Mom1 is a Semi-Dominant Modifier of Intestinal Adenoma Size and Multiplicity in *Min*/+ Mice

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ABSTRACT

The intestinal tumor multiplicity in mice heterozygous for Apc^{Min} is strongly modulated by genetic background. On the sensitive C57BL/6J (B6) background, mice develop large numbers of intestinal adenomas. The AKR/J (AKR) strain carries alleles that correlate with a strong reduction in tumor multiplicity. To study the effect of one of these modifiers, *Mom1*, we have generated a mouse line in which the AKR allele of *Mom1* is carried on the sensitive B6 genetic background. This strain was produced by using a marker-assisted selection method to eliminate unlinked AKR alleles more rapidly. The application and efficiency of this method are discussed. We used this strain to determine that *Mom1* affects both tumor multiplicity and tumor size in a semi-dominant fashion.

MIN mice are heterozygous for a germline mutation in the mouse *Apc* (adenomatous polyposis coli) gene. This nonsense mutation at codon 850 of the 2845 amino acid Apc protein leads to the development of adenomas throughout the intestinal tract (MOSER *et al.* 1990; SU *et al.* 1992). Genetic background strongly influences the number of tumors that develop in *Min*/+ mice (MOSER *et al.* 1992). *Min*/+ mice on the C57BL/6J background (B6-Min) develop an average of ~30 tumors in the regions of the intestine scored (MOSER *et al.* 1990). In contrast, *Min*/+ F_1 hybrid mice produced in crosses between AKR/J (AKR) and B6-Min develop only six tumors on average (MOSER *et al.* 1992). Analysis of the phenotypic variance in a [(AKR × B6) *Min*/+ F_1 × B6] backcross gave an estimate of 1.8 segregating genetic factors influencing tumor multiplicity (DIETRICH *et al.* 1993). In this cross, we mapped one locus influencing tumor multiplicity, *Mom1* (*Modifier of Min-1*), to a 15-cM region on distal mouse chromosome *4* (DIETRICH *et al.* 1993). In this backcross, heterozygosity for *Mom1* conferred a 1.5-fold reduction in average tumor multiplicity. The additional modifiers carried in the AKR strain are unmapped, but at least one must act in a dominant or semi-dominant fashion (DIETRICH *et al.* 1993). The presence of these modifiers prevented a rigorous analysis of the effect of heterozygosity and homozygosity for $Mom1^{AKR}$ on intestinal tumor multiplicity in crosses between AKR and B6-Min (DIETRICH *et al.* 1993). In these crosses between AKR and B6-Min, there was no evidence for any modifier loci at which the B6 allele conferred a reduction in tumor multiplicity (DIETRICH *et al.* 1993).

To study the AKR allele of *Mom1* on a defined genetic background free of additional AKR modifiers, we have constructed a B6.Mom1^{AKR} line. This strain carries a region of distal mouse chromosome *4* from the AKR strain on the B6 genetic background. Traditionally, congenic lines are generated through 10 generations of backcrossing. Heterozygosity unlinked to the region or gene of interest is reduced, on average, by 50% in each backcross generation. We have attempted to speed up the process of elimination of heterozygosity by using a marker-assisted selection (MAS) method (HILLEL *et al.* 1989; LANDE and THOMPSON 1989; HOSPITAL *et al.* 1992). The MAS method tries to achieve a more rapid reduction in heterozygosity by identifying and breeding the mice in each generation that have inherited <50% of remaining heterozygosity. This identification is accomplished by genotyping mice in each backcross generation with simple sequence length polymorphism (SSLP) markers to identify the animals most advantageous for breeding. Our experience indicates that the MAS method may prove to be useful.

Using our MAS-generated the B6.Mom1^{AKR} line, we have determined that *Mom1* is a semi-dominant modifier of intestinal adenoma multiplicity in *Min*/+ mice. Heterozygosity for $Mom1^{AKR}$ conferred a twofold reduction on intestinal adenoma multiplicity in Min mice relative to the $Mom1^{B6}$ homozygotes. Homozygosity for $Mom1^{AKR}$ conferred a fourfold reduction of intestinal adenoma multiplicity in Min mice relative to the $Mom1^{B6}$ homozygotes. We have also determined that *Mom1* is a semi-dominant modifier of intestinal adenoma size in Min mice.

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MATERIALS AND METHODS

Construction of the B6.Mom1^{AKR} strain: All mice were bred at the McArdle Laboratory for Cancer Research (Madison, WI) from AKR and B6 mice purchased from The Jackson Laboratory (Bar Harbor, ME). The F_1 generation was produced by crossing B6 females to AKR males. F_1 females were then backcrossed to B6 males to produce the N_2 generation. N_2 animals were typed for the markers *D4Mit9, D4Mit12, D4Mit13,* and *D4Smh6b* (referred to as *Mom1* markers) that flank the *Mom1* locus (see Figure 1). Mice heterozygous for all four of these markers were then typed with 35 markers (referred to as Set 1 markers) unlinked to *Mom1* that were scattered throughout the genome (see Figure 2). Mice that were heterozygous at the fewest number of Set 1 markers were bred to B6 mice to produce the N_3 generation. Mice from the N_3 generation were typed for the *Mom1* markers. Each animal heterozygous for all four *Mom1* markers was then typed for the subset of Set 1 markers that had remained heterozygous in its N_2 parent. This process was repeated through the N_4 generation.

Phenotyping crosses: To phenotype the B6.Mom1^{AKR} line, females from the N_3 or N_7 backcross generation were crossed to B6-*Min*/+ (N23-N24 or N29-N30, respectively) males to generate an F_1 generation. To generate mice homozygous for the AKR allele of *Mom1,* an F_2 generation was produced by crossing *Min*/+ N_7F_1 mice that were heterozygous for the entire *D4Mit9–D4Mit180* interval to +/+ (non-*Min*) mice produced in the same cross (N_7F_1 or N_8F_1 generation) that were also heterozygous for this interval.

