ANALYSIS OF THE *Mom1* MODIFIER OF INTESTINAL NEOPLASIA IN MICE

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□ *Although the methodology for mapping genes controlling susceptibility to tumor development in mice is becoming well established, it remains a formidable challenge to move from linkage to locus. Positional cloning, now commonly used in the identification of loci affecting a qualitative phenotype, has yet to be successfully applied to quantitative trait loci. This study describes the application of candidate gene testing, a method complementary to positional cloning. The method has been applied to evaluate candidates for the quantitative trait locus, Mom1, which modifies the susceptibility of* $Apc^{Min/+}$ *mice to spontaneous intestinal tumor development. The authors also discuss the further testing of one candidate, the phospholipase gene Pla2g2a, by transgenesis. Finally, studies on the mode of action of Mom1 are discussed in light of the evidence that Mom1 encodes this secretory phospholipase.*

Keywords *adenomatous polyposis coli (Apc), intestinal tumors, mice, modifier of Min-1 (Mom1), multiple intestinal neoplasia (Min), quantitative trait locus*

The intestinal epithelium, a common site of primary cancer in humans, is one of the most highly proliferative tissues within the human body [1, 2]. A single stem cell, residing near the base of each crypt, gives rise to the four differentiated cell types of the epithelium [1]. Asymmetric division of this stem cell produces one daughter cell that retains a limited stem cell capacity. A cohort of 4 to 16 such stem cells resides in each crypt [2]. The other daughter cell becomes highly proliferative and begins to migrate up the crypt, producing a clone of highly proliferative cells in the basal two-thirds of the crypt. As cells reach the top of this proliferative zone, they stop dividing and begin terminal differentiation. The differentiated enterocytes, goblet cells, and enteroendocrine cells continue an upward migration and populate the surface of the

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villi of the small intestine and the epithelial cuff in the large intestine. The Paneth cells move in the opposite direction, differentiating as they migrate downward to the base of the crypt.

Intestinal tumors are believed to be derived from stem cells within the crypt [3]. Analysis of adenomatous polyps and malignant carcinomas from humans have led to the hypothesis that specific somatic genetic and epigenetic alterations are causative factors in the development of these lesions [4]. One of the earliest detectable events is inactivation of the adenomatous polyposis coli (*APC*) gene. Most colorectal tumors (>70%) and colorectal tumor cell lines (>80%), carry no wild-type *APC* allele or gene product [5, 6].

THE *APC* GENE

The *APC* gene encodes a polypeptide of 2843 amino acids in length. Although the function of *APC* is unknown, the amino acid sequence of APC suggests a number of domains that may have functional significance. The amino-terminus of APC contains heptad repeat regions, believed to mediate homodimerization [7–11]. Two regions of sequence similarity with ral2 and the m3 muscarinic acetylcholine receptor (mAchR) suggest that APC may be involved in the regulation of G proteins. APC interacts with β-catenin through two distinct domains [12, 13]. The interaction suggests that APC may be involved in modulating both the transcriptional regulation and E-cadherin-dependent cell adhesion mediated by β-catenin [14–16]. APC also binds to DLG, the human homolog of the *Drosophila* discs large (*dlg*) tumor suppressor gene [17], a potential guanylate kinase homolog [18]. APC binds DLG in the putative kinase domain [17], suggesting that APC may regulate DLG kinase activity and thus the level of guanine nucleotides available to G proteins. The carboxy-terminal portion of APC contains a 200-amino acid basic domain [8]. This region has been hypothesized to mediate an association with cytoskeletal microtubules [19, 20].

GERMLINE MUTATION OF THE *APC* GENE

Humans heterozygous for a germline mutation in *APC* develop a condition known as familial adenomatous polyposis (FAP) [8, 9]. Classical FAP is characterized by the development of hundreds to thousands of colonic polyps, often by the second decade of life. A subset of these benign lesions invariably progress to carcinoma. As in sporadic tumors, adenomas and carcinomas in FAP patients have lost the wild-type allele of APC [5, 21].

The majority of disease-causing germline and somatic *APC* mutations are nonsense mutations within the coding region [22, 23]. In FAP, a number of features of the disease can be correlated with the position of the mutation.

For example, individuals carrying a germline nonsense mutation prior to amino acid 157 develop a mild form of FAP, known as attenuated adenomatous polyposis coli (AAPC), characterized by the development of fewer colonic polyps (1–100), which present later in life than those in classical FAP (described above) [24, 25]. In addition, occurrence of extracolonic lesions of the ocular fundus (CHRPEs), osteomas, desmoid tumors, and polyps of the stomach and small intestine, frequently associated with FAP, are also influenced by mutation position [26–28].

Genetic factors unlinked to *APC* may also influence adenoma multiplicity and the development of extracolonic manifestations in FAP patients. Within some FAP families striking variability in phenotype can be observed [29]. At least some of this variability is believed to be due to the segregation of modifying genetic factors. Recently, evidence for linkage of a modifier locus for FAP to markers on human chromosome 1p35–36 has been reported [30]. Interestingly, the subchromosomal region 1p35–36 is frequently deleted in colorectal cancer in humans [31].

THE *Min* MOUSE STRAIN

Min (multiple intestinal neoplasia) is a dominant mutation recovered following germline mutagenesis with the alkylating agent *N*-ethyl-*N*-nitrosourea (ENU). Mice heterozygous for *Min*, a nonsense mutation at codon 850 in the mouse *Apc* gene on chromosome 18, are predisposed to the development of adenomas throughout the intestinal tract [32–34]. Embryos that are homozygous for *Min* successfully undergo uterine implantation, but die prior to day 6.5 of gestation [35].

The human and mouse APC/Apc polypeptides are 90% identical at the amino acid level, and all potential functional domains are conserved between these two species [33]. The *Min* allele produces a stable 97-kD truncated protein product [16]. This truncated protein product contains only a subset of the heptad repeats as well as the ral2 and mAchR homologies. Thus, the product of the *Min* allele completely lacks both catenin binding domains, the microtubule association domain, and the DLG binding domain. This mutation is analogous to many germline mutations detected in FAP families in that the mutant gene product is predicted to retain homodimerization capabilities but lacks the majority of the potential functional domains.

Histologically, the adenomas in *Min* mice are similar to those in FAP patients. *Min*-induced neoplasms are largely comprised of undifferentiated cells [36]. However, isolated cells or clusters of cells expressing differentiation markers characteristic of the four differentiated cell types of the intestinal tract can be detected within the tumors [36]. The presence of all four differentiated cell types suggests that *Min*-induced adenomas are derived from a multipotential stem cell population within the crypt. As has been suggested

for adenomatous polyps in human FAP patients [37], adenomas from *Min* mice seem to be polyclonal in structure, containing cells from at least two somatic lineages [38].

