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Identification of *Mom7*, a Novel Modifier of *Apc*$^{Min/+}$ on Mouse Chromosome 18

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ABSTRACT

The *Apc*^{Min} mouse model of colorectal cancer provides a discrete, quantitative measurement of tumor multiplicity, allowing for robust quantitative trait locus analysis. This advantage has previously been used to uncover polymorphic modifiers of the Min phenotype: *Mom1*, which is partly explained by *Pla2g2a*; *Mom2*, a spontaneous mutant modifier; and *Mom3*, which was discovered in an outbred cross. Here, we describe the localization of a novel modifier, *Mom7*, to the pericentromeric region of chromosome 18. *Mom7* was mapped in crosses involving four inbred strains: C57BL/6J (B6), BTBR/Pas (BTBR), AKR/J (AKR), and A/J. There are at least two distinct alleles of *Mom7*: the recessive, enhancing BTBR, AKR, and A/J alleles and the dominant, suppressive B6 allele. Homozygosity for the enhancing alleles increases tumor number by approximately threefold in the small intestine on both inbred and F_1 backgrounds. Congenic line analysis has narrowed the *Mom7* region to within 7.4 Mb of the centromere, 28 Mb proximal to *Apc*. Analysis of SNP data from various genotyping projects suggests that the region could be as small as 4.4 Mb and that there may be five or more alleles of *Mom7* segregating among the many strains of inbred mice. This has implications for experiments involving *Apc*^{Min} and comparisons between different or mixed genetic backgrounds.

COLORECTAL cancer is the second leading cause of cancer death in the United States and the third in the world (Jemal *et al.* 2006). The multiple intestinal neoplasia (Min) mouse model has provided a means by which modifiers of the colorectal tumor phenotype can be readily identified. This line of mice harbors a truncating mutation at codon 850 of the *adenomatous polyposis coli* (*Apc*) gene, which is mutated in ~80% of all human colorectal tumors (Fearnhead *et al.* 2001). The discrete, countable nature of *Apc*^{Min} tumors has made the mouse a tractable model for quantitative studies.

Quantitative trait locus (QTL) analysis allows searches for polymorphic modifiers in either a backcross or an intercross population derived from different inbred strains. Identification of the first discovered modifier of *Apc*^{Min}, *Mom1*, included the use of three different strains (Dietrich *et al.* 1993). *Mom1* has two major alleles, resistant (*Mom1*^{R}) and sensitive (*Mom1*^{S}), and has been localized to a 5-cM region on chromosome 4. Cormier *et al.* (1997, 2000) showed that the gene *Pla2g2a*, in addition to a second locus distal to *D4Mit64*, is responsible for the *Mom1* effect. *Mom2*, discovered by Silverman *et al.* (2002), maps distal to the *Apc* locus on chromosome 18. Although the region has been narrowed to 7 cM, the underlying gene(s) remain to be identified (Silverman *et al.* 2003). In addition, Haines *et al.* (2005) identified a second polymorphic modifier locus linked to *Apc*, *Mom3*, the phenotype of which is affected by pregnancy (Suraweera *et al.* 2006). *Mom3* arose from an outbred stock, and the lack of known polymorphic markers has prevented its resolution beyond 25 cM.

Numerous other genetic modifiers have been described upon breeding known mutations onto the *Apc*$^{Min/+}$ background. A subset of modifiers has been shown to affect the pathway leading to loss of heterozygosity (LOH) at the *Apc* locus, which is believed to be the initiating process for tumorigenesis in the intestine. For example, *Blm*-deficient mice have a defect in maintaining genomic stability, including elevated somatic recombination rates that lead to increased tumor multiplicities in *Apc*$^{Min/+}$ mice (Luo *et al.* 2000; Goss *et al.* 2002; Suzuki *et al.* 2006). Haigis and Dove (2003) found that the Robertsonian translocation Rb(7.18)9Lub (Rb9) disrupted the somatic pairing of chromosome 18 homologs, thus decreasing the probability for somatic recombination and tumor multiplicity. We describe here the localization of a new Modifier of Min, *Mom7*, to within the first 7.4 Mb of chromosome 18. Noting its centromere-proximal position relative to *Apc*, we speculate that the modifier may directly regulate the loss of heterozygosity of distal elements.