Genomic DNA isolation: Mice were anesthetized with ether and blood was collected from the retro-orbital sinus. Genomic DNA was isolated from blood by using two distinct methods.

Method 1: This method is a modification of a protocol described previously (PHILLIPS and NADEAU 1984). Briefly, 250 μl of blood was collected into Eppendorf tubes containing 200 μl LST (29 mM Tris pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$). Two hundred fifty microliters 4XTNLB (5% sucrose, 4% NP-40 in LST) was added to the blood solution. Tubes were mixed by inversion and spun at 10,000 rpm for 3 min in a microfuge. The supernatant was decanted, and the pellet was air dried and resuspended in 500 μl ACE [50 mM sodium acetate, 10 mM EDTA (disodium salt) pH 5.1] with 10% SDS. This lysate was frozen and then extracted two times with phenol, two times with phenol/$CHCl_3$-isoamyl alcohol, and once with $CHCl_3$-isoamyl alcohol. The DNA was then precipitated with 2 vol of 100% ethyl alcohol and resuspended in 20 μl deionized water.

Method 2: DNA was prepared from 50 μl of blood as described previously (DIETRICH *et al.* 1993).

Chromosome 4 genotyping for the B6.Mom1^{AKR} line: Mice were typed by using methods described previously (DIETRICH *et al.* 1992) or as described below. After the N_6 generation, the marker *D4Mit180* was substituted for the marker *D4Smh6b.* Both markers map to the same position, but *D4Mit180* gave more reliable amplification.

The markers *D4Mit9, D4Mit12, D4Mit13, D4Mit180* were amplified under the following conditions: 0.55 μM of each primer, 2 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°), 0.1% Triton X-100, 0.2 mM (each) dATP, dCTP, dGTP, and dTTP, and 0.7 unit of Taq polymerase (Promega Corporation, Madison, WI). The marker *D4Smh6b* was used for amplification under the following conditions: 0.53 μM of each primer, 1.5 mM $MgCl_2$, 50 mM KCl, 10 mM Tris-HCl (pH 9.0 at 25°), 0.1% Triton X-100, 0.2 mM (each) dATP, dCTP, dGTP, and dTTP, and 0.7 unit of Taq polymerase. For chromosome *4* markers, 10 μl of DNA was amplified in a 25-μl reaction overlaid with 30 μl of mineral oil. Samples were amplified in an MJResearch Thermal Cycler (Watertown, MA) using the following profile: one cycle at 94° for 3 min followed by 30 cycles at 94° for 15 sec, 55° for 2 min, and 72° for 2 min, followed by one cycle at 72° for 7 min and 1 cycle at 15° for 1 min.

The products from these reactions were resolved by electrophoresis through 3.5–4% agarose (SeaKemLE, FMC Corp., Rockland, ME) gels and visualized by staining with ethidium bromide. The exception to this was *D4Smh6b,* which was resolved by electrophoresis through 0.8 mm 7% native polyacrylamide gels under standard conditions and visualized by staining with ethidium bromide.

Chromosome *4* genotyping for the B6.Mom1^{AKR} × B6 *Min*/+ crosses: Most mice were assigned a genotype at the *Mom1* locus based on genotyping at *D4Mit12* and *D4Mit13.* Mice that typed as heterozygous for both markers were designated as *Mom1$^{AKR/B6}$* heterozygotes. Mice homozygous for the AKR allele for both markers were designated as *Mom1$^{AKR/AKR}$* homozygotes. Likewise, mice homozygous for the B6 allele at *D4Mit12* and *D4Mit13* were designated as *Mom1$^{B6/B6}$* homozygotes. Mice that were recombinant in the *D4Mit12–D4Mit13* interval were genotyped at *D4Mit54,* a marker 4 cM proximal of *D4Mit13.* Since mapping data indicate that *Mom1* maps to the *D4Mit54–D4Mit13* interval, mice that were recombinant outside this interval could still be assigned a *Mom1* genotype (see GOULD *et al.* 1996). The marker *D4Mit54* was amplified under the same conditions as described for *D4Smh6b* above.

Set 1 markers: Thirty-five markers were used (see Figure 2). Mice were typed for Set 1 markers with DNA prepared via method 1 and PCR methods described previously (DIETRICH *et al.* 1992) or by using the methods described above for chromosome *4* markers.

Set 2 markers: Thiry-five markers were used (see Figure 2). Mice from the N_6 generation were typed for Set 2 markers from DNA prepared via method 1 and using PCR methods described previously (DIETRICH *et al.* 1992).

Min genotyping: Mice were genotyped to identify carriers of the *Min* mutation as described previously (DIETRICH *et al.* 1993)

Tumor scoring: All mice were sacrificed by CO_2 asphyxiation. The intestinal tract was removed, prepared, and scored for tumors as described previously (MOSER *et al.* 1990). In this method, three 4-cm sections of the small intestine, which is approximately one-third to one-half of the total length of the small intestine, and the entire large intestine were examined for tumors. The (B6.Mom1^{AKR} N_3 × B6) *Min*/+ F_1 animals were sacrificed at 150 days or when moribund. The (B6.Mom1^{AKR} N_7 × B6) *Min*/+ F_1 animals were sacrificed at 80, 120, or 200 days of age. The (B6.Mom1^{AKR} N_7 × B6) *Min*/+ F_2 animals were sacrificed at 120 days of age or when moribund. All *Min*/+ mice from crosses with the B6.Mom1^{AKR} strain were scored by K.A.G. to eliminate variation between observers.