Adenomas in *Min* mice on a number of genetic backgrounds show loss of wild-type *Apc*, although the mechanism of loss may vary [39–41]. Thus in *Min* mice, as in humans, tumor development is frequently, if not invariably, associated with loss of normal *Apc* function.

The *Min* phenotype is similar but not identical to FAP. In the congenic C57BL/6J(B6)-*Min* strain, polyps occur primarily, but not exclusively, in the small intestine [32]. Most extracolonic manifestations associated with FAP are not observed in B6 *Min* mice under normal conditions. The exception to this may be the desmoid tumor, which can be observed in older *Min* mice and in multiparous *Min* females [42]. Epidermoid cysts, FAP-associated skin lesions, are present in *Min* mice only as a result of treatment with ENU as a somatic mutagen [43]. Osteomas and CHRPE have not been detected in B6 *Min* mice [44, Dan Alberts, personal communication]. The differences in phenotypes of *Min* and FAP may be due to genetic, dietary, and/or environmental differences between mice and humans.

THE *Mom1* MODIFIER

Tumor multiplicity in *Min* mice is strongly modulated by genetic background. B6 *Min*/+ mice rarely live beyond 150 days of age and develop an average of 29 ± 10 tumors in the regions of the intestinal tract scored [32]. Crosses between B6 *Min*/+ mice and mice from other inbred strains such as AKR/J (AKR), MA/MyJ (MA), or *Mus musculus castaneus* (CAST) produce F1 hybrids that develop only 6–7 tumors on average and often live more than 1 year [36, 45]. These results suggest that the strains AKR, MA, and CAST each carry dominant resistance alleles that modify the *Min* phenotype.

To map these modifier loci, *Min*/+ F1 mice were crossed to B6 mice to produce a segregating backcross population. Analysis of the phenotypic variance in the [(AKR × B6) *Min*/+ F1 × B6] backcross estimated 1.8 segregating genetic factors influencing tumor multiplicity [45]. By genotyping 110 of these [(AKR × B6) *Min*/+ F1 × B6] backcross mice with a set of SSLP markers, evidence was obtained for linkage of a modifier locus, *modifier of Min 1* (*Mom1*), to markers on distal chromosome 4. Analysis of two additional backcrosses between B6 *Min* and MA or CAST, indicated that *Mom1* affected tumor multiplicity in these crosses as well. When the results of these three crosses were pooled, a combined LOD score of 14 in the interval between *D4Mit12* and *D4Mit13* was obtained [45]. This 20-cm *Mom1* region of chromosome 4 is syntenic with a region of human chromosome 1 that includes the 1p35–36 subregion frequently deleted in colorectal tumors [31] and hypothesized to contain a modifier of FAP [30].

Table 1 Effect of *Mom1* on Tumor Multiplicity in Backcrosses

Cross	F1 mice	Backcross	
		Mom1 heterozygotes	*Mom1* homozygotes
B6 × (SWR × B6 *Min*/+)	3.1 ± 2.0 (48)	8.5 ± 4.1 (26)	19.1 ± 8.9 (21)
B6 × (DBA × B6 *Min*/+)	14.0 ± 5.5 (25)	14.0 ± 7.5 (28)	29.0 ± 12.6 (30)
B6 × (BALB × B6 *Min*/+)	11.7 ± 5.5 (6)	18.5 ± 10.7 (23)	27.9 ± 16.6 (14)
B6 × (BTBR × B6 *Min*/+)	21.0 ± 9.7 (21)	40.0 ± 12.0 (38)	38.4 ± 14.8 (32)
B6 × (129 × B6 *Min*/+)	15.2 ± 6.7 (9)	27.9 ± 13.4 (11)	32.9 ± 19.9 (14)

Note. The average tumor multiplicity (± standard deviation) of each class is given. The number of *Min*/+ mice scored for each class is indicated in parentheses. B6 *Min*/+ mice develop, on average, 29 ± 10 tumors in the regions counted [32].

These crosses indicated that the AKR, CAST, and MA strains each carry a resistance allele at *Mom1*, whereas B6 carries a susceptibility allele [45]. The AKR, CAST, and MA strains also each carry resistance alleles at unmapped loci not linked to *Mom1* [41, 45]. In the 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 [45].

Determining the *Mom1* allele carried by another inbred strain involved two steps: (1) testing whether the strain carries any dominant modifiers of *Min* by comparing tumor number in F1 progeny with the B6 *Min* mice and (2) testing by backcross analysis whether any dominant modifier maps to the same region as *Mom1*. Mapping a modifier to the *Mom1* region in this way strongly suggests that it is identical with *Mom1*. By contrast, no information about *Mom1* phenotype can be drawn based on tumor multiplicity in F1 mice, since modifiers unlinked to *Mom1* clearly exist [41, 45]. Thus, it is crucial to use a backcrosses or intercross to assign allelic status in a polygenic trait, in which phenotypic modification could reflect the action of any of several unlinked loci. The situation requires considerably more care than for simple mendelian traits with distinct qualitative phenotypes.

To characterize the inbred strains SWR, DBA, BALB, 129, and BTBR, we crossed females of the given strain to B6 *Min*/+ males and counted tumors in the resulting *Min*/+ F1 progeny [46]. To determine whether any reduction in tumor multiplicity mapped to the *Mom1* region, we backcrossed the *Min*/+ F1 mice to B6 and compared the distribution of tumor number in backcross progeny heterozygous across the *Mom1* region with that observed in progeny homozygous for the B6 alleles across this region.

The F1 *Min*/+ mice showed a significant reduction in average tumor multiplicity with four strains: SWR, DBA, BALB, and 129 [46] (Table 1), indicating the presence of a dominant modifier allele. No reduction of tumor number was seen in the F1 progeny with BTBR. In three of the four strains showing resistance in the F1 progeny, the backcross revealed a clear effect

attributable to genotype in the *Mom1* region (Table 1). These results indicate that SWR, DBA, and BALB each carry a dominant modifier that maps to the *Mom1* region. By contrast, the *Mom1* region had no detectable effect on tumor multiplicity in the backcross with 129. The most likely explanation for this observation is that the dominant resistance seen in the (129 × B6) F1 progeny is due to unlinked modifier loci (Table 1). The results of the 129 cross illustrate the necessity of the backcross test in determining the allelic status at *Mom1*. Finally, the *Mom1* region had no detectable effect on tumor multiplicity in the backcross with BTBR. Based on these analyses, we conclude that AKR, MA, CAST, SWR, DBA, and BALB each carry semi- or fully dominantly acting resistance alleles ($Mom1^R$), while B6, BTBR, and 129 carry recessively acting susceptibility alleles ($Mom1^S$) [46].