MATERIALS AND METHODS

Mice: The mice used to generate segregating populations were C57BL/6J, AKR/J, and C57BL/6-Chr 18$^{A/J}$ (B6.18$^{A/J}$)

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consomics (The Jackson Laboratory, Bar Harbor, ME) and BTBR/Pas (Pasteur Institute, Paris). Congenic lines bred in our laboratory are described in the text. These strain names have been registered with the Mouse Genomic Nomenclature Committee: B6.AKR-*Mom7*$^{AKR/J}$ (B6.*Mom7*^{AKR}), AKR.B6-*Mom7*$^{C57BL/6J}$ (AKR.*Mom7*^{B6}), AKR.B6-(*D4Mit13-D4Mit54*) (abbreviated as AKR.*Mom1*^{S}), BTBRPas.B6-*Mom7*$^{C57BL/6}$ (BTBR.*Mom7*^{B6}), and C57BL/6-Chr 18$^{BTBR/Pas}$ (B6.18^{BTBR}). Mice were housed in a standard facility with automatic watering and access to Purina 5020 chow (Purina, St. Louis). B6.*Apc*$^{Min/+}$ and BTBR.*Apc*$^{Min/+}$ mice were sacrificed between 2 and 4 months of age. Since AKR.*Apc*$^{Min/+}$ mice showed no significant difference in tumor multiplicity between 6 and 11 months of age (data not shown), tumor counts over this age range were combined. Intestinal preparation and tumor counts were performed as previously described (Haigis and Dove 2003).

Statistical analysis: Pairwise *P*-values for the B6.*Mom7* congenic strains were determined by a Wilcoxon rank-sum test and subjected to a Bonferroni correction for multiple comparisons (see supplemental Table 1 at http://www.genetics.org/supplemental/). LOD scores and maps were obtained using the Mapmaker3 program (Lander *et al.* 1987; Lincoln *et al.* 1992).

LOH analysis: DNA extraction from tumors and Pyrosequencing assays (Biotage, Upsala, Sweden) were performed as described previously (Amos-Landgraf *et al.* 2007). Tumors taken from AKR mice were all >2 mm in diameter. Primers used were forward (TTT TGA CGC CAA TCG ACA TG) and reverse (biotinylated) (GAT GGT AAG CAC TGA GGC CAA TA); sequencing (CGT TCT GAG AAA GAC AGA AG). An LOH/maintenance of heterozygosity (MOH) cutoff value of 31.4% was determined by adapting the normal distribution mixture technique previously described (Shoemaker *et al.* 1998): we used the method of maximum likelihood to fit a mixture of two normal curves to data on single nucleotide polymorphism (SNP) signal intensity. Tumor data were considered to arise from either an LOH distribution normal component or an MOH normal distribution component, according to some mixing probability; control data, normal tissue from a heterozygous animal, consists of known MOH components. Using the fitted mixture model, a critical intensity value, c, was determined so that Prob[intensity $> c \mid$ LOH] $= 0.05$. $N = 348$ tumor values and $n = 22$ control values were used for maximum-likelihood calculations. In the estimated normal mixture, the LOH component had a mean of 17.3 and a standard deviation of 6.5; the MOH component had a mean of 43.2 and a standard deviation of 5.1. From this, the critical signal value was $c = 31.4$. Modes of the likelihood surface at the boundary of the parameter space were avoided. Computations were done in R software (R Development Core Team 2005).

***In silico* SNP analysis and original markers:** *In silico* SNP data from the recent Perlegen strain resequencing project covering 16 inbred strains were obtained from the Mouse Phenome Database (http://phenome.jax.org/pub-cgi/phenome/mpdcgi?rtn=snpsdvsd/door). The first 3 Mb of chromosome 18 is an unassembled centromeric sequence with no SNP data; the next 0.5 Mb (positions 3.0–3.5 Mb on MGSC Build 36) were also excluded due to a high degree of homology to regions on chromosomes 4, 6, and 16. Therefore, only the region between 3.5 and 7.4 Mb was considered, encompassing 2171 informative SNPs (~1 SNP/2 kb). Novel primers were designed to amplify SSLPs using sequences found through the Tandem Repeat Finder program (Benson 1999).

RESULTS

An AKR.*Mom1*$^{S/S}$ congenic line demonstrates the presence of other AKR modifiers: It was previously shown that the AKR genetic background strongly reduces tumor multiplicity compared with the B6 background (Shoemaker *et al.* 1998). This effect is due in part to the semidominant resistant *Mom1*^{R} allele carried by AKR. Accordingly, the Mom1 effect was removed by breeding a line of AKR mice congenic for the sensitive *Mom1*^{S} allele from B6. Marker-assisted selection was employed so that progeny are considered congenic by the N4 generation (Hospital *et al.* 1992). Genome scans were performed on mice from N2–N6, selecting for those with the highest percentage of AKR homozygosity for 55 markers, with at least two markers/chromosome. At N4, 53/55 (94.5%) markers were homozygous for AKR, not including the regions surrounding the B6-derived *Mom1*^{S} allele between *D4Mit13* and *D4Mit54* and the B6-derived *Apc*^{Min} allele between *D18Mit111* and *D18Mit24*. All data presented here involved mice from the N6 to N9 generations to ensure congenic status. AKR.*Apc*$^{Min/+}$; *Mom1*$^{S/S}$ mice developed an average of 16 intestinal tumors, 1.5-fold more than *Mom1*$^{R/S}$ and 4-fold more than *Mom1*$^{R/R}$ mice (Table 1A, *Mom7* congenic lines), consistent with the effects of *Mom1* found in previous studies with B6.*Mom1*^{R} congenic mice (Gould *et al.* 1996a). Therefore, additional modifiers must be present in the AKR background, since tumor counts among AKR.*Apc*$^{Min/+}$;*Mom1*$^{S/S}$ mice (16) were sevenfold less than among B6-*Apc*$^{Min/+}$;*Mom1*$^{S/S}$ mice (118; Figure 1A).