Tumor diameter measurements: After tumor multiplicity was assessed at sacrifice, the intestines were fixed overnight in 10% buffered formalin. After fixation, intestines were rinsed in three overnight incubations in 70% ethanol. Samples were stored in 70% ethanol until tumors were measured. The maximum diameter of tumors was measured with a calibrated eye piece reticle on a dissecting microscope.

Statistics: All statistics were performed with the Wilcoxon rank sum test. One-sided *P* values are given, except where indicated otherwise.

RESULTS

Construction of the B6.Mom1^{AKR} strain: LOD scores suggest that *Mom1* most likely maps to the 15-cM inter-

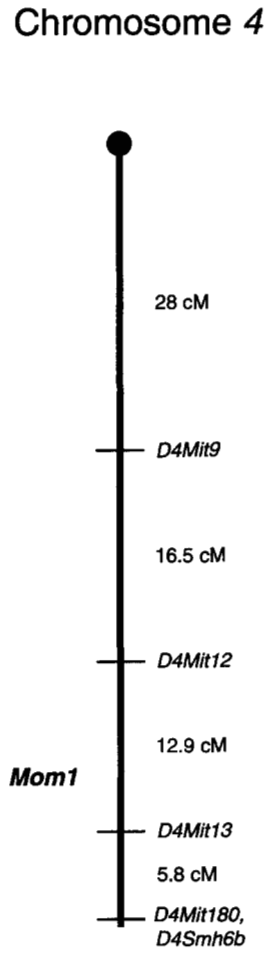

FIGURE 1.—Chromosomal distribution of *Mom1* markers. The distribution of markers on chromosome *4* that were used to select for the *Mom1* region from the AKR strain is shown. The distance between markers is given in cM.

val defined by *D4Mit12* and *D4Mit13*. To ensure that the B6.Mom1^{AKR} strain would carry the *Mom1* locus, even if it mapped outside the *D4Mit12–D4Mit13* interval, we chose to generate a line carrying more than just this interval. Using the MAS strategy, we constructed a strain in which we selected for a 35-cM region encompassing the *D4Mit12–D4Mit13* interval (Figure 1). The distribution of the 35 SSLP markers (Set 1) used for counter-selection through the N_4 generation is shown in Figure 2. The selection efficiency in the construction of the B6.Mom1^{AKR} line using 35 SSLP markers (Set 1) is shown in Table 1. In each backcross generation, a small number of animals were selected on the basis of being heterozygous in the *Mom1* region and having inherited the fewest AKR alleles unlinked to *Mom1*. In the N_4 generation, we identified mice that were homozygous for B6 alleles at all 35 Set 1 markers.

At the N_6 generation we performed a second round of selection with a new group of 35 SSLP markers located in regions not covered by the Set 1 markers (Figure 2). Three animals typed were homozygous for the B6 allele at all 35 Set 2 markers, and one of these was chosen to produce the N_7 generation. Twenty-two animals typed were still heterozygous for one to four of the Set 2 markers. Combining markers from Set 1 and Set 2, we attempted to have a marker placed approximately every 20 cM. However, in some regions, gaps greater than 20 cM do exist. This result is due to the fact that the map positions of some markers were refined after their use. The only gaps greater than 30 cM are on chromosome *1* (44 cM) and on chromosome *10* (34 cM). From the genotyping data, we estimate that mice from the N_6 generation carry, on average, 36 cM of AKR genome unlinked to chromosome *4*.

The effect of heterozygosity for *Mom1*^{AKR} on tumor multiplicity: To determine whether the construction of the B6.Mom1^{AKR} strain was successful in isolating the Mom1^{AKR} allele on the B6 background free of other AKR modifiers, (B6.*Mom1*^{AKR} × B6-Min) F_1 animals were produced. One set of F_1s was produced by crossing *Mom1*$^{AKR/B6}$ females from the N_3 generation with B6 *Min*/+ males. The average tumor number of *Mom1*$^{AKR/B6}$ *Min*/+ mice was 15.9. This is significantly different from the average of 32.1 observed in the *Mom1*$^{B6/B6}$ *Min*/+ mice ($P = 0.009$) (Figure 3A). These results suggest that the region of chromosome *4* from AKR in the B6.Mom1^{AKR} strain does carry *Mom1*^{AKR}. A second set of (B6.*Mom1*^{AKR} × B6-Min) F_1 animals was produced by crossing *Mom1*$^{AKR/B6}$ females from the N_7 generation with B6 *Min*/+ males. As shown in Figure 3B, for *Mom1*$^{AKR/B6}$ *Min*/+ mice, the average number of tumors is 13.1. This value is significantly different from the average of 26.1 observed in their *Mom1*$^{B6/B6}$ *Min*/+ littermates ($P = 3.5 \times 10^{-6}$) (Figure 3B). These results suggest that heterozygosity for the *Mom1*^{AKR} allele confers approximately a twofold reduction in average tumor multiplicity in *Min*/+ mice.

At the N_3 and N_7 generations, there was no significant difference in either the means (two-sided $P = 0.50$) or the variances (two-sided $P = 0.11$) of the tumor multiplicity distributions from *Mom1*$^{AKR/B6}$ *Min*/+ mice. Likewise, the means (two-sided $P = 0.20$) and variances (two-sided $P = 0.26$) of the tumor multiplicity distributions in the *Mom1*$^{B6/B6}$ *Min*/+ mice from the N_3 and N_7 crosses do not differ significantly. The fact that the tumor multiplicity distributions in *Min*/+ mice of a given *Mom1* genotype does not change from the N_3 to the N_7 generation suggests that all AKR modifiers other than *Mom1* have been removed by the N_3 generation. This hypothesis is supported by the fact that the average tumor multiplicities in *Mom1*$^{B6/B6}$ *Min*/+ mice from neither the N_3 nor N_7 crosses differ from the average of 27.2 observed in a control group of congenic B6 *Min*/+ mice ($P = 0.19$ and 0.25, respectively).