THE B6.$Mom1^{AKR}$ STRAIN

To study *Mom1* further, it was essential to obtain a strain carrying a resistance allele at *Mom1* and free of other modifier alleles. At the time this work was initiated, none of the inbred strains characterized met these criteria. Therefore, we produced a mouse line in which the *Mom1* region from the *AKR* strain ($Mom1^{AKR}$) is carried on the sensitive B6 genetic background [47]. This B6.$Mom1^{AKR}$ strain has permitted further characterization of *Mom1* and provided a valuable tool for subsequent biological experiments relating to *Mom1*.

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 attempted to speed up the process of elimination of heterozygosity by using a marker-assisted selection (MAS) method [48–50]. The MAS method tries to achieve a more rapid reduction in heterozygosity by identifying and breeding the mice in each generation that have inherited less than 50% of remaining heterozygosity. The identification of animals most advantageous for breeding is accomplished by genotyping mice in each backcross generation with SSLP markers.

Using the MAS strategy, we constructed a strain in which we selected for a 35-cM region encompassing the *D4Mit12–D4Mit13* interval hypothesized to contain *Mom1* [47]. A total of 69 SSLP markers, approximately 3 markers per chromosome, were used for counterselection through the N6 generation. In each backcross generation, a small number of *Mom1* heterozygotes were selected on the basis having inherited the fewest AKR alleles unlinked to *Mom1*. At the N6 generation, one *Mom1* heterozygote that was homozygous for the B6 allele at all 69 counterselection markers was chosen to produce the N7 generation of the B6.$Mom1^{AKR}$ strain.

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*) F1 animals were produced by crossing N7 $Mom1^{AKR/B6}$ females with B6 *Min*/+ males. The average tumor number in $Mom1^{AKR/B6}$ *Min*/+ mice was 13.1. This value is significantly different from the average of 26.1 observed in their $Mom1^{B6/B6}$ *Min*/+ littermates [47]. These results suggest that the region of chromosome 4 from AKR in this strain does carry $Mom1^{AKR}$ and that this allele confers approximately a twofold reduction in average tumor multiplicity in *Min*/+ mice. Similar results were obtained in crosses at the N3 generation of the B6.*Mom1 AKR* line, suggesting that all AKR modifiers unlinked to *Mom1* had been removed by the N3 generation.

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. The average tumor multiplicity of the $Mom1^{AKR/B6}$ *Min*/+ mice from this F2 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 of the F1 generation described above [47]. This indicates that the B6.$Mom1^{AKR}$ line does not carry out 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*/+ F2 mice is the same as the average of 26.1 tumors in the $Mom1^{B6/B6}$ *Min*/+ F1 mice above. The F2 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 [47]. Thus, homozygosity for $Mom1^{AKR}$ confers a fourfold reduction in tumor multiplicity relative to $Mom1^{B6}$ homozygotes.

To determine whether $Mom1^{AKR}$ influences tumor size, we measured tumors from the *Min*/+ F2 mice described above. The size of each tumor was estimated by measuring its maximum diameter. There was a significant correlation between *Mom1* genotype and tumor size in the small intestine. Average maximum 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 [47]. This result suggests that *Mom1* affects the size of tumors of the small intestine in a semidominant fashion. The average size of tumors from the large intestine of *Min*/+ mice was not influenced by the *Mom1* genotype [47]. 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.

Since *Mom1* affects average tumor size, we hypothesized that the number of visible tumors in mice carrying $Mom1^{AKR}$ would increase over time. Indeed, the average tumor multiplicity in $Mom1^{AKR/B6}$ *Min*/+ increased sig-

nificantly from 10.7 at 80 days of age, to 14.7 at 120 days of age to 15.3 at 200 days of age [47]. No significant increase in tumor multiplicity was observed in B6 *Min* mice over this time frame. To begin to assess whether the *Mom1* genotype may affect net growth rate, we determined the average maximum diameter of small intestinal tumors from $Mom1^{AKR/B6}$ and $Mom1^{B6/B6}$ mice at 60, 80, 100, and 120 days of age. Over these time points, the rate of increase in net tumor size in $Mom1^{AKR}$ heterozygotes was considerably slower than in B6 *Min* mice, suggesting that *Mom1* may affect the net growth rate of adenomas in *Min*/+ mice.

EVALUATION OF CANDIDATE GENES FOR *Mom1*

Many genes map to distal mouse chromosome 4 and could be considered as candidates for the *Mom1* locus. MacPhee et al. have suggested an attractive candidate gene: the secretory phospholipase *Pla2g2a* [51], which is expressed in Paneth cells and might affect prostaglandin biosynthesis or defense mechanisms against intestinal [52, 53]. The genetic evidence initially offered in favor of *Pla2g2a* as *Mom1* was limited to two observations: *Pla2g2a* maps to the same region of approximately 30 Mb containing *Mom1* and has a frameshift mutation in B6 but not in AKR, MA, and CAST. However, the reported *Mom1* region is likely to contain several hundred to a thousand genes, and many are likely to have mutations or variants specific to B6. Indeed, another interesting candidate gene in the region, the GTPase-activating protein Rap-1*GAP*, also maps to the region and shows amino acid variants specific to B6.

Formal genetic evaluation of candidate genes for a quantitative modifier locus is a challenging problem that has received little attention. Given a candidate gene that maps to the correct region and shows a specific mutation (or variant) in strains carrying a particular allele at the modifier locus, one can begin to test the candidate in two ways: (1) study additional strains to determine if genotype at the candidate accurately predicts the allelic state of the modifier locus; and (2) perform fine-structure mapping experiments to test whether the candidate gene and the modifier locus can be separated by recombination.

To this end, we compared the *Mom1* phenotypes of the nine mouse strains we had previously characterized to the genotypes at the *Pla2g2a* and Rap-1*GAP* genes. Using a PCR assay to distinguish these alleles, we determined the genotypes of the strains in which the *Mom1* allele had been characterized. The six strains (AKR, MA, CAST, SWR, DBA, and BALB) with a resistance allele $Mom1^{R}$ all carried the wild-type $Pla2g2a^{+}$ allele, while the three strains (B6, BTBR, and 129) with the susceptible allele $Mom1^{S}$ all carried the mutant $Pla2g2a^{-}$ allele [46] (Table 2).

