hampered by the fact that such natural variation is often polygenic. Fortunately, with the advent of dense genetic linkage maps, genetic dissection of polygenic traits is becoming practical (Lander and Botstein, 1989) and offers a potentially powerful way to study physiological traits such as susceptibility to cancer, hypertension, atherosclerosis, and diabetes, for which considerable natural variation is known to exist.

Genetic Identification of *Mom-1*, a Major Suppressor of *Min*-Induced Neoplasia

The *Apc* gene is a particularly interesting locus for which to identify genetic modifiers, since human familial colon cancer kindreds carrying *APC* mutations can show substantial phenotypic variability. *Min*/+ mice show variability in tumor number, in regional distribution between small and large intestine, and in mammary involvement.

Starting with the *Min* mutation on the relatively sensitive B6 genetic background, we examined F1 hybrids with other mouse strains in order to screen for modifier alleles that could act in a dominant fashion to confer partial resistance to *Min*-induced tumorigenesis. Hybrids with three different mouse strains, AKR, MA, and CAST, showed evidence of significant dominant reduction of tumor multiplicity, and backcrosses to the B6 strain showed a phenotypic distribution consistent with the segregation of a relatively small number of major modifier loci (Moser et al., 1992; Table 2).

By studying the inheritance of genetic markers throughout the genome, we found strong evidence (LOD score > 14) in two intraspecific backcrosses (AKR and MA) for a major modifier locus on distal mouse chromosome 4, which we have called *Mom-1*. Animals heterozygous for *Mom-1* show a reduction of about 12 tumors, with no statistically significant difference between the two crosses. The differences in the proportion of variance explained and in the LOD scores between the two crosses reflect effects of other weaker modifier loci. Although qualitative modifier loci have long been known in the mouse, this study identifies a quantitative modifier locus affecting a monogenic mouse model of human disease.

A third backcross involving the different subspecies M. m. castaneus also showed evidence for an effect of *Mom-1*, but the magnitude of the effect was significantly smaller. This is perhaps not surprising in view of the considerable evolutionary distance between the CAST and laboratory mice.

Mom-1 is not the only genetic factor influencing *Min*-induced tumorigenesis. This is evident from the fact that animals with the genotype $Mom\text{-}1^{AKR}/Mom\text{-}1^{B6}$ in the (AKR × B6-Min)F1, the (AKR × B6-Min) × B6 BC1 progeny, and the (AKR × B6-Min) × AKR BC1 progeny show different average tumor incidences. In addition, *Mom-1* explains only about 50% of the genetic variance in the intraspecific backcrosses. There must thus be further modifier loci with weaker effects, falling below the threshold for detection. In particular, QTLs causing a reduction of fewer than about seven tumors would not be detected in these crosses (Lander and Botstein, 1989), partly because the strong effect of *Mom-1* obscures the ability to detect weaker effects. More generally, it is unlikely that all quantitative modifier loci segregating in a cross can be found for any trait.

To study the effects of *Mom-1* more precisely and to search for other modifier loci, it would be useful to construct a congenic strain, B6.Mom-1^{AKR}, carrying a small genetic region around a resistance allele of *Mom-1* in an otherwise sensitive B6 background. Crosses between B6.Mom-1^{AKR} and B6 (with *Min* carried by one of the parents) could be used for examining the effects of *Mom-1*, since the effect of other modifiers would be eliminated. Such crosses also would allow finer localization and positional cloning of *Mom-1* (Jacob et al., 1991); the current localization of *Mom-1* to a region of about 10 cM is close to the maximal resolution that can be obtained from crosses of reasonable size, in the presence of other segregating modifiers. Crosses between B6.Mom-1^{AKR} and AKR (with *Min* carried by the former parent) could be used to map other, weaker modifiers, since the effect of *Mom-1* would be eliminated.

Finally, it is striking that the effects of *Min* can be almost entirely eliminated in the backcross to the resistant AKR strain: the mean tumor number is only 1.7, with one-third of the progeny tumor free in the regions examined. The results hint that homozygosity for *Mom-1* accounts for some of this reduction, but also point to the existence of further modifiers. It will be interesting to determine whether further generations of backcrosses to AKR would completely eliminate tumors in all *Min*/+ progeny or whether *Min* would continue to have some non-zero penetrance even when fully introgressed onto the AKR genetic background.