The effect of homozygosity for *Mom1*^{AKR} on tumor multiplicity: To determine the effect of two copies of the *Mom1*^{AKR} allele on intestinal adenoma multiplicity in Min mice, we crossed *Mom1*$^{AKR/B6}$ *Min*/+ mice with *Mom1*$^{AKR/B6}$ mice. Most litters were produced from

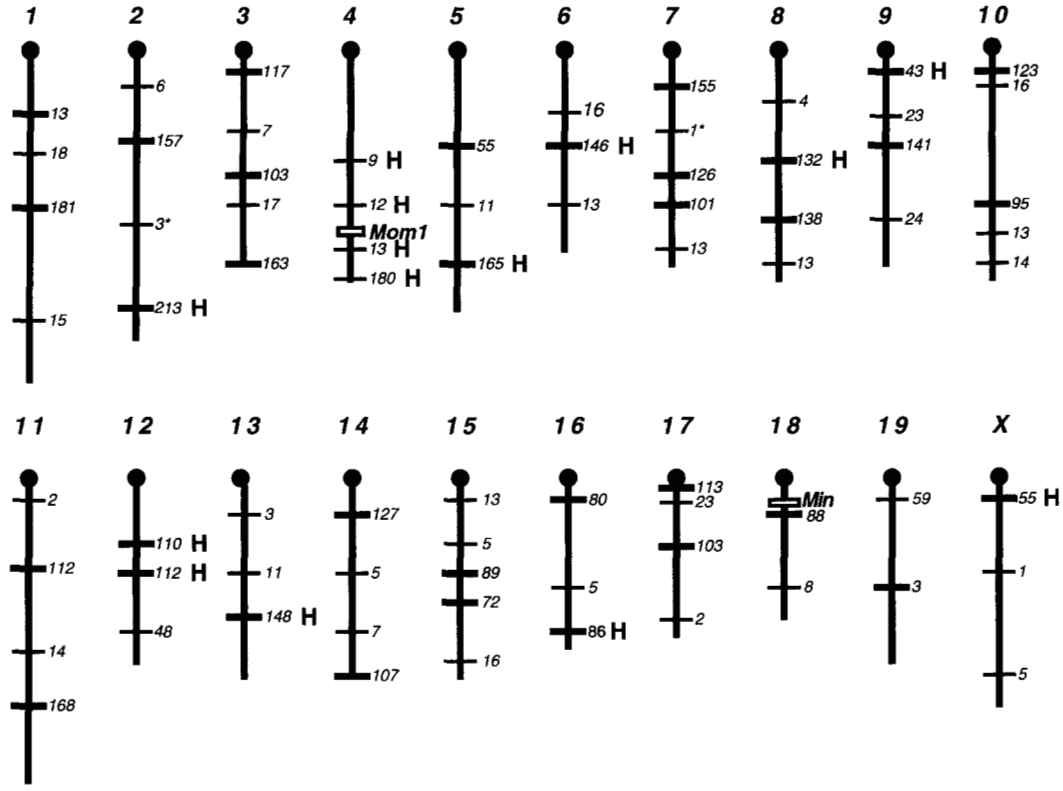

FIGURE 2.—Chromosomal distribution of *Mom1* markers, Set 1 markers, and Set 2 markers. This diagram shows the distribution of Set 1 and Set 2 markers throughout the genome. The narrow hash marks indicate the positions of the Set 1 markers. The wide hash marks indicate the positions of the Set 2 markers. H indicates the markers at which heterozygosity was detected in the N_6 generation. The number beside each hash mark indicates the marker used (*i.e.*, chromosome *1* marker indicated by number 13 is *D1Mit13*). * indicates that this marker is an *Nds* rather than an *Mit* marker. The distances between markers as well as chromosome lengths are drawn to scale.

crosses in which *Min* was carried by the male. The *Mom1*$^{AKR/B6}$ *Min*/+ females had a very poor breeding performance: they produced fewer and smaller litters than non-Min females that were also heterozygous in the *Mom1* interval (K.A.G., data not shown).

The average tumor multiplicity of the *Mom1*$^{AKR/B6}$ *Min*/+ mice from this F_2 generation was 15.0. This result is not significantly different from the average of 13.1 tumors found in the *Mom1*$^{AKR/B6}$ *Min*/+ mice from the F_1 generation described above ($P = 0.15$). Thus the B6.Mom1^{AKR} line does not carry any detectable recessive AKR modifiers unlinked to the *Mom1* region. This assertion is supported by the observation that the average tumor multiplicity of 26.1 in the *Mom1*$^{B6/B6}$ *Min*/+ F_2 mice is the same as the average of 26.1 tumors in the *Mom1*$^{B6/B6}$ *Min*/+ F_1 mice above. The F_2 mice homozygous for the AKR allele of *Mom1* developed on average only 7.8 tumors. This average tumor load is significantly lower than the average of 15.0 tumors observed in the *Mom1*$^{AKR/B6}$ heterozygotes ($P = 2.3 \times 10^{-5}$) (Figure 4A). Homozygosity for *Mom1*^{AKR} conferred a fourfold reduction in tumor multiplicity relative to *Mom1*^{B6} homozygotes.