Table 2 Summary of analysis of inbred strains

Inbred strain	*Mom1*	*Pla2s*	*Rap-1GAP*
B6	S	–	B6
AKR	R	+	AKR
CAST	R	+	AKR
MA	R	+	AKR
SWR	R	+	AKR
DBA	R	+	AKR
BALB	R	+	AKR
BTBR	S	–	B6
129	S	–	B6

While a single instance of discordance between *Pla2g2a* genotype and *Mom1* genotype would exclude *Pla2g2a* as a candidate, perfect concordance in the nine strains does not provide definitive support for the hypothesis. The *Pla2g2a* mutation almost surely arose on a single ancestral chromosome that was subsequently transmitted to B6, BTBR, and 129. The actual *Mom1* susceptibility allele may be a different variation on this ancestral chromosome, at a locus close enough to have remained in linkage disequilibrium with *Pla2g2a* [54]. In fact, linkage disequilibrium can be seen for another gene that maps to the *Mom1* region, Rap-*1GAP*, which encodes a GTPase-activating protein specific for p21Rap1, a member of the Ras superfamily of GTPases [55]. Analysis of the nine inbred strains studied revealed that, just as for *Pla2g2a*, there was perfect concordance between genotype at Rap-1*GAP* and *Mom1* phenotype in these strains [46] (Table 2).

To localize more finely the position of *Mom1* on distal chromosome 4 and to test the *Pla2g2a* and Rap-*1GAP* candidates more thoroughly, we generated a fine-structure map of $Mom1^{AKR}$. To carry out fine-structure mapping of *Mom1*, we used mice from the B6. $Mom1^{AKR}$ line to perform a B6 $Mom1^{AKR/B6}$ × B6 cross and identified progeny in which recombination had reduced the AKR region present.

Lines carrying each recombinant chromosome (in a heterozygous state over B6) were established and tested to determine whether the recombinant chromosome carried the resistance allele from AKR or susceptibility allele from B6. Specifically, females from each recombinant line were crossed to B6 *Min*/+ males, and tumors were counted in *Min*/+ progeny. In contrast to the situation for simple Mendelian traits, one must test a population of progeny to ascertain the *Mom1* allele of a recombinant chromosome. If progeny inheriting the recombinant chromosome showed a distribution of tumor multiplicities that was not significantly different from that of sibs carrying the B6 chromosome (but was significantly higher than that seen in age-matched B6 $Mom1^{AKR/B6}$ *Min*/+ mice produced from the B6.$Mom1^{AKR}$ line), the recombinant chromosome was inferred to carry the susceptibility

allele *Mom1*^{B6}. Conversely, if these progeny showed a distribution of tumor multiplicities that was significantly lower than that for sibs inheriting the B6 chromosome (but was not significantly different from that of age-matched B6 *Mom1*$^{AKR/B6}$ *Min*/+ controls), then the recombinant chromosome was inferred to carry the resistance allele *Mom1*^{AKR}. In this fashion, *Mom1* was genetically mapped relative to the breakpoints of the recombinant chromosomes.

Eight lines carrying recombinant chromosomes were characterized [46] (Figure 1). The *Mom1* allele carried on each recombinant chromosome was determined as described above. For six of the lines, the phenotype was consistent with carrying either *Mom1*^{AKR} or *Mom1*^{B6} – that is, it was consistent with either a twofold reduction or no reduction in tumor number. Lines 2, 6, 26, and 29 were determined to carry *Mom1*^{AKR} [46] (Table 3). Lines 20 and 21 were determined to carry *Mom1*^{B6} [46] (Table 3). From the *Mom1* genotype of the recombinant chromosomes, the *Mom1* locus must lie between the recombination breakpoints of lines 2 and 20. In particular, *Mom1* maps to the 4-cM interval between *D4Mit54* and *D4Mit13* [46] (Figure 1).

To further evaluate *Pla2g2a* and Rap-1*GAP*, we used genetic mapping. We first used an (AKR × B6) F2 intercross and an (AKR × B6) × B6 backcross to localize both of these genes to the 4-cM interval defined by *D4Mit54* and *D4Mit13* [46] (Figure 1). We then used the recombinant lines described above to map the candidate genes relative to *Mom1*. *Pla2g2a* was recombinationally inseparable from *Mom1* in these lines, with *Pla2g2a*$^{+}$ being present in the lines carrying *Mom1*^{R}, and *Pla2g2a*$^{-}$ being present in those carrying *Mom1*^{S} [46] (Figure 1). In contrast, Rap-1*GAP* had recombined with *Mom1* in one recombinant line: line 2 carries the B6 allele at Rap-1*GAP* but carries the *Mom1*^{R} allele [46] (Figure 1). This single recombination event eliminates Rap-1*GAP* as a candidate for *Mom1*. This result underscores the fact that a perfect correlation of phenotype in inbred mouse strains with allelic status at a candidate gene does not provide reliable proof of identity.

Two recombinant lines (25 and 32) failed to give a result that was fully consistent with carrying either *Mom1*^{AKR} or *Mom1*^{B6}: the average tumor multiplicity in the recombinant heterozygotes was intermediate to, but significantly different from, both age-matched, contemporaneous *Mom1*^{B6} and *Mom1*$^{AKR/B6}$ controls [46] (Table 3). Since line 32 is a derivative of line 25, we assume that both lines 25 and 32 carry the same recombination breakpoint in the interval between *D4Mit64* and *D4Mit284*. The intermediate phenotype in line 25 and its derivative line 32 raises the possibility that *Mom1* may represent a complex locus consisting of two or more genes. The recombination event between *D4Mit64* and *D4Mit284* carried in these two lines may have separated these genes, such that the recombinant chromosomes would carry AKR alleles at only one (or some) of these genes and thus exhibit a partial effect of *Mom1*^{AKR}. Further investigation of these lines is important,