Identification of a modifier of intestinal tumor multiplicity near the chromosome 18 centromere in crosses between B6 and AKR: To identify such additional polymorphic modifiers between AKR and B6, (B6 × AKR) *Mom1*$^{S/S}$ F_1 progeny were backcrossed to AKR.*Apc*$^{Min/+}$; *Mom1*$^{S/S}$ mice to generate an N2 population. The genomes of 162 phenotyped N2 animals were scanned with 71 markers, with a total of 6598 informative genotypes (see supplemental Table 2 at http://www.genetics.org/supplemental/). A LOD score of 21 (supplemental Figure 1 at http://www.genetics.org/supplemental/) corresponding to *D18Wis1* indicated the presence of a strong modifier, which we have designated "*Mom7*," linked to the position at 3.7 Mb (1 cM) as measured on MGSC Build 36. Although the LOD interval is maximal between *D18Wis1* and *D18Mit64*, a more centromeric location cannot be excluded, owing to the absence of more proximal markers. Strikingly, the *D18Wis1*$^{AKR/AKR}$ class developed 3-fold more small intestinal tumors than the *D18Wis1*$^{B6/AKR}$ class (genomewide $P \ll 0.01$; Table 1A, Mapping crosses). In addition, 90 F_2 mice were generated by intercrossing *Mom1*$^{S/S}$ (B6 × AKR) F_1 progeny. A complete genome scan was not carried out on this population; however, we found that tumor multiplicities *vs.* genotypes at *D18Wis1* confirmed this position: the *D18Wis1*$^{AKR/AKR}$ class developed 1.8-fold more tumors than the *D18Wis1*$^{B6/AKR}$ class (Table 1A, Mapping crosses). We note that the AKR grandparent always transmitted the *Apc*^{Min} allele, which is linked to *D18Wis1*^{AKR}. Therefore, it was rare to obtain the *D18Wis1*$^{B6/B6}$;*Apc*$^{Min/+}$ class;

TABLE 1

Effect of the *Mom7* alleles on *Apc*$^{Min/+}$ small intestinal tumor counts

Genetic background	Tumor count [mean ± SD (*N*)] *Mom7*$^{B6/AKR}$	Tumor count [mean ± SD (*N*)] *Mom7*$^{AKR/AKR}$	Fold effect of *Mom7*$^{AKR/AKR}$
	A. Effect of the AKR allele		
Mapping crosses			
(B6 × AKR) × AKR *Mom1*$^{S/S}$ N2	17 ± 10 (75)	50 ± 22 (87)	3.0*
(B6 × AKR) × (B6 × AKR) *Mom1*$^{S/S}$ F_2	53 ± 35 (51)	97 ± 48 (39)	1.8*
Mom7 congenic lines			
AKR.*Mom1*$^{S/S}$	ND	16 ± 7.5 (9)	ND
AKR.*Mom1*$^{R/S}$	5.4 ± 3.4 (18)	10 ± 5.8 (23)	1.9*
AKR.*Mom1*$^{R/R}$	1.0 ± 0.8 (8)	3.5 ± 2.6 (42)	3.5*
(B6 × AKR).*Mom1*$^{S/S}$ F_1	37 ± 10 (10)	116 ± 15 (4)	3.1*
(B6 × AKR).*Mom1*$^{R/S}$ F_1	19 ± 10 (15)	69 ± 10 (4)	3.6*
Genetic background	Tumor count [mean ± SD (*N*)] *Mom7*$^{B6/BTBR}$	Tumor count [mean ± SD (*N*)] *Mom7*$^{BTBR/BTBR}$	Fold effect of *Mom7*$^{BTBR/BTBR}$
	B. Effect of the BTBR allele		
Mapping cross			
(B6 × BTBR) × BTBR N2	175 ± 74 (12)	557 ± 165 (28)	3.2*
Congenic line			
BTBR.*Apc*$^{Min/+}$	307 ± 116 (53)	625 ± 104 (75)	2.0*

The *Mom7* allele was determined using marker *D18Wis1* in the crosses with AKR, and marker *D18Mit110* in the crosses with BTBR. *$P < 0.01$, Wilcoxon rank-sum test; ND, not done.

one such animal was obtained but was omitted from statistical analysis. Thus, the AKR allele of this modifier appears to be a recessive enhancer of tumor multiplicity.