TABLE 1

Selection with set 1 markers

Generation	No. selected/no. genotyped	Markers heterozygous
F_1	NA	35/35 (100)
N_2	6/96	11-14/35 (35)
N_3	3/63	2-4/35 (9)
N_4	7/62	0/35 (0)

For the N_2–N_4 backcross generations, the number of animals selected for breeding compared to the total number of animals genotyped in each generation is given. The number of Set 1 markers heterozygous for the AKR allele is given. NA, not applicable. For each backcross generation, the average percentage of alleles remaining heterozygous in mice selected for breeding is shown in parentheses. The average heterozygosity for the B6.Mom1^{AKR} line was empirically determined based on calculations using the SSLP genotyping data.

The effect of *Mom1*^{AKR} on total tumor multiplicity is a reflection of changes in both the small and large intestine. In a set of age-matched F_2 mice, there was a significant increase in average small intestinal tumor multiplicity from 7.0 in *Mom1*$^{AKR/AKR}$ mice, to 13.2 in *Mom1*$^{AKR/B6}$ mice, to 20.9 in *Mom1*$^{B6/B6}$ mice ($P < 1 \times 10^{-6}$). A similar trend was observed in the large intestine, where the average tumor multiplicity increased from 1.3 in *Mom1*$^{AKR/AKR}$ mice, to 2.5 in *Mom1*$^{AKR/B6}$ mice, to 3.9 in *Mom1*$^{B6/B6}$ mice ($P = 0.002$). Thus, *Mom1* genotype affects tumor multiplicity in both the small and large intestine of *Min*/+ mice.

The effect of *Mom1*^{AKR} on tumor size: To determine whether *Mom1*^{AKR} influences tumor size, we measured tumors from *Min*/+ mice produced by crossing *Mom1*$^{AKR/B6}$ *Min*/+ mice with *Mom1*$^{AKR/B6}$ mice. The size of a tumor was estimated by measuring its maximum diameter. For this analysis, tumors were measured only from animals sacrificed at 120 days of age. There was a significant correlation between *Mom1* genotype and tumor size in the small intestine. Average maximum

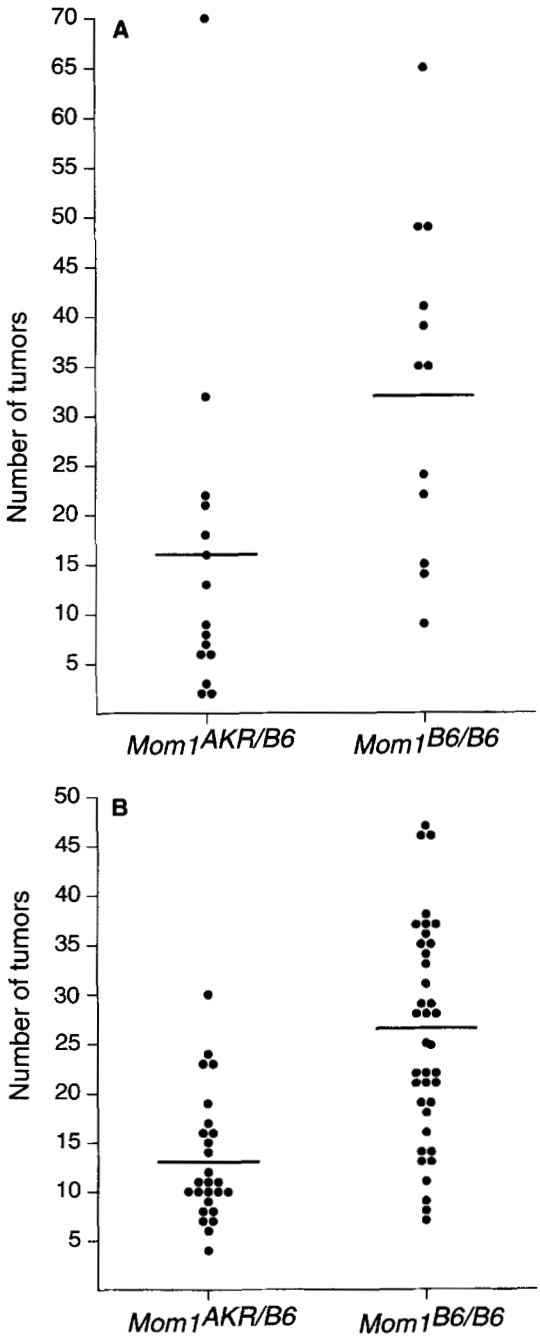

FIGURE 3.—The effect of *Mom1* on adenoma multiplicity in F_1 mice. The horizontal bar indicates the position of the mean of each distribution. The scatter plot in A shows the distribution of tumor multiplicities in *Min*/+ mice produced in the B6.*Mom1*^{AKR} N_3 × B6 *Min*/+ cross. The scatter plot in B shows the distribution of tumor multiplicities in *Min*/+ mice produced in the B6.*Mom1*^{AKR} N_7 × B6 *Min*/+ cross.