Is *Mom-1* a Tumor Suppressor Gene?

Modifier genes identified by their ability to reduce the incidence of *Min*-induced tumors in vivo could, in principle, act in any of a variety of ways. In cancer genetics, the term "tumor suppressor gene" is reserved for genes for which loss of function contributes to the neoplastic transformation of one or more cell lineages. By this definition, *Mom-1* need not be a tumor suppressor gene, notwithstanding the fact that it causes suppression of tumors at the level of the whole organism. Indeed, *Mom-1* action could involve factors either extrinsic or intrinsic to the tumor cell lineage (Heston and Dunn, 1951; Shapiro and Kirschbaum, 1951; Kemp et al., 1989).

Actions extrinsic to the tumor cell lineage are illustrated by male-dependent hepatic carcinogenesis. Male mice of some inbred strains show greatly increased susceptibility to chemically induced liver tumors compared with female mice. *Tfm* mutations (in the androgen receptor) suppress this susceptibility at the level of the whole organism, but studies using mosaic female mice heterozygous for the X-linked *Tfm* mutation show that tumor development does not depend on the *Tfm* genotype of the tumor cell lineage, indicating that androgen-mediated enhancement involves action extrinsic to the tumor lineage (Kemp et al., 1989). A similar approach could be used to test in chimeras whether the interaction between *Min* and *Mom-1* is extrinsic or intrinsic to the tumor cell lineage.

Actions intrinsic to tumor cell lineage might involve mutational inactivation of $Mom\text{-}1^{AKR}$ (i.e., the "tumor suppressor" paradigm, which could be tested by testing for loss of heterozygosity in [AKR × B6-Min]F1 animals) or different allelic effects on an important cell function in tumorigenesis (fidelity of DNA replication, speed of cell cycle, formation of endogenous carcinogens) without mutational inactivation being required for tumor development.

It is interesting that the *Mom-1* region of the mouse genome is syntenic with the human chromosomal segment 1p35-36, a region that shows frequent somatic loss in a variety of human tumors, including colon cancer (Matthew et al., 1987; Bale et al., 1989; Leister et al., 1990). Although this might be a coincidence, it is tempting to speculate that the mouse *Mom-1* gene involved in inherited variation in tumor number and the human gene in 1p35-36 responsible for somatic progression of tumors might be evolutionary homologs. Potential candidate genes include the p58-GTA protein kinase (Eipers et al., 1991) and the helix-loop-helix protein *heir-1* (Ellmeier et al., 1992).

Conclusion

Genetic background can have a profound effect on the mouse mutations that serve as models of human disease (Smithies, 1993; Shedlovsky et al., 1993). Indeed, if the *Min* mutation had arisen on an AKR genetic background, we would probably not have detected the mutant phenotype. Smithies (1993) has argued that one can use such genetic background effects to "fine-tune the phenotype."

The feasibility of this suggestion depends on the ability to map the salient modifier system so that the fine-tuned strain can be propagated efficiently. Here, we report the genetic mapping of a quantitative modifier affecting a mouse model of human disease.

The search for "disease" loci can be complemented by the search for modifier loci. With an appropriate mouse model for a familial disease syndrome, one can scan the gene pool of the mouse for modifiers. In general, it should be much easier to obtain statistically significant evidence for modifier loci in mouse crosses than in human disease kindreds. Given a candidate region identified in the mouse, one can examine the corresponding region in the human to test for significant association with a severity index of the familial disease in individual human kindreds.

Experimental Procedures

Mouse Breeding

All mice were bred at the McArdle Laboratory for Cancer Research of the University of Wisconsin. The *Min* mutation was isolated on an (AKR × B6)F1 background during an ethylnitrosourea germline mutagenesis experiment (Moser et al., 1990). The Min pedigree has been maintained by backcrossing *Min*/+ males to B6 females; the *Min* mutation causes a recessive lethal phenotype and thus is maintained in a heterozygous condition. Parents for these experiments were from generations 4 through 16 (N4–N16) of this backcross program; even at N4, the strain carried the B6 rather than the AKR allele at all polymorphic loci tested, suggesting that the vast majority of the genome is derived from B6. Accordingly, we refer to the strain as B6-Min.

