Gastrointestinal Tumor Phenotypes in Offspring of Collaborative Cross Mice and a Sensitized Line

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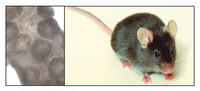
ABSTRACT

Stomach, small intestine, colon, and rectal cancers affect hundreds of thousands of people worldwide each year. Gastrointestinal (GI) cancer initiation, growth, and progression depend on genetic and environmental factors. The Adenomatous Polyposis Coli *(APC)* gene is one of the top 5 genes somatically-mutated in colon and rectal cancers. Germline mutations in the *APC* gene cause Familial Adenomatous Polyposis (FAP), an autosomal dominant disorder; patients with FAP develop hundreds to thousands of colon and rectal adenomas, which if left untreated, progress to cancer. Adenoma number and time of onset can vary between family members carrying the same mutation in the *APC* gene. The Collaborative Cross (CC) strains were developed as a powerful resource to dissect genetic factors affecting complex disorders. We designed a screen to determine whether the genomic diversity in selected CC strains could result in novel GI tumor phenotypes. Females from 10 CC strains were mated with males from the sensitized, double congenic 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + line which we generated. These congenic mice have a long life span and develop few adenomas with a high probability of progressing to adenocarcinomas. Hybrid F1 offspring were aged and the GI tract evaluated for tumor phenotypes, including location, number, shape, and size. The results revealed a diversity of GI tumor phenotypes, which develop due to combinations of alleles in the CC strains and the double congenic line. These findings indicate the presence of tumor-suppressing and tumor-promoting modifier loci in the genomes of CC strains. This exploratory work builds a foundation for future studies to identify new modifier genes that effectively suppress polyposis and potentially serve as therapeutic targets for GI cancers. Research supported by NCI R21 CA202496 to LDS.

MOUSE MODELS OF MUTANT *Apc*-INDUCED INTESTINAL POLYPOSIS AND MODIFIER LOCI



C57BL/6J (B6) *Apc^{Min}*/+
80-100 polyps

129S1/SvImJ (129) *Apc^{Min}*/+
10-15 polyps

Figure 1. Genetic background influences tumorigenesis in *Apc^{Min}*/+ mice. C57BL/6J (B6) *Apc^{Min}*/+ mice exhibit 80-100 polyps by 90-110 days of age within the entire intestinal tract, with the highest incidence in the distal small intestine and distal colon. B6 *Apc^{Min}*/+ mice usually die at 5-6 months of age due to severe anemia and intestinal blockage. 129S1/SvImJ (129) mice congenic for the *Apc^{Min}* mutation exhibit 10-15 polyps by 153-205 days of age and have a much longer lifespan than B6 *Apc^{Min}*/+ mice.

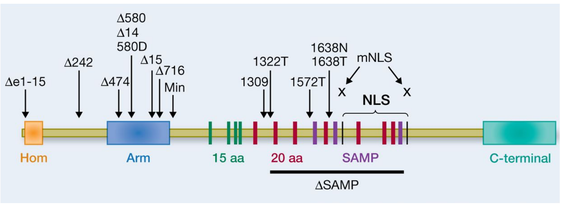


Figure 2. Structure of the murine Apc protein and mutation sites in common mouse models. Arrows indicate the locations of different *Apc* mutations. The *Apc^{Min}* mouse was the product of a chemical mutagenesis screen that produced a germline transversion in codon 850, resulting in premature protein truncation. Domains of Apc are Hom, homodimerization; Arm, Armadillo repeats; serine–alanine–methionine–proline (SAMP), axin binding; NLS, nuclear localization signals and C-terminal. The Mutation Cluster Region (MCR) is located between codons 1250 and 1464. (Zeineldin and Neufeld, *Cancer Research*, 2013)

Modifier of *Min 1 (Mom1)* Locus: The *Mom1* phenotype is due to a mutation in the *Pla2g2a* gene on mouse chromosome 4. One wildtype *Pla2g2a^{Mom1R}* allele decreases polyp number by 50% in *Apc^{Min}*/+ mice.

Modifier of *Min 2 (Mom2*) Locus: The *Mom2* phenotype is due to a mutation in the *Atp5a1* gene on mouse chromosome 18. One mutant *Atp5a1^{Mom2R}* allele decreases polyp number by 90% in B6 *Apc^{Min}*/+ mice. The resistant *Mom2* allele functions only when in *cis* with the *Apc^{Min}* mutation.