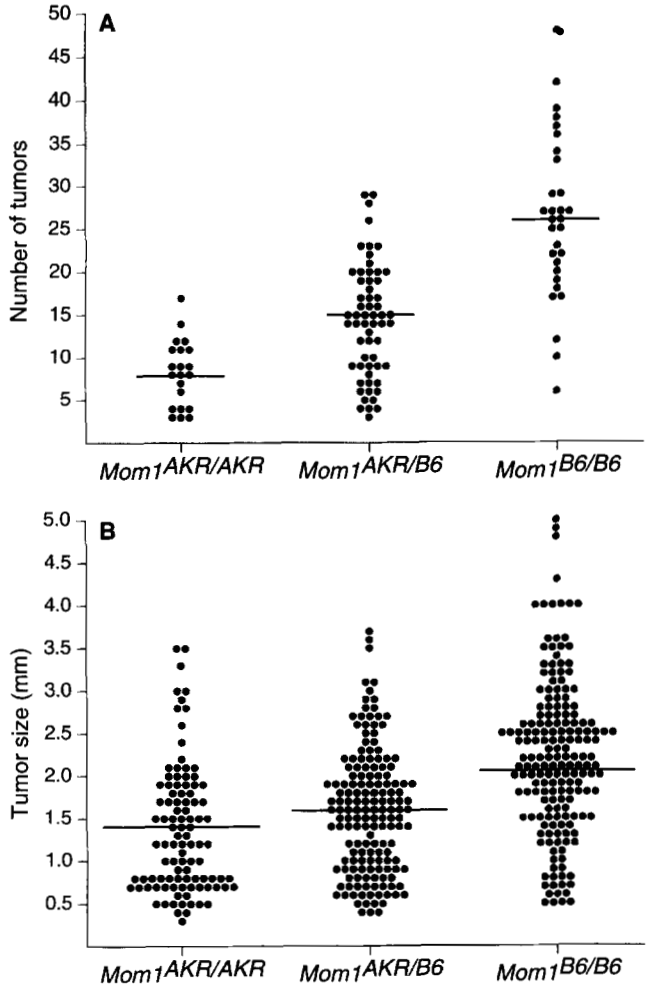

FIGURE 4.—The effect of *Mom1* on adenoma size and multiplicity in F_2 mice. The horizontal bar indicates the position of the mean of each distribution. The scatter plot in A shows the distribution of tumor multiplicities in *Min*/+ mice produced in the B6.*Mom1*$^{AKR/B6}$ × B6 *Mom1*$^{AKR/B6}$ *Min*/+ cross. The scatter plot in B shows the distribution of the sizes of tumors from *Min*/+ mice produced in the B6.*Mom1*$^{AKR/B6}$ × B6. *Mom1*$^{AKR/B6}$ *Min*/+ cross.

tumor diameter increased from 1.41 mm in *Mom1*^{AKR} homozygotes, to 1.57 mm in the *Mom1*^{AKR} heterozygotes, to 2.16 mm in the *Mom1*^{B6} homozygotes ($P < 1 \times 10^{-6}$). This result suggests that *Mom1* affects the size of tumors of the small intestine in a semi-dominant fashion. The average size of tumors from the large intestine of *Min*/+ mice was not influenced by the *Mom1* genotype ($P = 0.36$).

Since *Mom1* affects average tumor size, we hypothesized that perhaps the number of visible tumors in mice carrying *Mom1*^{AKR} would increase over time. This question was addressed by comparing the average tumor multiplicity in congenic B6 *Min*/+ mice and *Mom1*$^{AKR/B6}$ *Min*/+ mice sacrificed at three time points: 80, 120, and 200 days. No increase in average tumor multiplicity in a control population of congenic B6 *Min*/+ mice was observed over this time course. In these mice, the average tumor multiplicity decreased with increasing age, most likely due to a selective, early lethality of mice with high tumor multiplicity. No B6 *Min*/+ mice survived to the 200-day time point. The average tumor multiplicity in the B6 *Min*/+ mice decreased from 29.2 (80 days, $N = 27$) to 25.0 (120 days, $N = 28$) to 19.7 (>140 days, $N = 10$). The difference

in average tumor multiplicity between the 80- and 120-day time points fell short of significance (two-sided $P = 0.07$). The difference in average tumor multiplicity between the earliest (80 day) and latest timepoint (>140 days) was significant (two-sided $P = 0.02$). The average tumor multiplicity in *Mom1*$^{AKR/B6}$ *Min*/+ mice increased from 10.7 (80 days, $N = 11$) to 14.7 (120 days, $N = 14$) to 15.3 (200 days, $N = 7$). The difference in average tumor multiplicity between the 80- and 120-day time points fell short of significance (two-sided $P = 0.06$). Thus, there is no evidence that tumor multiplicity is significantly altered over this time period. However, the difference in average tumor multiplicity between the 80- and 200-day time points did achieve significance (two-sided $P = 0.05$).

To begin to assess whether *Mom1* genotype may affect net growth rate, we determined the average area of small intestinal tumors from *Mom1*$^{AKR/B6}$ and *Mom1*$^{B6/B6}$ mice at 60, 80, 100 and 120 days of age. Plotting these data, we observed that for both genotypic classes, the change in tumor area over time was approximately linear (data not shown). This result was surprising, given that tumors are generally considered to grow exponentially. However, mathematical models suggest that under certain circumstances tumors may grow in a linear fashion (TOMLINSON and BODMER 1995). Using linear regression, we calculated the rate of change in average tumor diameter over time (slope). This slope in each case provides an estimate for the net growth rate. For the *Mom1*$^{B6/B6}$ data, we obtained a slope of 0.05. This value was higher that the slope of 0.02 obtained from the *Mom1*$^{AKR/B6}$ data. This difference suggests that *Mom1* may affect the net growth rate of adenomas in *Min*/+ mice.

DISCUSSION

The AKR strain carries several alleles that reduce the average tumor multiplicity in mice heterozygous for the *Min* mutation (DIETRICH *et al.* 1993). To study one of these modifiers, *Mom1*, in the absence of other AKR modifiers, we constructed a B6.Mom1^{AKR} mouse strain. In this line, a 35-cM region of the AKR chromosome *4* containing *Mom1*^{AKR} was selectively introduced onto the B6 background.