To test whether the AKR allele of this modifier region alone is sufficient to produce the modified phenotype, we bred a reciprocal pair of congenic lines in which B6 mice carried a proximal chromosome 18 segment from AKR, and AKR mice carried the same region from B6. The interval selected extends from *D18Wis1* to *Apc*. We were aware that, if the modifier were proximal to *D18Wis1*, it could be lost by recombination. Therefore, we always confirmed its presence by phenotyping candidate congenic carriers.

After at least eight generations of backcrossing, these heterozygous *Mom7* congenics carrying either *Apc*$^{Min/+}$ or *Apc*$^{+/+}$ were intercrossed. On the B6 background, all three *Mom7* genotypes were analyzed. Homozygotes for the AKR *Mom7* allele developed an average of 322 ± 31 small intestinal tumors (Figure 1E), 2.5-fold more than either *Mom7* heterozygotes (Figure 1, G and H)

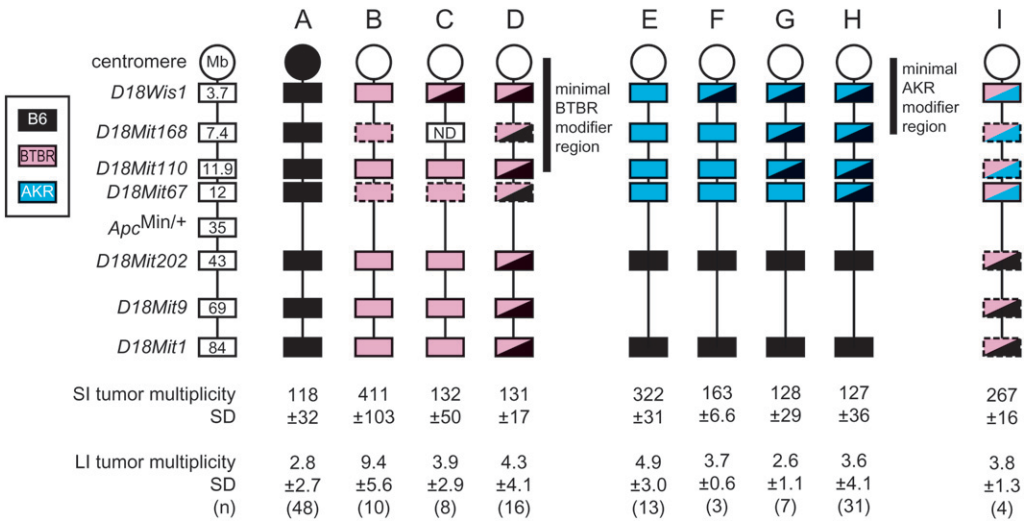

FIGURE 1.—Mapping of the *Mom7* modifier in B6 *Apc*$^{Min/+}$ mice. Genotypes on chromosome 18 are shown; all other chromosomes are homozygous B6. Boxes outlined by dashes represent inferred genotypes based on either flanking markers or obligate genotypes. The congenic sublines C and F define a minimal region for the BTBR and AKR alleles of the *Mom7* locus, respectively. SI, small intestinal; LI, large intestinal. For line I, *D18Mit19* was used as a surrogate for *D18Wis1*. All mice were sacrificed between 2 and 4 months of age. ND, not done.

or B6-*Apc*$^{Min/+}$ controls (Figure 1A); further, there was no significant difference between the heterozygotes and the controls (supplemental Table 1 at http://www.genetics.org/supplemental/).

On the AKR background, *Mom7*$^{B6/B6}$ animals were not generated; however, *Mom7* AKR homozygotes developed two- to threefold more small intestinal tumors than heterozygotes (Table 1A, *Mom7* congenic lines). This effect is independent of *Mom1*, suggesting that the two modifiers act independently. As reported previously, tumors did not develop in the colon (Shoemaker *et al.* 1998). To determine whether a fully heterozygous genetic background influenced the effects of the modifier and to confirm the lack of interaction between *Mom1* and *Mom7*, (B6 × AKR) *Apc*$^{Min/+}$ F_1 mice with different *Mom1* and *Mom7* genotypes were generated by crossing B6.*Mom7*$^{AKR/B6}$;(*Mom1*$^{S/S}$); *Apc*$^{Min/+}$ congenics to AKR.(*Mom7*$^{AKR/AKR}$);*Mom1*$^{R/S}$ congenics. Again, homozygosity for the AKR allele of *Mom7* enhanced tumor number more than threefold relative to AKR/B6 heterozygosity (Table 1A, *Mom7* congenic lines), and this effect was independent of the *Mom1* genotype. Thus, on three different genetic backgrounds—B6, AKR, and (B6 × AKR) F_1—the AKR allele of *Mom7* enhanced tumor multiplicity approximately more than threefold in a manner fully recessive to the B6 allele and independent of *Mom1* status.