METHODS: CROSS CC FEMALES WITH MALES FROM A NEWLY ESTABLISHED SENSITIZED CONGENIC LINE

- C57BL/6J (B6) have a high polyp incidence which causes complications, such as gastrointestinal blockage and anemia, and consequently they die relatively young in life at 5-6 months of age.
- The need for a long-lived mouse model to study tumor progression in the context of aging led us to create the 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + line.
- The whole intestinal tract of mice of the 4 possible genotypes was evaluated to quantify tumor characteristics such as number, size, polyp burden. Note: only mice with 2 genotypes *Apc^{Min}*/+ and *Apc^{Min} Atp5a1^{Mom2R}*/+ + develop polyps in the intestinal tract.

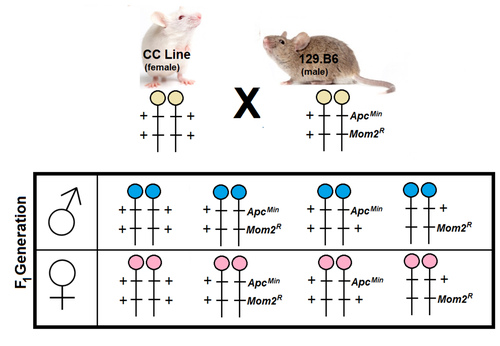


Figure 3. F1 offspring genotypes of interest. Congenic 129.B6 *Apc^{Min} Mom2^{R}*/+ + mice were created by sequential backcrossing of B6 *Apc^{Min} Mom2^{R}*/+ + mice with mice from the 129S1/SvImJ inbred strain. For the polyposis phenotype screen, females from different CC strains were outcrossed with 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + males to produce the 4 classes of hybrid offspring shown above.

AVERAGE POLYP NUMBER IN THE SMALL INTESTINE OF 129.B6 AND CC HYBRIDS

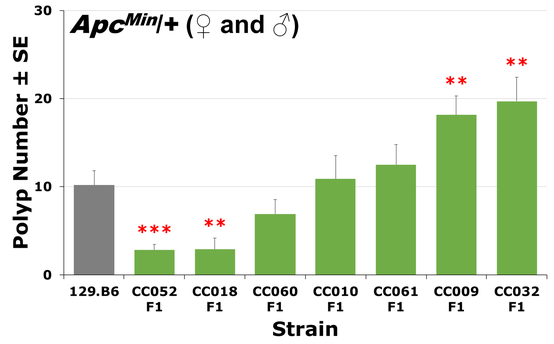


Figure 4. Average polyp number in hybrid CC offspring compared to 129.B6 *Apc^{Min}*/+ control. F1 mice (♀ and ♂) from crosses of CC strain females to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + males were aged to 250+ days. A total of 88 mice were analyzed, with 6 to 15 mice per group. The whole intestinal tract was dissected and analyzed under a microscope. Quantitation of polyp number in mice is shown above. **GREY** bar shows 129.B6 *Apc^{Min}*/+ mice and **GREEN** bars show F1 *Apc^{Min}*/+ mice. Data are average polyp number ± standard error. The Student's t-test was used for comparisons of polyp number in F1 mice to 129.B6 *Apc^{Min}*/+ controls.

[*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$]

AVERAGE POLYP SIZE IN THE SMALL INTESTINE OF 129.B6 AND CC HYBRIDS

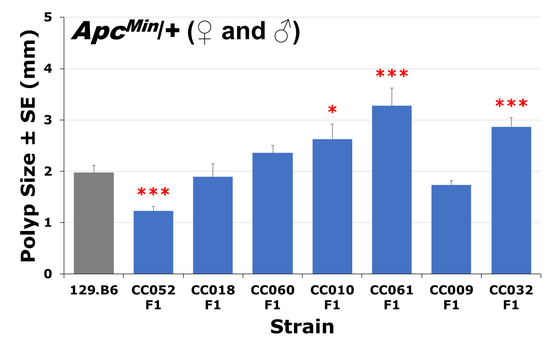


Figure 6. Average polyp size of hybrid CC offspring compared to 129.B6 *Apc^{Min}*/+ control. F1 mice (♀ and ♂) from crosses of CC strain females to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + males were aged to 250+ days. A total of 80 mice were analyzed, with 6 to 15 mice per group. The whole intestinal tract was dissected and analyzed under a microscope. Quantitation of polyp size in mice is shown above. **GREY** bar shows 129.B6 *Apc^{Min}*/+ mice and **BLUE** bars show F1 *Apc^{Min}*/+ mice. Data are average polyp size ± standard error. The Student's t-test was used for comparisons of polyp number in F1 mice to 129.B6 *Apc^{Min}*/+ controls.