F1 animals were produced by mating B6 *Min*/+ males (B6-Min; N4–N16) to AKR/J (AKR), CAST/EiJ (CAST), or MA/MyJ (MA) females. The backcross progeny were then produced by crossing *Min*/+ F1 animals with B6 or AKR mice. The backcrosses to B6 were performed in both parental orientations. The genotype of the F1 parent at the *Apc* locus (i.e., *Min*/+ or +/+) was not known at the time of mating, but was determined in retrospect by progeny testing (i.e., observing whether they transmitted the Min phenotype to their offspring) and by inspection of the intestines at sacrifice. The genotype was subsequently confirmed by DNA typing using allele-specific hybridization for the *Min* mutation (Su et al., 1992). The genotypes of the backcross and intercross progeny were determined either by allele-specific hybridization or by a direct polymerase chain reaction (PCR) assay (see below).

Scoring of Tumors

Animals were sacrificed by CO_2 asphyxiation when moribund or at 300 days of age. The intestinal tract from duodenum to colon was removed. Sections of 4 cm in length from duodenum, jejunum, and ileum were harvested, opened along the longitudinal axis, and washed in phosphate-buffered saline (Moser et al., 1990). The entire colon was treated in an identical manner. All tumors in each section of the intestines were counted under a dissecting microscope at 5× magnification. The smallest tumors that could be counted were approximately 0.5 mm in diameter (Moser et al., 1992). Errors are most likely for the highest tumor numbers, owing to the obscuring of small tumors. All animals were scored by a single investigator (A. R. M.) to minimize variation due to the observer. Animals were scored without prior knowledge of genotype.

Genotype Analysis

Some animals were genotyped prior to sacrifice, with DNA prepared from peripheral blood by a modification of a protocol of Phillips and Nadeau (1984). Blood (50 μl) was collected in 200 μl of solution A (29 mM Tris [pH 7.4], 10 mM NaCl, 3 mM $MgCl_2$) in an Eppendorf tube and mixed by inversion; 200 μl of 5% sucrose (w/v), 4% NP-40 (v/v) in solution A was added to each tube, and the contents were mixed by inversion until clear. Tubes were then centrifuged for 3 min at 10,000 rpm in a microfuge, the supernatant was discarded, and the pellet was resuspended in 100 μl of deionized H_2O. Samples were vortexed and then frozen. Just prior to use, the samples were boiled for 3 min. For most PCR assays, 10 μl of the sample was used. Upon sacrificing, tissues were harvested from all animals and DNA was prepared by a modification of the method of Blin and Stafford (1976).

The *Min* mutation was identified by allele-specific hybridization (Su et al., 1992) or by allele-specific PCR. The allele-specific PCR assay used the primer MAPC MT (5′-TGAGAAAGACAGAAGTTA-3′), whose 3′-most nucleotide is complementary to the *Min* nonsense mutation at nucleotide 2549 of *Apc*, and the primer MAPC 15 (5′-TTCCACTTTGGCATAAGGC-3′). The MAPC MT primer is efficiently extended when annealed to the *Min* allele of *Apc*, but extension does not occur when annealed to the wild-type allele because of the 3′ A:A mismatch (Sommer et al., 1992). A PCR product of 327 bp is generated in the presence of the *Min* allele, but no product is generated in its absence. As an internal positive control for the PCR, a third primer, MAPC 9 (5′-GCCATCCCTTCACGTTAG-3′), was included in the reaction. MAPC 9 and MAPC 15 together generate a 618 bp product surrounding the *Min* nonsense mutation (Su et al., 1992); this 618 bp product is generated in +/+ and *Min*/+ animals. The PCR was carried out as follows. Genomic DNA (100 ng) was amplified in a 25 μl reaction containing 4 μM MAPC MT, 1 μM MAPC 15, 0.02 μM MAPC 9, 500 μM (each) dCTP, dGTP, dTTP, and dATP, 3.5 mM $MgCl_2$, 10 mM Tris–HCl (pH 9.0 at 25°C), 50 mM KCl, 0.1% Triton X-100, and 1.25 U of Taq polymerase (Promega). All reactions were overlaid with 30 μl of mineral oil (Sigma). For reactions containing DNA isolated from tissues, amplification conditions with a Coy Thermal Cycler or an MJ Research Thermal Cycler PTC-100 fitted for a 96-well microtiter plate were 30 cycles at 94°C for 1 min, 56°C for 2 min, and 72°C for 3 min, followed by a final extension at 72°C for 10 min. For the amplification of DNA from blood, 10 μl of the DNA solution was used per reaction. The amplification conditions were the same as listed above when done with the Coy Thermal Cycler. With the MJ Research Thermal Cycler, an annealing temperature of 55°C was necessary for amplification of blood DNA. The PCR products were visualized by electrophoresis through 3% agarose (Sea-Kem), followed by staining with ethidium bromide.