Identification of a modifier of intestinal tumor multiplicity near the chromosome 18 centromere in crosses between B6 and BTBR: It had previously been shown that the inbred BTBR strain, like B6, carries the sensitive *Mom1*^{S} allele. Further, BTBR does not appear to carry other dominant modifiers of Min (Gould *et al.* 1996b). However, after backcrossing the *Apc*$^{Min/+}$ allele for >10 generations onto the BTBR background, a significant enhancing effect was observed: mice became moribund by 60 days of age and were found to have in excess of 600 tumors/animal (Table 1B, Congenic line). To identify the enhancing modifier, we used the QTL strategy described in the AKR analysis. A partial genome scan of DNA from the N2 generation identified a highly significant modifier locus linked to the *D18Mit110* marker (11.9 Mb): *D18Mit110*$^{BTBR/BTBR}$ mice developed 3.2-fold more tumors than *D18Mit110*$^{B6/BTBR}$ mice (Table 1B, Mapping cross).

Using marker-assisted selection, a B6.18$^{BTBR/BTBR}$ consomic line was obtained with marker *D18Wis1* (3.7 Mb) at the most proximal end and marker *D18Mit1* (84 Mb) at the most distal end. Both *Apc*$^{Min/+}$ and *Apc*$^{+/+}$ were bred to produce two lines, each heterozygous for chromosome 18, that could be intercrossed to obtain animals homozygous for all the BTBR alleles in the region, except for the *Apc*^{Min} allele. Animals homozygous for the BTBR alleles developed significantly more tumors in both the small intestine and the colon (Figure 1B) than either heterozygotes or B6-*Apc*$^{Min/+}$ controls (supplemental Table 1 at http://www.genetics.org/supplemental/; Figure 1, A and D; $P < 0.05$ for colon); there was no significant difference between lines A and D. A congenic strain carrying the B6 *Mom7* region on the BTBR background was also bred. Homozygotes for the *Mom7* BTBR alleles developed twofold more tumors than heterozygotes (Table 1B, Congenic line). Thus, on both the B6 and BTBR genetic backgrounds, the BTBR allele of *Mom7* is an enhancer, fully recessive to the B6 allele.

The AKR and BTBR modifiers are not statistically different from one another: Although both the AKR and BTBR modifiers are linked to *D18Wis1*, perhaps they represent separate factors within the *Mom7* region. To further characterize the relationship of these alleles, (BTBR × AKR) *Apc*^{Min};*Mom1*$^{S/S}$ F_1 mice were backcrossed to the AKR (*Mom1*$^{R/R}$) strain. *D18Mit19* (5.0 Mb) was used as a surrogate marker for *D18Wis1*, which is not polymorphic between the two strains. Results from 41 N2 progeny indicated that the tumor multiplicity for *Mom7* heterozygotes (18 ± 17, $n = 4$) is not statistically different from that of AKR homozygotes (27 ± 16, $n = 36$; $P = 0.3$). To confirm this initial result, we crossed B6.*Mom7*^{AKR} congenics to B6.*Mom7*^{BTBR} consomics. These results indicate that the B6.*Mom7*$^{AKR/BTBR}$ progeny (Figure 1I) develop tumor multiplicities greater than B6.*Apc*$^{Min/+}$ controls and not significantly different from AKR or BTBR *Mom7* homozygotes (supplemental Table 1 at http://www.genetics.org/supplemental/). Similarly, lines B and E did not differ significantly from each other (supplemental Table 1 at http://www.genetics.org/supplemental/). Importantly, there is an overlap in tumor distributions for lines B, E, and I (194–541, 279–392, and 232–307, respectively). Thus, it appears that the AKR and BTBR alleles are additive in *trans* and probably represent a single *Mom7* modifier factor.

The major AKR *Mom7* enhancer lies within the proximal 7.4 Mb of chromosome 18: Several recombinant sublines were established. One (Figure 1F) is a B6 subline homozygous for AKR alleles within the *Mom7* region except for loci proximal to *D18Mit168* (7.4 Mb). A second subline (Figure 1G) is homozygous for the *Mom7* AKR alleles except for loci proximal to *D18Mit67* (12 Mb). Tumor counts on the small sample of mice from these sublines are statistically indistinguishable from B6 controls and full heterozygotes (Figure 1, A and H), yet line G is significantly different from B6.*Mom7*$^{AKR/AKR}$ congenics (Figure 1E; supplemental Table 1 at http://www.genetics.org/supplemental/). Although line F is not statistically different from the full congenic line E (supplemental Table 1 at http://www.genetics.org/supplemental/), this is likely owing to the low number of mice assayed for line F. Furthermore, the individual tumor distributions for lines E and F do not overlap (279–392 *vs.* 156–169, respectively), which suggests that they are indeed different. Therefore, the major *Mom7* determinant most likely lies proximal to 7.4 Mb. Similar results were obtained with sublines carrying

TABLE 2

Haplotype block groups between 3.5 and 4.4 Mb seen among the 16 strains in the Perlegen mouse resequencing project

Group 1	Group 2[a]	Group 3	Group 4[b]	Group 5
C57BL/6J	C3H/HeJ	AKR/J	FVB/NJ	CAST/EiJ
NOD/LtJ	BALB/cByJ	A/J	DBA/2J	MOLF/EiJ
KK/HlJ		BTBR T⟨+⟩ tf/J		PWD/PhJ
WSB/EiJ		NZW/LacJ		CZECHII/EiJ[c]
129S1/SvImJ				

Actual data are given in supplemental Table 4 at http://www.genetics.org/supplemental/.