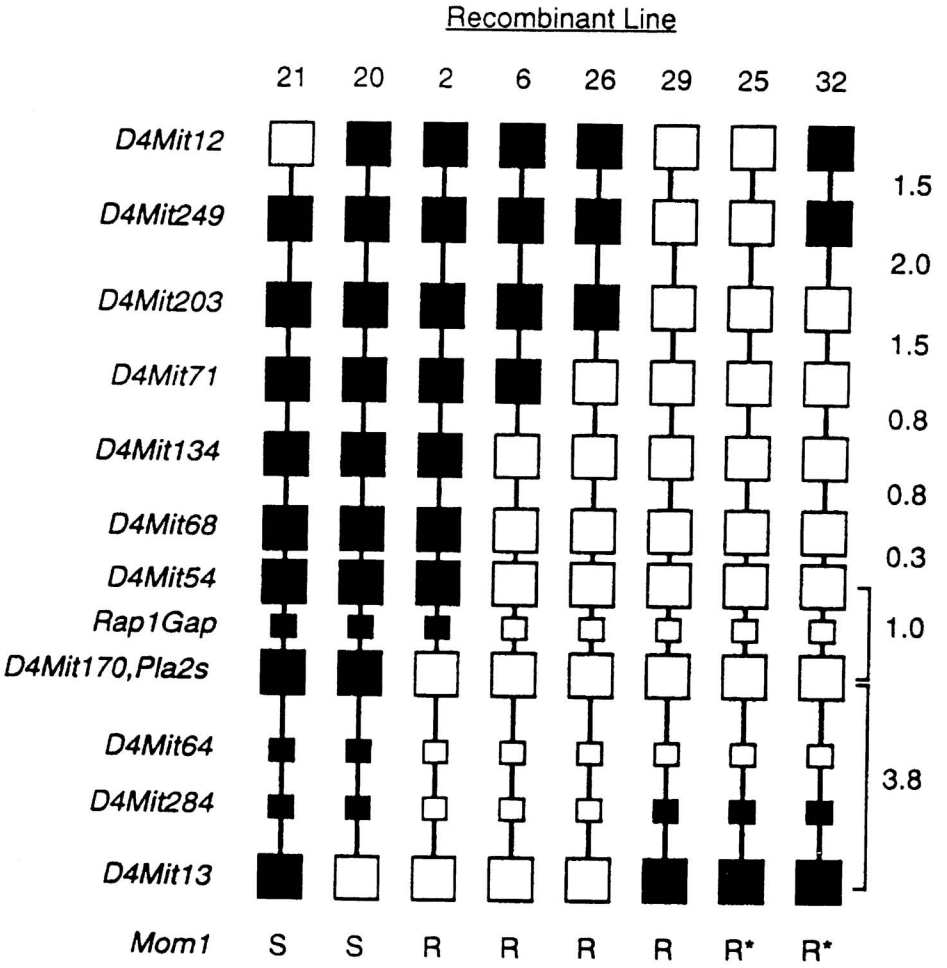

Figure 1 Genotypes of recombinant lines in the *Mom1* region. For each recombinant line, the open squares indicate positions where each line is heterozygous AKR/B6 and the filled squares indicate positions at which each line is homozygous B6. Small squares indicate a gene or marker that is known to map within the interval shown, but the precise position within the interval is not known. For each line, the deduced *Mom1* phenotype is classified as sensitive (S), resistant (R), or partially resistant (R*). The genetic distance (in cM) for each interval is given on the right of the figure.

since any complexity of *Mom1* would affect both the design and interpretation of further tests of any single candidate locus. Based on the recent mapping of two additional genes encoding secretory phospholipases to the *Mom1* region [56], it is of particular interest to investigate the possibility that these genes may influence tumor multiplicity in *Min* mice.

To test more directly the hypothesis that *Mom1* encodes *Pla2g2a*, a transgenic mouse line in which *Pla2g2a*^{AKR} is carried on the B6 genetic background was generated [57]. The transgene, a 41.1-kb cosmid clone from an AKR genomic library, contains the entire *Pla2g2a* gene and at least 10.9 kb of

Table 3 Tumor Multiplicity Data from Crosses with the Recombinant Lines

Cross	Recombinant heterozygotes	Aged-matched controls: Recombinant homozygotes	p value	Congenic heterozygotes	p value	*Mom1* phenotype
Rec21 × Min	30.3 ± 12.8 (17)	32.2 ± 7.1 (14)	0.23	15.0 ± 7.8 (77)	$< 1 \times 10^{-6}$	S
Rec20 × Min	24.9 ± 8.6 (34)	29.6 ± 10.2 (24)	0.04[a]	10.7 ± 5.8 (11)	2.2×10^{-5}	S
Rec2 × Min	10.7 ± 4.7 (23)	24.6 ± 10.8 (29)	$<1 \times 10^{-6}$	10.7 ± 5.8	0.35	R
Rec6 × Min	13.0 ± 5.9 (24)	31.2 ± 10.6 (13)	6×10^{-6}	15.0 ± 7.8	0.15	R
Rec26 × Min	12.1 ± 6.9 (12)	27.9 ± 9.2 (11)	5×10^{-4}	10.7 ± 5.8	0.43	R
Rec29 × Min	17.8 ± 10.5 (18)	30.2 ± 13.3 (25)	3×10^{-4}	15.0 ± 7.8	0.494	R
Rec25 × Min	21.5 ± 6.9 (20)	30.1 ± 12.8 (14)	0.025[b]	15.0 ± 7.8	3.4×10^{-3}	R*
Rec32 × Min	15.8 ± 5.9 (12)	23.4 ± 8.5 (10)	9×10^{-3}	10.7 ± 5.8	0.02[c]	R*

Note. The average tumor multiplicity (± standard deviation) of each class is given. The number of *Min*/+ mice scored from each class is indicated in parentheses. Mice from crosses with Rec lines 21, 6, 29, and 25 were scored at 120 days of age. Mice from Rec lines 20, 2, 26, and 32 were scored at 80 days of age. The deduced *Mom1* phenotype of each line is indicated as S (sensitive), R (resistant), or R* (partially resistant).

[a] To determine whether this result was significant or not, we compared tumor multiplicities between Rec20 heterozygotes and a large group ($N = 73$) of age-matched $Mom1^{B6/B6}$ mice. The difference in tumor multiplicities between these two groups was not significant (24.2 versus 26.5, $p = 0.17$).

[b] To determine whether this result was significant or not, we compared tumor multiplicities between Rec25 heterozygotes and a large group ($N = 55$) of age-matched $Mom1^{B6/B6}$ mice. The difference in tumor multiplicities between these two groups was significant (21.5 vs 31.4, $p = 2.5 \times 10^{-4}$).

[c] To determine whether this result was significant or not, we compared tumor multiplicities between Rec32 heterozygotes and a large group ($N = 73$) of age-matched $Mom1^{B6/B6}$ mice. The difference in tumor multiplicities between these two groups was significant (15.8 vs 26.5, $p = 1.4 \times 10^{-4}$).

flanking sequence in each direction. The transgenic line carries approximately nine copies of the cosmid. Northen blot and Western blot analysis suggests that transgenic mice express $Pla2g2a^{AKR}$, which is absent in B6 mice. The spatial pattern of expression of *Pla2g2a* within the intestine was determined by immunohistochemistry with an antibody directed against *Pla2g2a*. The pattern of *Pla2g2a* expression within the intestinal crypts of transgenic mice was similar but not identical to that in non transgenic $Mom1^{AKR}$ heterozygotes.