This B6.Mom1^{AKR} line was generated with MAS. The efficacy of this method in the construction of a mouse line is determined by the number of markers used, the number of animals screened, and the number of animals selected for breeding from each backcross generation. Based on the results presented in Table 1, screening 60–100 animals in the first two or three backcross generations with a set of 35 markers allows a more rapid initial reduction of residual heterozygosity than backcrossing without counterselection. Experiments with an MAS-generated line can therefore be performed several generations earlier than with a line generated by the standard method. In fact, tumor multiplicity data from crosses with the B6.Mom1^{AKR} strain at the N_3 and the N_7 backcross generation is fully consistent with the hypothesis that *Mom1*^{AKR} is the only detectable dominant or semi-dominant modifier segregating in the congenic line by the N_3 generation.

To maintain the benefit of MAS beyond the second or third generation, it is necessary to continue selection at a high level of intensity. This requires one to select with at least a constant number of markers in each generation rather than reducing the number of markers used in each generation. This strategy enhances the probability of identifying the few small regions of remaining heterozygosity. By relaxing the intensity in our selection after initial generations, we found that by the N_6 backcross generation, our MAS line does not carry a significantly different amount of residual heterozygosity from what one would have expected had we used backcrossing without selection.

In the MAS and standard methods, the residual heterozygosity is reduced by one-half, on average, in each backcross generation. Within each backcross generation, the amount of residual heterozygosity varies from animal to animal, and some mice will have substantially more or substantially less heterozygosity than average. In our MAS line, mice from each generation identified as carrying the least amount of residual heterozygosity were chosen for breeding (Table 2). For example, in our MAS line, the N_6 mouse chosen for breeding had been shown by genotypic analysis to be homozygous for the B6 allele at all 70 markers unlinked to chromosome *4*. Thus the N_7 generation will also be homozygous for the B6 allele at these 70 markers. In a non-MAS line, the mice chosen from each generation are selected at random and therefore may contain more than the average amount of heterozygosity (Table 2). Therefore, although mice from the N_6 generation of a non-MAS line will be heterozygous, *on average,* for 2/70 markers, the mice selected at random for breeding may be heterozygous at more than two markers. Thus, the actual amount of residual heterozygosity in a non-MAS line at the N_7 generation is not fixed and may be considerably higher from what that in the B6.*Mom1*^{AKR} MAS line at N_7.

In general, our results generating the B6.Mom1^{AKR} line with MAS closely resembles the predictions for the efficacy of MAS based on modelling and computer simulations (HILLEL *et al.* 1989; LANDE and THOMPSON 1989; HOSPITAL *et al.* 1992). These results indicate that use of the MAS method can reduce the number of generations required to produce a genetically defined line of laboratory mice.

Crosses between the B6.Mom1^{AKR} strain and the B6-Min strain indicate that *Mom1* is a semi-dominant modifier of tumor multiplicity. Mice heterozygous for the AKR allele of *Mom1* develop on average twofold fewer tumors than the *Mom1*^{B6} homozygotes. Mice homozygous for *Mom1*^{AKR} develop fourfold fewer tumors than their siblings homozygous for *Mom1*^{B6}. It is unclear at

TABLE 2

Standard *vs.* MAS method

	Average number of markers heterozygous in breeders			
	Standard congenic		B6.Mom1^{AKR} strain	
Gen	Set 1	Set 2	Set 1	Set 2
F_1	35	35	35	35
N_2	18	18	13*	18
N_3	9	9	3*	9
N_4	5	5	0*	5
N_5	3	3	0*	3
N_6	2	2	0*	0*
N_{10}	0	0	0*	0

For each backcross generation, the average percentage of alleles remaining heterozygous in mice chosen for breeding is based on statistical probability except where indicated by an asterisk (*). Values followed by an asterisk were determined empirically based on calculations from the SSLP genotyping data.

this time whether *Mom1*^{AKR} functions actively to reduce tumor number, whether *Mom1*^{B6} functions actively to increase tumor number, or both.

Crosses between the B6.Mom1^{AKR} strain and the B6-Min strain also reveal that *Mom1* is a semi-dominant modifier of tumor size within the small intestine. Since *Mom1* reduces tumor multiplicity in both the small and large intestine, the fact that *Mom1* does not detectably influence tumor size in the colon is surprising. This observation may be a reflection of the fact that tumor diameter is a less accurate measure of the size of the pedunculate adenomas of the colon than of the nonpedunculate adenomas of the small intestine. The effect of *Mom1* on tumor size suggests that tumors in *Mom1*$^{AKR/B6}$ *Min*/+ mice may have been initiated later or grow more slowly than tumors in *Mom1*$^{B6/B6}$ mice. Both of these hypotheses are consistent with the observation that the average number of visible tumors in B6.*Mom1*$^{AKR/B6}$ mice increases between 80 and 200 days of age. The effect of *Mom1* on tumor multiplicity may be mediated through its effect of tumor size. The increase in average tumor multiplicity in B6.*Mom1*$^{AKR/B6}$ mice over time contrasts with previously reported data indicating that the number of visible tumors in (AKR × B6) F_1 *Min*/+ mice is not a function of age (MOSER *et al.* 1992). The most likely explanation for this apparent discrepancy is that mice were examined over different time periods (80–200 days *vs.* 105–365 days) or that the presence of additional modifier loci in the AKR strain masks this effect of *Mom1*. The average size of small intestinal tumors in *Mom1*$^{B6/B6}$ *Min*/+ and *Mom1*$^{AKR/B6}$ *Min*/+ mice increases between 60 and 120 days of age. The fact that the rate of increase in the size of tumors in *Mom1*$^{B6/B6}$ *Min*/+ was higher than in *Mom1*$^{AKR/B6}$ *Min*/+ mice is consistent with the hypothesis that *Mom1* may effect the net growth rate of tumors in *Min*/+ mice.