[a] Group 2 shares a haplotype block with group 1 up through position 3827436 and then has a unique sequence beyond (see supplemental Tables 5 and 6 at http://www.genetics.org/supplemental/ for additional evidence and group members).

[b] Group 4 differs from group 3 by only a single SNP (rs13483184) at position 3796540 (see supplemental Tables 5 and 6 at http://www.genetics.org/supplemental/ for additional evidence and group members).

[c] Informative 537 SNPs, most of which are from other sequencing projects.

the BTBR modifier: animals that are homozygous BTBR for the complete chromosome 18 (Figure 1B) develop greater than threefold more tumors than do partial consomics (Figure 1C) that are heterozygous only for loci proximal to *D18Mit110* (11.9 Mb). These proximal heterozygotes are phenotypically the same as heterozygotes for the entire chromosome (Figure 1D). Thus, the positions of both the AKR and BTBR alleles of *Mom7* have been refined to the most pericentromeric region of chromosome 18.

The heterozygous B6.*Mom7*^{AKR} sublines F, G, and H also allowed us to determine whether the *Mom7* effect differs when the B6 allele is in *cis* or in *trans* to *Apc*^{Min}. Lines F and G carry the B6 allele in *cis* to *Apc*^{Min} and the AKR allele is in *trans*, while line H has the opposite orientation. Since no significant difference in multiplicity exists when F and G are combined and compared to H ($P = 0.22$), the B6 *Mom7* allele is dominant in both a *cis* or *trans* configuration to *Apc*^{Min}.

Preliminary data (see supplemental Table 3 at http://www.genetics.org/supplemental/) suggest that a fourth strain, A/J, also carries an enhancing *Mom7* allele. This result is consistent with the identical proximal chromosome 18 haplotype of strains A/J, AKR, and BTBR (see Table 2 and below).

BTBR and AKR share a pericentromeric haplotype distinct from B6: SNP data have become instrumental in reducing critical QTL intervals by identifying the regions most likely to contain relevant polymorphisms (Park and Hunter 2003). An *in silico* analysis was therefore performed on high-density SNP data in the minimal 7.4-Mb *Mom7* region. Only the region between 3.5 and 7.4 Mb was considered (see materials and methods). Setting AKR, B6, and MOLF as reference strains, extensive sequence homology among the 16 strains identified at least five segregating haplotypes (supplemental Table 4 at http://www.genetics.org/supplemental/). In the region between 3.5 and 4.4 Mb, the strains could be classified into five broad groups (Table 2). Importantly, AKR, BTBR, and A/J grouped together and separately from B6 in this region, but not distal to 4.4 Mb, where BTBR and A/J shared a haplotype with B6 (supplemental Table 4 at http://www.genetics.org/supplemental/). We independently verified the haplotype data using seven novel SSLPs (sequences and positions given in Table 3) and *D18Mit19* (5.0 Mb) for B6, AKR, BTBR, and A/J (Table 3). Thus, AKR, BTBR, and A/J share at least a 0.9-Mb haplotype block (positions 3.5–4.4 Mb), which is distinct from that of B6 and which may extend into the centromere.

Cross-referencing the Perlegen SNP data with the 48-strain SNP survey (Pletcher *et al.* 2004), we found that 103 informative SNPs further supported the division between groups 3 and 4 and added 34 more putative members to the five groups (supplemental Table 5 at http://www.genetics.org/supplemental/). A smaller set of 15 informative SNPs from the Broad Institute and Applied Biosystems provided preliminary data on an additional 33 strains (supplemental Table 6 at http://www.genetics.org/supplemental/).

The rate of loss of heterozygosity in *Mom7* congenics: To determine the effects of the *Mom7* locus on LOH at *Apc*, we developed a Pyrosequencing assay to quantify allelic ratios. On the B6 background, the percentage of tumors showing LOH did not differ significantly between any of the five B6.*Mom7* classes carrying B6, AKR, or BTBR alleles ($P > 0.1$, Fisher's exact test; Table 4). However, it is notable that homozygotes for both the AKR and the BTBR *Mom7* alleles showed LOH in every tumor tested.