The methods and SSLP markers used for genotype analysis are essentially as described previously by Dietrich et al. (1992, 1993). *Mom-1* was localized relative to the mouse gene map by genotyping 46 progeny from a (B6 × SPRET) F1 × B6 interspecific backcross (Copeland and Jenkins, 1991).

Estimate of Number of Loci Controlling Tumor Number

The apparent (or effective) number n of QTLs controlling tumor number in a backcross was estimated by the classical formula of Wright (1968):

$$n = (\mu_{F1} - \mu_P)^2/4\sigma^2_G,$$

where μ_P and μ_{F1} are the mean phenotypes of the recurrent backcross parent and the F1 progeny, respectively, and σ^2_G is the genetic variance of the phenotype in the backcross. The genetic variance was estimated as

$$\sigma^2_G = (\sigma^2_{BC} - \sigma^2_E),$$

where σ^2_{BC} is the total phenotypic variance and σ^2_E is the environmental component of the phenotypic variance in the backcross. The environmental component of the variance was inferred from the parental generation: $\sigma^2_E = {}^2_P$. The proportion of the total phenotypic variance due to all genetic factors is estimated as $1 - (\sigma^2_E/\sigma^2_{BC})$. Wright's formula is based on certain simplifying assumptions: namely, that each animal's phenotype results from addition of independent contributions of n unlinked genetic factors, together with random environmental noise with a normal distribution having constant variance σ^2_E and that the resistant strain carries all "resistance" alleles and the susceptible strain carries all "susceptibility" alleles at each of the n loci. The quantity n can be interpreted as the estimated number of unlinked, additive modifier genes with equal phenotypic effects necessary to explain the phenotypic variation in the cross. If the actual modifier genes have unequal phenotypic effects, as will likely be true in practice, the quantity will underestimate the number of genes that play a role (and, thus, it might be better named the minimum number of genetic factors). Nonetheless, the estimate can provide a good indication of the approximate

number of major genes controlling the trait (Lander and Botstein, 1989).

The assumptions underlying Wright's formula imply that the phenotype of the backcross progeny should be the average of the phenotypes in the recurrent backcross parent and the F1 progeny (i.e., $\mu_{BC} = [\mu_P + \mu_{F1}]/2$) and that the distributions in the recurrent backcross parent and F1 progeny should be approximately normal with equal variances (i.e., $\sigma^2_P = \sigma^2_{F1}$). If these conditions are not approximately satisfied, it is necessary to apply a transformation to the data (Wright, 1968). In fact, the tumor multiplicity failed to meet these criteria: it was roughly distributed as a Poisson variable (as might be expected for a count statistic based on rare events occurring in independent cell lineages). The data were transformed by using a square-root transformation, which has the property of stabilizing the variance of a Poisson distribution (Kendall et al., 1983) and has previously been applied to the genetic analysis of tumor multiplicity (Bloom and Falconer, 1964). The transformed data fit the assumptions of additivity among loci, normality, and equal variance reasonably well, and all analyses were performed on this "transformed phenotype."

Linkage Analysis and QTL Mapping

Linkage analysis of the SSLPs relative to each other was performed using the MAPMAKER computer package, as described (Lander et al., 1987; Dietrich et al., 1992). QTL analysis was performed using the MAPMAKER-QTL computer package (Lander and Botstein, 1989; Paterson et al., 1988) as described previously (Paterson et al., 1991).

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