In *Mom1*$^{AKR/B6}$ *Min*/+ mice, the increase in the number of visible tumors between the 80- and 200-day time points did achieve significance. However, the increase in the number of visible tumors between 80- and 120-day time points did not. Based on this, we conclude that pooling data between *Mom1*$^{AKR/B6}$ *Min*/+ mice of different ages is acceptable within certain, but not all age ranges. For example, in the B6.*Mom1*^{AKR} N_3 × B6 *Min*/+ cross, the average age at sacrifice was 103 ± 23 days for *Mom1*$^{B6/B6}$ mice and 130 ± 30 days for *Mom1*$^{AKR/B6}$. The average tumor multiplicity in the *Mom1*$^{AKR/B6}$ *Min*/+ mice sacrificed at later time points may have been slightly higher than it would have been had they been sacrificed earlier (see RESULTS). However, the fact that the average tumor multiplicity in these mice was significantly lower than that in their younger *Mom1*$^{B6/B6}$ *Min*/+ littermates indicates that the difference in age at sacrifice does not alter the conclusions that can be drawn from this experiment. If anything, these results indicate that had the *Mom1*$^{AKR/B6}$ *Min*/+ mice been sacrificed at the same ages at the *Mom1*$^{B6/B6}$ *Min*/+ mice, an even more significant difference in average tumor multiplicity would have obtained. Therefore, pooling data from mice sacrificed at different time points in this experiment is acceptable.

The B6.Mom1^{AKR} line has been a useful tool in the process of identifying the *Mom1* locus. A series of mouse lines derived from this strain have been used to generate a fine structure map of the *Mom1* region. This series of derivative pedigrees has been used to narrow the *Mom1* region to a 4-cM interval (GOULD *et al.* 1996). This series of derivatives provides a particularly valuable resource of material for evaluating candidate genes for the *Mom1* locus, including the nonpancreatic secretory phospholipase A_2 gene, *Pla2g2a,* which has been proposed as a candidate for *Mom1* (MACPHEE *et al.* 1995; GOULD *et al.* 1996).

The B6.Mom1^{AKR} line can also be used in a variety of experiments to investigate the role of *Mom1*. One issue to resolve is whether the *Mom1* effect on *Min*-induced intestinal neoplasia is an allele-specific interaction. Experiments can also be performed to determine whether *Mom1* interacts with the *Dnmt* locus, the only other gene currently known to modify the intestinal tumor phenotype of Min mice (LAIRD *et al.* 1995). With the B6.Mom1^{AKR} line, one now can determine the effect of *Mom1* on nonintestinal phenotypes associated with the *Min* mutation (MOSER *et al.* 1995).

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LITERATURE CITED

DIETRICH, W. F., H. KATZ, S. E. LINCOLN, H.-S. SHIN, J. FRIEDMAN *et al.*, 1992 A genetic map of the mouse suitable for typing intraspecific crosses. Genetics **131:** 423–447.

DIETRICH, W. F., E. S. LANDER, J. S. SMITH, A. R. MOSER, K. A. GOULD *et al.*, 1993 Genetic identification of *Mom-1*, a major modifier locus affecting *Min*-induced intestinal neoplasia in the mouse. Cell **75:** 631–639.

GOULD, K. A., C. LUONGO, A. R. MOSER, M. K. MCNELEY, N. BORENSTEIN *et al.*, 1996 Genetic evaluation of candidate genes for the *Mom1* modifier of intestinal neoplasia in mice. Genetics **144:** 1777–1785.

HILLEL, J., T. SCHAAP, A. HABERFELD, A. J. JEFFREYS, Y. PLOTZKY *et al.*, 1989 DNA fingerprints applied to gene introgression in breeding programs. Genetics **124:** 783–789.

HOSPITAL, F., C. CHEVALET and P. MULSANT, 1992 Using markers in gene introgression breeding programs. Genetics **132:** 1199–1210.

LAIRD, P. W., L. JACKSON-GRUSBY, A. FAZELI, S. DICKINSON, W. E. JUNG *et al.*, 1995 Suppression of intestinal neoplasia by DNA hypomethylation. Cell **81:** 197–205.

LANDE, R., and R. THOMPSON, 1989 Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics **124:** 743–756.

MACPHEE, M., K. P. CHEPENIK, R. A. LIDDELL, K. K. NELSON, L. D. SIRACUSA *et al.*, 1995 The secretory phospholipase A2 gene is a candidate for the Mom1 locus, a major modifier of Apc^{Min}-induced intestinal neoplasia. Cell **81:** 957–966.

MOSER, A. R., H. C. PITOT and W. F. DOVE, 1990 A dominant mutation that predisposes to multiple intestinal neoplasia in the mouse. Science **247:** 322–324.

MOSER, A. R., W. F. DOVE, K. A. ROTH and J. I. GORDON, 1992 The *Min* (Multiple Intestinal Neoplasia) mutation: its effect on gut epithelial cell differentiation and interaction with a modifier system. J. Cell Biol. **116:** 1517–1526.

MOSER, A. R., C. LUONGO, K. A. GOULD, M. K. MCNELEY, A. R. SHOEMAKER *et al.*, 1995 Apc^{Min}: a mouse model for intestinal and mammary tumorigenesis. Eur. J. Cancer **31A:** 1061–1064.

PHILLIPS, S., and J. NADEAU 1984 Research News: DNA preparation. Mouse Newsletter **70:** 83.

SU, L.-K., K. W. KINZLER, B. VOGELSTEIN, A. C. PREISINGER, A. R. MOSER *et al.*, 1992 Multiple intestinal neoplasia caused by a mutation in the murine homolog of the APC gene. Science **256:** 668–670.

TOMLINSON, I. P. M., and W. F. BODMER, 1995 Failure of programmed cell death and differentiation as causes of tumors: some simple mathematical models. Proc. Natl. Acad. Sci. USA **92:** 11130–11134.

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