[*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$]

AVERAGE POLYP BURDEN IN THE SMALL INTESTINE OF 129.B6 AND CC HYBRIDS

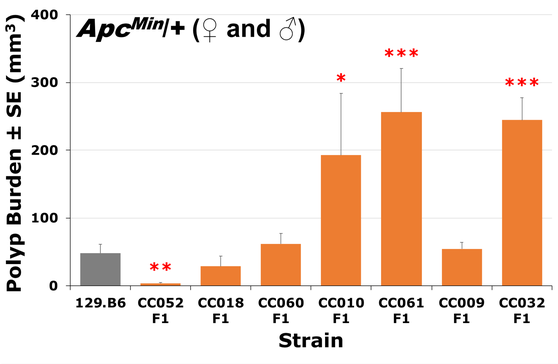


Figure 8. Average polyp burden of hybrid CC offspring compared to 129.B6 *Apc^{Min}*/+ control. F1 mice (♀ and ♂) from crosses of CC strain females to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + males were aged to 250+ days. A total of 80 mice were analyzed, with 6 to 15 mice per group. The whole intestinal tract was dissected and analyzed under a microscope. Quantitation of polyp burden in mice is shown above. **GREY** bar shows 129.B6 *Apc^{Min}*/+ mice and **ORANGE** bars show F1 *Apc^{Min}*/+ mice. Data are average polyp burden ± standard error. The Student's t-test was used for comparisons of polyp number in F1 mice to 129.B6 *Apc^{Min}*/+ controls.

[*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$]

DATABASES

- Mouse Genome Informatics www.informatics.jax.org/
- Mouse Phylogeny Viewer msub.csbio.unc.edu/
- UNC Systems Genetics csbio.unc.edu/CCstatus/index.py

AVERAGE POLYP NUMBER IN THE SMALL INTESTINE OF 129.B6 AND CC HYBRIDS

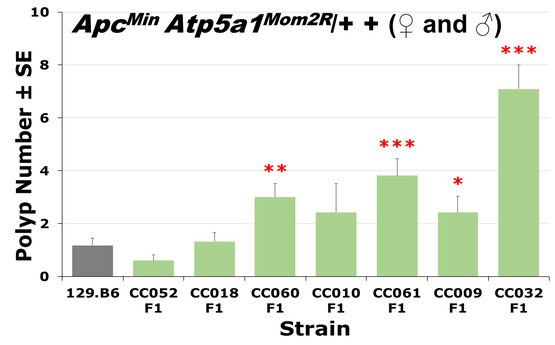


Figure 5. Average polyp number in hybrid CC offspring compared to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + control. F1 mice (♀ and ♂) from crosses of CC strain females to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + males were aged to 250+ days. A total of 89 mice were analyzed, with 6 to 20 mice per group. The whole intestinal tract was dissected and analyzed under a microscope. Quantitation of polyp number in mice is shown above. **GREY** bar shows 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice and **LIGHT GREEN** bars show F1 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice. Data are average polyp number ± standard error. The Student's t-test was used for comparisons of polyp number in F1 mice to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + controls.