On the AKR background, *Mom7* homozygous congenics did not differ from heterozygotes in the percentage of LOH tumors (Table 4; compare AKR.*Mom1*$^{R/S}$;*Mom7*$^{AKR/AKR}$ to AKR.*Mom1*$^{R/S}$;*Mom7*$^{B6/AKR}$). By contrast, one or two copies of *Mom1*^{S} on AKR.*Mom7*$^{AKR/AKR}$ significantly increased the percentage of tumors with LOH from 33% (a percentage consistent with Shoemaker *et al.* 1998) to 86 and 70%, respectively ($P < 0.05$;

TABLE 3

Primers used to genotype the first 14 Mb of assembled pericentromeric chromosome 18 and their amplicon lengths

Primer name	Forward primer	Reverse primer	Chromosome 18 position (bp)	AKR	A/J	BTBR	B6
D18Wis2	AAGTCCTGGTCCCCTACCTC	ATCCACTCATGGCACAAACA	3478885	~200–210	~200–210	~200–210	192
D18Wis3	GGCAGGCAAGACTTCAATGT	CTGGAAGCAAGGATGGGTAA	3591908	~194	~194	~194	209
D18Wis1	TAAGGCAGCAGGGAGAGAAA	CCCTGAGACAGGAGAACTGG	3669044	~155	~155	~155	171
D18Wis4	ATTGGGCGACTAGCAAGAGA	AGGACACCCAGGGCTATACA	3670134	~255–260	~255–260	~255–260	249
D18Wis5	TTCCCAAACTACGAAGGTGAA	GAAGGCTCAGGCTCTTCCAT	4185518	~245–250	~245–250	~245–250	234
D18Wis6	AAACCTGCAGAAAGGCTTGA	ATCCTTCCATCTCCCTGTCC	4351585	~235	~235	~235	243
D18Wis7	GGGGGAAAGTATAGCTGAAGG	AGAGTTGGCCCTTTTGCTTTT	4373408	~145	~145	~145	162
D18Mit19	ATTGGGTGTTCAGGTGCAG	ATGCACAATAGCTCATAGCTTCT	4941117	138	158	~150	150

Sequence positions and PCR product sizes for the B6 strain were taken from MGSC Build 36 (http://www.ncbi.nlm.nih.gov). All primers except *D18Mit19* are novel. Product sizes for D18Mit19 were taken from Mouse Genome Informatics (http://www.informatics.jax.org). All other products were sized in our laboratory using 3% agarose gels.

Table 4). However, even two copies of $Mom1^{S}$ did not raise the AKR LOH percentage to the 100% observed in B6.$Apc^{Min/+}$;$Mom7^{AKR/AKR}$ mice ($P < 0.05$).

DISCUSSION

In this study, we identify a novel modifier of the Apc^{Min} tumor phenotype by a classical genetic approach. We used a previously described tumor-resistant mouse strain, AKR (Shoemaker *et al.* 1998), and the BTBR strain, which was found to be tumor sensitive. To our surprise, both the AKR and BTBR strains carry a strong recessive enhancer linked to the *D18Wis1* marker (3.7 Mb) on chromosome 18. We have given one name to this factor: "*Mom7*" (Modifier of Min 7). Congenic and consomic sublines refined the majority of the *Mom7* effect to 7.4 Mb between the centromere and *D18Mit168*. The enhancers were found to have a consistent effect on different inbred backgrounds and to be independent of the *Mom1* modifier effect. Preliminary results indicate that the AKR and BTBR alleles are noncomplementing (Figure 1). Possible interpretations of these observations are discussed below.

In principle, a modifier can control tumor viability, growth, or initiation. For example, a modifier linked to Apc^{Min} could reduce tumor multiplicity by inducing cellular lethality after the lethal allele undergoes somatic homozygosis. *Mom1* and *Dnmt* have been shown to control the net growth rate of tumors (Cormier and Dove 2000). The Rb9 modifier prevents tumor initiation by disrupting the pairing of chromosome 18 homologs (Haigis and Dove 2003). Which of these modes of action applies to *Mom7*? The cellular lethality model is eliminated since homozygotes for each allele of *Mom7* are viable. Support for the growth model awaits analysis of tumor size, which is currently underway. An effect on tumor initiation is consistent with our results, but requires confirmation at the genomic level.

Classically, the recessive nature of the *Mom7* enhancer alleles would be interpreted as a loss of function of a

TABLE 4

LOH at the *Apc* locus in $Apc^{Min/+}$ B6.*Mom7* and AKR.*Mom1;Mom7* congenic small intestinal tumors

Genetic background	Frequency of tumors showing LOH (%)	No. of mice
B6.$Mom7^{B6/B6}$	77/80 (96)	4
B6.$Mom7^{B6/AKR}$	86/86 (100)	4
B6.$Mom7^{AKR/AKR}$	85/85 (100)	5
B6.$Mom7^{B6/BTBR}$	47/53 (89)	7
B6.$Mom7^{BTBR/BTBR}$	56/56 (100)	7
AKR.$Mom1^{S/S}$;$Mom7^{AKR/AKR}$	33/47 (70)	5
AKR.$Mom1^{R/S}$;$Mom7^{B6/AKR}$	13/16 (81)	3
AKR.$Mom1^{R/S}$;$Mom7^{AKR/AKR}$	18/21 (86)	2
AKR.$Mom1^{R/R}$;$Mom7^{AKR/AKR}$	4/12 (33)	4