To determine the effect of the transgene on the tumor phenotype in *Min* mice, transgenic males were crossed to tB6 $Mom1^{AKR/B6}$ *Min*/+ females. The resulting *Min*/+ progeny carrying either the $Mom1^{AKR}$ allele or the transgene displayed a two fold reduction in tumor multiplicity and a decrease in tumor size as compared to their $Mom1^{B6}$ homozygous siblings. Thus, the qualitative and quantitative effects of the transgene on the tumor phenotype of *Min* mice are similar to that of $Mom1^{AKR}$. Given this, it is tempting to conclude that the transgene contains *Mom1*, and that *Mom1* is *Pla2g2a*. However under this hypothesis, it is surprising that the transgene, with its nine copies of the *Pla2g2a*-containing cosmid, does not confer a larger reduction in tumor multiplicity than a single copy of $Mom1^{AKR}$. Similarly, the observation that tumor multiplicity in B6 *Min* mice carrying both the transgene and $Mom1^{AKR}$ does not differ significantly from mice carrying either factor alone is also unexpected, given that *Mom1* has been shown to be semidominant. These discrepancies may be due to some feature of the transgene such as abnormal spatial or temporal expression of *Pla2g2a*. Although this explanation may be the most plausible, it is possible that these observations reflect complexity at the *Mom1* locus or the disruption of dosage-sensitive enhancer of the *Min* phenotype due to transgene integration. Analysis of additional *Pla2g2a* transgenic lines and a targeted mutation of the wild-type allele of *Pla2g2a* may facilitate resolution of this issue.

Mom1 AND *Pla2g2a*

The mechanism by which *Pla2g2a* might reduce the size and number of adenomas in *Min* mice is unclear. A priori, based on the known biological activities of *Pla2g2a*, a number of possibilities exist. For example, the effect of *Pla2g2a* may be mediated by its role in promoting the production of prostaglandins. However, inhibition of prostaglandin synthesis reduces tumor size and multiplicity in mice with *Apc* mutations [58, 59]. Given this, one would expect that the mutant *Pla2g2a* allele in the B6 strain would correlate with resistance to tumor development rather than sensitivity.

Biological experiments investigating the mechanism of action of *Mom1* may shape our thinking about how *Pla2g2a* might affect the *Min* phenotype. For example, using intestinal isografts, we have shown that *Mom1* action is

not dependent on ingested material or the presence of bacteria within the gut [60]. Thus, any effect of *Pla2g2a* on tumor multiplicity could not be mediated through direct metabolism of dietary lipids and would not be dependent on its bactericidal properties [53]. These experiments also indicated that *Mom1* is not a systematically acting factor [60]. This observation suggests that the ability of *Pla2g2a* to influence tumor multiplicity is due to its expression in Paneth cells and not to other cell types, such as mast cells, neutrophils, and fibroblasts, that also secrete this phospholipase [52, 61]. These conclusions would also apply to the two linked phospholipase genes, if they are indeed components of *Mom1*.

The effects of $Mom1^{AKR}$ thus appear to be autonomous to the small intestine at the level of the whole tissue [60]. However, these experiments do not assess autonomy of action at the cellular level. Therefore, if either of these genes is notautonomous at the cellular level, it would involve a short range of action, acting in a paracrine or autocrine manner within the intestine. To investigate this possibility, further, we have generated two complementary sets of mouse aggregation chimeras that differ with respect to which cell population ($Min/+$ or $+/+$) is homozygous for $Mom1^{AKR}$ and which cell population ($Min/+$ or $+/+$) is homozygous for $Mom1^{B6}$: $Mom1^{B6/B6}$ $Min/+ \leftrightarrow Mom1^{AKR/AKR}+/+$ and $Mom1^{AKR/AKR}$ $Min/+ \leftrightarrow Mom1^{B6/B6}+/+$.) (62). Tumors from these chimeras were enumerated and the percentage of $Min/+$ cells in the intestine of each chimera was determined using a quantitative PCR-based assay for genotyping at the *Apc* locus. From these data, we calculated an expected tumor multiplicity for a composition of 100% $Min/+$ cells. These normalized tumor multiplicity distributions were compared to populations of control $Mom1^{AKR/AKR}$ $Min/+$ and $Mom1^{B6/B6}$ $Min/+$ mice. The $Mom1^{AKR/AKR}$ $Min/+$ and $Mom1^{AKR/AKR}$ $Min/+ \leftrightarrow Mom1^{B6/B6}+/+$ mice showed a significant reduction in tumor number relative to both $Mom1^{B6/B6}$ $Min/+$ and $Mom1^{B6/B6}$ $Min/+ \leftrightarrow Mom1^{AKR/AKR}+/+$ mice [62]. These comparisons indicate that the presence of $Mom1^{AKR}$ in the $Min/+$ lineage results in a reduction in the number of *Min*-induced tumors in these mice. By contrast, the $Mom1^{AKR}$ allele in the $+/+$ lineage does not result in any detectable reduction in the number of tumors that develop from the juxtaposed $Min/+$ lineages ($Mom1^{B6/B6}$) [62]. Based on the fact that two lineages are highly intermixed in the chimeras, we conclude that *Mom1* acts to influence tumor multiplicity in a localized fashion and not over substantial distances within the intestine.

Within the intestinal epithelium, it is difficult to distinguish between a gene that acts in a non-cell-autonomous fashion between cells derived from a single crypt and a gene that acts in a strictly cell autonomous fashion. Remembering that crypts are clonal units, the most conservative interpretation of our result is that *Mom1* acts autonomously within the lineage derived from a crypt (crypt lineage autonomous).