[*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$]

AVERAGE POLYP SIZE IN THE SMALL INTESTINE OF 129.B6 AND CC HYBRIDS

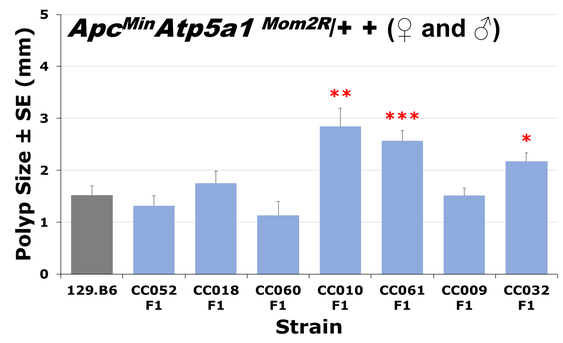


Figure 7. Average polyp size of hybrid CC offspring compared to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + control. F1 mice (♀ and ♂) from crosses of CC strain females to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + males were aged to 250+ days. A total of 74 mice were analyzed, with 5 to 20 mice per group. The whole intestinal tract was dissected and analyzed under a microscope. Quantitation of polyp size in mice is shown above. **GREY** bar shows 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice and **LIGHT BLUE** bars show F1 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice. Data are average polyp size ± standard error. The Student's t-test was used for comparisons of polyp number in F1 mice to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + controls.

[*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$]

AVERAGE POLYP BURDEN IN THE SMALL INTESTINE OF 129.B6 AND CC HYBRIDS

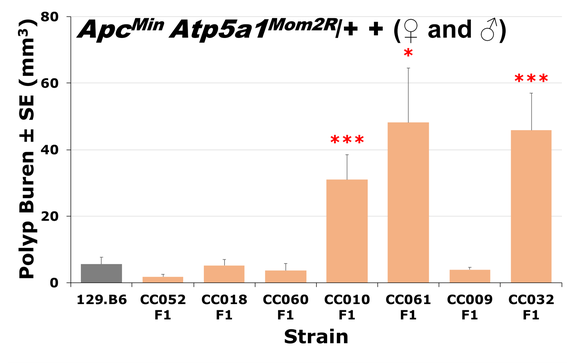


Figure 9. Average polyp burden of hybrid CC offspring compared to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + control. F1 mice (♀ and ♂) from crosses of CC strain females to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + males were aged to 250+ days. A total of 74 mice were analyzed, with 5 to 20 mice per group. The whole intestinal tract was dissected and analyzed under a microscope. Quantitation of polyp burden in mice is shown above. **GREY** bar shows 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice and **LIGHT ORANGE** bars show F1 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice. Data are average polyp burden ± standard error. The Student's t-test was used for comparisons of polyp number in F1 mice to 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + controls.

[*$p \le 0.05$, **$p \le 0.01$, ***$p \le 0.001$]

DEDICATION

In loving memory of **Liza Michaela Dorfman, M.S.** July 25, 1989 - February 9, 2019. As a dedicated graduate student at Thomas Jefferson University, Liza made significant contributions to this project.

Apc^{Min}/+ vs *Apc^{Min} Atp5a1^{Mom2R}*/+ + POLYPOSIS IN CC F1 HYBRID MICE

- There was a 89% decrease in polyp number in 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice compared to 129.B6 *Apc^{Min}*/+ mice. The reduction in polyp number for all hybrid CC *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice compared to their *Apc^{Min}*/+ littermates ranged from 54 - 87%.
- There was a 23% decrease in polyp size in 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice compared to 129.B6 *Apc^{Min}*/+ mice. The decrease in polyp size for hybrid CC *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice compared to their *Apc^{Min}*/+ littermates ranged from 7 - 52% for CC018, CC060, CC061, CC009, and CC032. In contrast, an 8.5% increase in polyp size in *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice compared to *Apc^{Min}*/+ mice was noted for CC052 and CC010.
- There was a 88% reduction in polyp burden in 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice compared to 129.B6 *Apc^{Min}*/+ mice. The reduction in polyp burden for hybrid CC *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice compared to their *Apc^{Min}*/+ littermates ranged from 52 - 94%.

COLON POLYPS

Table 1. Characteristics of Colon Polyps in Hybrid CC F1 Offspring

#	Strain	Genotype	Days of Age	# of Mice	Average # of Polyps	Average Size of Polyps (mm)
1	129.B6	*Apc^{Min}*/+	250+	5	2	1.3
2	CC010 F1	*Apc^{Min}*/+	250+	1	1	1.7
3	CC061 F1	*Apc^{Min} Atp5a1^{Mom2R}*/+ +	250+	1	1	0.7
4	CC032 F1	*Apc^{Min}*/+	250+	1	1	2.0
		Apc^{Min} Atp5a1^{Mom2R}/+ +	250+	1	1	3.4

YELLOW highlights colon polyps in the congenic 129.B6 line.