trans-acting gene product. Similar effects are seen in knockouts for the Recql4 helicase, the normal function of which is to suppress aneuploidy by maintaining sister-chromatid cohesion (Mann *et al.* 2005). Alternatively, the *Mom7* locus could modulate tumor phenotypes in *cis* by affecting the recombination substrates on chromosome 18 and the consequent rate of LOH at the distal *Apc* locus. In such a *cis*-acting model, the *Mom7* alleles could represent polymorphisms that alter pericentromeric sequences required for the efficiency of somatic recombination hotspots. For example, the ribosomal DNA (rDNA) repeats on centromeric chromosome 18 could be recombinogenic due to the high fidelity and copy number of homologous sequence and to the presence of transcription termination sequences and replication fork barriers (Gerber *et al.* 1997; Lopez-estrano *et al.* 1998). If *Mom7* does act in *cis*, it is important to note that its phenotype differs from that of the Rb9 rearrangement (Haigis and Dove 2003), which has a semidominant effect on tumor multiplicity, and from the action of sequence heterozygosity (Shao *et al.* 2001), which would be predicted to have an overdominant effect. Although our preliminary evidence (Table 4) does not reach statistical significance, it is at least consistent with a direct effect of *Mom7* on the frequency of somatic recombination. A more sensitive assay is required to investigate this hypothesis.

It will be important to determine whether the *Mom7* effect is attributable to one or many genes. At present, the first ~3 Mb of chromosome 18 remain unassembled, owing to the highly repetitive nature of the centromere, which includes the rDNA repeats. Thus, determining the sequence identity of the *Mom7* modifier(s) remains a challenging prospect. Toward this end, more recombinant sublines are being identified and analyzed to further narrow the *Mom7* region before candidate testing. All congenic sublines have been backcrossed >10 generations, providing a genetic resource with which to identify the salient pericentromeric sequence associated with the *Mom7* effect.

Intriguingly, the sequence of the *Mom7* genetic region provides insight into its evolutionary nature. AKR, BTBR, and A/J all share the same pericentromeric haplotype, distinct from B6, providing evidence that the major *Mom7* determinant likely lies proximal to the 4.4-Mb position. Although the SNP analysis indicates that there may be as many as five haplotype blocks in the *Mom7* region, the *Mom7* allelic status of other inbred strains remains to be determined experimentally. The Perlegen SNP analysis also indicates that there are no nonsynonymous coding region polymorphisms among AKR, BTBR, or B6 for any of the annotated genes (Crem, Cul2, Bambi, Lyzl1, Map3k8, Papd1) in the interval between 3 and 4.4 Mb. This does not rule out regulatory or splice mutations or mutations in unannotated genes. Finally, none of the genes in the minimal modifier region were found to be mutated in the recent survey of human colorectal cancer mutations (Sjoblom *et al.* 2006). Nevertheless, copy number variations, epigenetic or structural differences, or more proximal mutations may underlie the *Mom7* determinant.

An important aspect of modifiers is that they can act either additively or synergistically (Cormier and Dove 2000). We have shown that *Mom7* acts independently of *Mom1*; the two modifiers therefore affect tumor multiplicity through separate, noncomplementary pathways. This independence creates an even wider disparity in relative tumor multiplicity between B6 and AKR mice: upon factoring out the effects of *Mom1* and *Mom7*, there remains a 15-fold difference. Although the N2 genome scan did identify a second polymorphic modifier on chromosome 11 with a LOD score of 3.3 (supplemental Figure 1 at http://www.genetics.org/supplemental/), congenic line analysis has not borne out a significant effect (L. N. Kwong, data not shown). Similarly, the higher tumor multiplicity of the congenic line BTBR.*Mom7*$^{B6/BTBR}$ compared to the partial consomic lines shown in Figure 1, C and D, or in (BTBR × B6) F_1 mice (data not shown) suggests the presence of additional recessive enhancers in the BTBR strain.

The demonstration that different alleles of *Mom7* segregate among inbred lines will have an impact on studies involving *Apc*^{Min}. Currently, the decision to use particular inbred strains must take into account the status of the *Mom1* alleles; future *Apc*^{Min} experiments will also require knowledge of the pertinent *Mom7* allelic genotypes when comparing strains or using mixed genetic backgrounds. Unlike *Mom1*, where screening for the *Pla2g2a* mutation can give information regarding an untested strain's allelic status, no specific mutation has yet been found for *Mom7*. However, it would be relatively simple to cross the strain of interest to a genotypically informative *Mom7* congenic line (*i.e.*, one with polymorphic markers) and determine its allelic status phenotypically.

The identification of a novel gene underlying *Mom7* would add another target for chemoprevention. The demonstration of somatic recombination hotspots would also be valuable, as similar genetic determinants could exist for human colon cancer patients. What is now needed is to determine whether humans harbor a polymorphic modifier in the region orthologous to *Mom7* on human chromosome 5.

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