If the *Mom1* gene product is *Pla2g2a*, we predict a non-cell-autonomous action for *Mom1*. Therefore, under the hypothesis that *Mom1* encodes this secretory molecule, the result of analysis of chimeric mice requires that *Pla2g2a* influences tumor development from within the tumorigenic crypt lineage. How might the local level of *Pla2g2a* secreted by the Paneth cells within the crypt influence the development of adenomas in *Min* mice? If tumors in *Min* mice arise from the single, ultimate stem cell within the crypt, the only local source of *Pla2g2a* would be the small number of differentiated Paneth cells within the tumor. In this scenario, there would be selection for allelic loss at *Pla2g2a*. However, heterozygosity in the *Mom1* region, and at *Pla2g2a* specifically, is maintained in adenomas from *Min* mice [44, 46]. Alternatively, if tumors in *Min* mice arise from just one of several multipotential precursor cells present within the crypt, a second local but clonally related source of *Pla2g2a* would exist; normal multipotential precursor cells within the crypt would generate the Paneth cells that secrete *Pla2g2a* from the base of the crypt. In these nonneoplastic cells, there would be no selection for loss of the resistance allele of *Mom1*. This interpretation connects the current data regarding the mechanism of action of *Mom1*, the evidence that *Mom1* encodes a secretory phospholipase, and the fact the *Mom1* region, and *Pla2g2a* in particular, maintain heterozygosity in *Min*-induced adenomas.

REFERENCES

1. Cheng H, Leblond CP. Origin, differentiation, and renewal of the four main epithelial cell types in the mouse small intestines, V: unitarian theory of the origin of the four epithelial cell types. Am J Anat. 1974;141:537–562.
2. Potten CS, Loeffler M. Stem cells: attributes, cycles, spirals, pitfalls and uncertainties: lessons for and from the crypt. Development. 1990;110:1001–1020.
3. Kirkland S. Clonal origin of columnar, mucous, and endocrine cell lineages in human colorectal epithelium. Cancer. 1987;61:1359–1363.
4. Vogelstein B, Fearon ER, Hamilton SR, et al. Genetic alterations during colorectal-tumor development. N Engl J Med. 1988;319:525–532.
5. Powell SM, Zilz N, Beazer-Barclay Y, et al. *APC* mutations occur early during colorectal tumorigenesis. Nature. 1992;359:235–237.
6. Smith KJ, Johnson KA, Bryan TM, et al. The *APC* gene product in normal and tumor cells. Proc Natl Acad Sci USA. 1993;90:2846–2850.
7. Bourne HR. Consider the coiled coil. Nature. 1991;351:188–190.
8. Groden J, Thliveris A, Samowitz W, et al. Identification and characterization of the familial adenomatous polyposis coli gene. Cell. 1991;66:589–600.
9. Kinzler KW, Nilbert MC, Su L-K, et al. Identification of FAP locus genes from chromosome 5q21. Science. 1991;253:661–665.
10. Joslyn G, Richardson DS, White R, Alber T. Dimer formation by an N-terminal coiled coil in the APC protein. Proc Natl Acad Sci USA. 1993;90:11109–11113.
11. Su L-K, Johnson KA, Smith KJ, Hill DE, Vogelstein B, Kinzler KW. Association between wild type and mutant APC gene product. Cancer Res. 1993;53:2728–2731.
12. Rubinfeld B, Souza B, Albert I, et al. Association of *APC* gene product with β-catenin. Science. 1993;262:1731–1734.
13. Su L-K, Vogelstein B, Kinzler KW. Association of APC tumor suppressor protein with catenins. Science. 1993;262:1734–1737.

14. Korinek V, Barker N, Morin PJ, et al. Constitutive transcriptional activation by a β-catenin-Tcf complex in *APC*-/-colon carcinoma. Science. 1996;275:1784–1787.
15. Morin PJ, Sparks AB, Korinek V, et al. Activation of β-catenin-Tcf signalling in colon cancer by mutations in β-catenin or *APC*. Science. 1996;275:1787–1790.
16. Shoemaker AR, Gould KA, Luongo C, Moser AR, Dove WF. Studies of neoplasia in the Min mouse. Biochim Biophys Acta. 1996;1332:f25–f48.
17. Matsumine A, Ogai A, Senda T, et al. Binding of APC to the human homolog of the *Drosophila* discs large tumor suppressor protein. Science. 1996;272:1020–1023.
18. Woods DF, Bryant PJ. The discs-large tumor suppressor gene of *Drosophila* encodes a guanylate kinase homolog localized at septate junctions. Cell. 1995;66:451–464.
19. Munemitsu S, Souza B, Müller O, Albert I, Rubinfeld B, Polakis P. The *APC* gene product associates with microtubules in vivo and promotes their assembly in vitro. Cancer Res. 1994;54:3676–3681.
20. Smith KJ, Levy DB, Maupin P, Pollard TD, Vogelstein B, Kinzler KW. Wild-type but not mutant *APC* associates with the microtubule cytoskeleton. Cancer Res. 1994;54:3672–3675.
21. Nishisho I, Nakamure Y, Miyoshi Y, et al. Mutations of chromosome 5q21 genes in FAP and colorectal cancer patients. Science. 1991;253:665–669.
22. Miyoshi Y, Nagase H, Ando H, et al. Somatic mutations of the *APC* gene in colorectal tumors: mutation cluster region in the *APC* gene. Hum Mol Genet. 1992;1:229–233.
23. Nagase H, Nakamura Y. Mutations of the *APC* (adenomatous polyposis coli) gene. Hum Mutat. 1993;2:425–434.
24. Leppert M, Burt R, Hughes JP, et al. Genetic analysis of an inherited predisposition to colon cancer in a family with a variable number of adenomatous polyps. N Engl J Med. 1990;322:904–908.
25. Spirio L, Olschwang S, Groden J, et al. Alleles of the *APC* gene: an attenuated form of familial polyposis. Cell. 1993;75:1–20.
26. Olschwang S, Tiret A, Laurent-Puig P, Muleris M, Parc R, Thomas G. Restriction of ocular fundus lesions to a specific subgroup of *APC* mutations in adenomatous polyposis coli patients. Cell. 1993;75:959–968.
27. Dobbie Z, Spycher M, Mary J-L, et al. Correlation between the development of extracolonic manifestations in FAP patients and mutations beyond codon 1403 in the *APC* gene. J Med Genet. 1996;33:274–280.
28. Caspari R, Olschwang S, Friedl W, et al. Familial adenomatous polyposis: desmoid tumours and lack of opthalmic lesions (CHRPE) associated with *APC* mutations beyond codon, 1444. Hum Mol Genet. 1995;4:337–340.
29. Paul P, Letteboer T, Gelbert L, Groden J, White R, Coppes MJ. Identical *APC* exon 15 mutations result in a variable phenotype in familial adenomatous polyposis. Hum Mol Genet. 1993;2:925–931.
30. Tomlinson IPM, Neale K, Talbot IC, et al. A modifying locus for familial adenomatous polyposis may be present on chromosome 1p35-p36. J Med Genet. 1996;33:268–273.
31. Leister I, Weith A, Brüderlein S, et al. Human colorectal cancer: high frequency of deletions at chromosome 1p35. Cancer Res. 1990;50:7232–7235.
32. Moser AR, Pitot HC, Dove WF. A dominant mutation that pedisposes to multiple intestinal neoplasia in the mouse. Science. 1990;247:322–324.
33. Su L-K, Kinzler KW, Vogelstein B, et al. Multiple intestinal neoplasia caused by a mutation in the murine homolog of the *APC* gene. Science. 1992;256:688–670.
34. Luongo C, Gould KA, Su LK, et al. Genomics. 1993;15:3–8.
35. Moser AR, Shoemaker AR, Connelly CS, et al. Homozygosity for the *Min* allele of *Apc* results in disruption of mouse development prior to gastrulation. Dev. Dynamics. 1995;203:422–433.
36. Moser AR, Dove WF, Roth KA, Gordon, JI. The *Min* (multiple intestinal neoplasia) mutation: its effect on gut epithelial cell differentiation and interaction with a modifier system. J Cell Biol. 1992;116:1517–1526.
37. Novelli MR, Williamson JA, Tomlinson IP, et al. Polyclonal origin of colonic adenomas in an XO/XY patient with FAP. Science. 1996;272:1187–1190.
38. Merritt AJ, Gould KA, Dove WF. Polyclonal structure of intestinal adenomas in $Apc^{Min/+}$ mice with concomitant loss of Apc^{+} from all tumor lineages. Proc Natl Acad Sci USA. 1997;94:13927–13931.
39. Luongo C, Moser AR, Gledhill S, Dove WF. Loss of Apc^{+} in intestinal adenomas from Min mice. Cancer Res. 1994;54:5947–5952.