F1 *Apc^{Min}*/+ and *Apc^{Min} Atp5a1^{Mom2R}*/+ + POLYP PHENOTYPES COMPARED TO 129.B6 MICE

Table 2. *Apc^{Min}*/+ and *Apc^{Min} Atp5a1^{Mom2R}*/+ + Polyp Phenotypes in Hybrid CC F1 Offspring Compared to Control 129.B6 Mice

Strain	Days of Age	*Apc^{Min}*/+ POLYP PHENOTYPE			*Apc^{Min} Atp5a1^{Mom2R}*/+ + POLYP PHENOTYPE		
		# of Polyps	Size of Polyps	Burden of Polyps	# of Polyps	Size of Polyps	Burden of Polyps
129.B6	250+	Control	Control	Control	Control	Control	Control
CC052 F1	250+	Down	Down	Down	-	-	-
CC018 F1	250+	Down	-	-	-	-	-
CC060 F1	250+	-	-	-	Up	-	-
CC010 F1	250+	-	Up	Up	-	Up	Up
CC061 F1	250+	-	Up	Up	Up	Up	Up
CC009 F1	250+	Up	-	-	Up	-	-
CC032 F1	250+	Up	Up	Up	Up	Up	Up

YELLOW highlights polyp phenotypes in 129.B6 controls. GREEN with "Down" - Average polyp number in the intestinal tract of hybrid CC mice is lower than in 129.B6 *Apc^{Min}*/+ *mice*. RED with "Up" - Average polyp number in the intestinal tract of hybrid CC mice is higher than in 129.B6 *Apc^{Min}*/+ controls. "-" No difference in polyp number, size, or burden between hybrid CC mice and 129.B6 mice.

CONCLUSIONS

- **We used an unbiased approach to screen CC strains to expand the range of known intestinal polyposis phenotypes, with the goal of identifying unique models for human cancer.**
- **Our sensitized congenic 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + line crossed to mice from seven CC strains achieved the goal of having long-lived (250+ days of age) *Apc^{Min}*/+ and *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice which develop polyposis to serve as models for human cancer, since most sporadic gastrointestinal tumors in humans occur in the later decades of life.**
- **Hybrid CC052 mice exhibited the lowest amount of polyposis of all CC strains tested, with consistently lower polyp number, size, and burden than 129.B6 *Apc^{Min}*/+ and 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice.**
- **Hybrid CC032 mice exhibited the highest number of polyps of all CC strains tested, with consistently larger polyp number, size, and burden than 129.B6 *Apc^{Min}*/+ and 129.B6 *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice.**
- **The most dramatic differences in polyp number were observed between CC052 F1 (low) and CC032 F1 (high) mice. There was a 7-fold difference between the *Apc^{Min}*/+ groups and a 12-fold difference between the *Apc^{Min} Atp5a1^{Mom2R}*/+ + groups.**
- **The most dramatic (3-fold) difference in polyp size was observed between CC052 F1 (low) and CC061 (high) F1 mice between the *Apc^{Min}*/+ groups; the same 3- fold difference in polyp size was observed between CC060 F1 (low) and CC010 (high) F1 mice between the *Apc^{Min} Atp5a1^{Mom2R}*/+ + groups.**
- **The most dramatic differences in polyp burden were observed between CC052 F1 (low) and CC061 (high) F1 mice. There was a 69-fold difference between the *Apc^{Min}*/+ groups and a 27-fold difference between the *Apc^{Min} Atp5a1^{Mom2R}*/+ + groups.**
- **Interestingly, although CC009 F1 *Apc^{Min}*/+ and *Apc^{Min} Atp5a1^{Mom2R}*/+ + mice exhibited high polyp numbers, these mice had smaller polyps which consequently led to low polyp burdens.**
- **Stomach polyps were rare and observed only in CC061 F1 mice (Data not shown).**
- **Colon polyps were observed in a small subset of 129.B6 mice as well as in some CC010, CC061, and CC032 F1 mice.**
- **This exploratory work builds a foundation to identify new modifier genes that can effectively suppress polyposis and potentially serve as novel targets for therapeutic intervention.**

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