40. Luongo C, Dove WF. Somatic genetics events linked to the *Apc* locus in intestinal adenomas of the Min mouse. Genes, Chrom Cancer. 1996;17:194–198.
41. Shoemaker AR, Moser AR, Midgley CA, Dove WF. Alternative pathways of adenoma formation in $Apc^{Min/+}$ mice: *Apc* silencing versus dominant enhancement of *Apc* allele loss. Submitted.
42. Dove WF, Gould KA, Luongo C, Moser A, Shoemaker AR. Emergent issues in the genetics of intestinal neoplasia. Cancer Surveys. 1995;25:335–355.
43. Shoemaker AR, Moser AR, Dove WF. *N*-Ethyl-*N*-nitrosourea treatment of multiple intestinal neoplasia (Min) mice: age-related effect on the formation of intestinal adenomas, cystic crypts, and epidermoid cysts. Cancer Res. 1995;55:4479–4485.
44. Dove WF, Luongo C, Connelly CS, et al. The adenomatous polyposis gene of the mouse in development and neoplasia. In: Cold Spring Harbor Symposia on Quantitative Biology: The Molecular Genetics of Cancer. Cold Spring Harbor: Cold Spring Harbor Press; 1994:501–508.
45. Dietrich WF, Lander ES, Smith JS, et al. Genetic identification of *Mom1*, a major modifier locus affecting *Min*-induced intestinal neoplasia in the mouse. Cell. 1993;75:631–639.
46. Gould KA, Luongo C, Moser AR, et al. Genetic evaluation of candidates genes for the *Mom1* modifier of intestinal neoplasia in mice. Genetics. 1996;144:1777–1785.
47. Gould KA, Dietrich WF, Borenstein N, Lander ES, Dove WF. *Mom1* is semi-dominant modifier of intestinal adenoma size and multiplicity in *Min*/+ mice. Genetics. 1996;144:1769–1777.
48. Lande R, Thompson R. Efficiency of marker-assisted selection in the improvement of quantitative traits. Genetics. 1989;124:743–756.
49. Hillel J, Schaap T, Haberfeld A, et al. DNA fingerprints applied to gene introgression in breeding programs. Genetics. 1989;124:783–789.
50. Hospital F, Chevalet C, Mulsant P. Using markers in gene introgression breeding programs. Genetics. 1992;132:1199–1210.
51. MacPhee M, Chepenik KP, Liddell RA, Nelson KK, Siracusa LD, Buchberg AM. The secretory phospholipase A2 gene is a candidate for the *Mom1* locus, a major modifier of Apc^{Min}-induced intestinal neoplasia. Cell. 1995;81:957–966.
52. Mulherkar R, Desai SJ, Rao RS, Wagle AS, Deo MG. Expression of enhancing factor gene and its localization in mouse tissues. Histochemistry. 1991;96:367–370.
53. Harwig SS, Tan L, Qu XD, Cho Y, Eisenhauer PB, Lehrer RI. Bactericidal properties of murine intestinal phospholipase A_2. J Clin Invest. 1995;95:603–610.
54. Frankel W. Taking stock of complex trait genetics in mice. Trends Genet. 1995;11:471–477.
55. Rubinfeld B, Munemitsu S, Clark R, et al. Molecular cloning of GTPase activating protein specific for the Krev-1 protein $p21^{rap1}$. Cell. 1991;65:1033–1042.
56. Tischfield JA, Xia Y-R, Shih DM, et al. Low-molecular weight, calcium dependent phospholipase A_2 genes are linked and map to homologous chromosome regions in mouse and human. Genomics. 1996;32:328–333.
57. Cormier RT, Hong K, Halberg RB, et al. Secretory phospholipase Pla2g2a confers resistance to intestinal tumorigenesis. Nature Genet. 1997;17:88–91.
58. Boolbol SK, Dannenberg AJ, Chadburn A, et al. Cyclooxygenase-2 overexpression and tumor formation are blocked by sulindac in a murine model of familial adenomatous polyposis. Cancer Res. 1996;56:2556–2560.
59. Jacoby RF, Marshall DJ, Newton MJ, et al. Chemoprevention of spontaneous intestinal adenomas in the Apc^{Min} mouse model by the nonsteroidal anti-inflammatory drug piroxicam. Cancer Res. 1996;56:710–714.
60. Gould KA, Dove WF. Action of *Min* and *Mom1* in ectopic intestinal grafts. Cell Growth and Differ. 1996;7:1361–1368.
61. Kudo I, Murakami M, Hara, Shuntaro, Inoue K. Mammalian non-pancreatic phospholipases A_2. Biochim Biophys Acta. 1993;117:217–231.
62. Gould KA, Dove WF. Localized Action of *Min* and *Mom1* in intestinal neoplasia. Proc Natl Acad Sci USA. 1997;94:5848–5853.