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SHORT COMMUNICATION

System genetic analysis of intestinal cancer and periodontitis development as influenced by aging and diabesity using Collaborative Cross mice

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Abstract

It is increasingly recognized that young, chow-fed inbred mice poorly model the complexity of human carcinogenesis. In humans, age and adiposity are major risk factors for malignancies, but most genetically engineered mouse models (GEMM) induce carcinogenesis too rapidly to study these influences. Standard strains, such as C57BL/6, commonly used in GEMMs, further limit the exploration of aging and metabolic health effects. A similar challenge arises in modeling periodontitis, a disease influenced by aging, diabesity, and genetic architecture. We propose using diverse mouse populations with hybrid vigor, such as the Collaborative Cross (CC)×*Apc*^{Min} hybrid, to slow disease progression and better model human colorectal cancer (CRC) and comorbidities. This perspective highlights the advantages of this model, where delayed carcinogenesis reveals interactions with aging and adiposity. Unlike *Apc*^{Min} mice, which develop cancer rapidly, CC×*Apc*^{Min} hybrids recapitulate human-like progression. This facilitates the identification of modifier loci affecting inflammation, diet susceptibility, organ size, and polyposis distribution. The CC×*Apc*^{Min} model offers a transformative platform for studying CRC as a disease of adulthood, reflecting its complex interplay with aging and comorbidities. The insights gained from this approach will enhance early detection, management, and treatment strategies for CRC and related conditions.

KEYWORDS
Aging and intestinal cancer, gene identification of aging and cancer, gene maping, type 2 diabetes and intestinal cancer

1 | INTRODUCTION

Genome-wide association studies (GWAS) have identified numerous common alleles that affect cancer susceptibility.[1,2] Still, there is accumulating evidence that the majority of the risk for heritable diseases cannot be explained by the combined impact of these genes. This implies that the hereditary component of cancer risk cannot be unraveled using only approaches designed to identify the main effects of individual alleles in human populations.[2] Examining the effects of genetic modifiers is best done using genetically engineered mouse models (GEMM) of cancer.[2] Modifier loci are those that exhibit epistatic behavior with identified vulnerability loci. Because of

the huge sample size needed, epistasis in human GWAS is difficult to identify. On the contrary, it is relatively easier to employ GEMMs with an established vulnerability alteration that increases disease risk. Modifier loci can be mapped by breeding the altered mouse with a set of mice with distinct genetic backgrounds of naturally occurring diversity.[2]

Adenomatous polyposis coli (*Apc*) is a classical tumor suppressor protein located on band q21 of the long arm of human chromosome 5 (5q21) and encodes a large protein.[1] The *Apc* gene product indirectly regulates transcription of several critical cell proliferation genes by interacting with the transcription factor β-catenin.[3–5] *Apc* also interacts with numerous actin and microtubule-associated proteins.[4,5] Roles for *Apc* in cell migration have been demonstrated in vitro and in mice.[5,6] *Apc* mutations are detected in the germline of cases of famelial adenomatous polyposis (FAP) and in more than 80% of sporadic colorectal adenomas.[2,7] Adenomas are the precursors of the majority of colorectal malignancies (colorectal cancer [CRC]).[8,9] Numerous mouse strains exhibiting heterozygous germline mutations of *Apc*, which develop intestinal polyps, exist.[2,4,10,11] Variations in illness severity are associated with distinct alterations in polyp formation. For instance, $Apc^{Min/+}$ mice typically develop ~100 polyps between 3 and 4 months of age, whereas the *Apc* (1322 T) model produces more aggressive polyps at a faster rate. In contrast, the *Apc* (1638 N) model develops only a few polyps, often at more than a year of age.[10–12] Additionally, *Apc*-mutant females exhibit poor maternal behavior, necessitating the use of males for breeding before they become affected by the polyp burden.[13]

Although the cell biology of *Apc* in mice almost perfectly recapitulates that of human *Apc*, there are two major limitations of the $Apc^{Min/+}$ mouse model. First, in human FAP, polyposis and carcinogenesis primarily occur in the colon, whereas in the mouse model, tumors primarily form in the small intestine.[14] Second, in human FAP, the average age of onset of malignancy is 39—polyposis, when monitored aggressively, can be observed in teenagers.[15] Thus, although being born heterozygous for a mutation in *Apc* converts CRC from a disease of the aged to one of young adulthood, the corresponding mutation in mice produces polyposis at 4 weeks, malignancies at the onset of reproductive maturity and cancer cachexia by ~16 weeks.[16,17] Thus, although it is abundantly clear that age and adiposity are major contributors to human colon cancer,[18] this has not been effectively modeled in mice. CRC predominantly affects adults, with aging and metabolic dysfunction as critical drivers. Existing models, such as Apc^{Min} mice, fail to capture this gradual progression due to their rapid onset of advanced disease. This limitation restricts their utility for studying the interplay between aging, sarcopenia, and CRC. The Collaborative Cross (CC)×Apc^{Min} hybrid model addresses this gap, leveraging hybrid vigor to slow carcinogenesis and mimic human-like disease progression. This model offers a unique platform to explore the genetic and environmental modifiers of CRC in the context of aging.

Previous studies using CC×Apc^{Min} hybrids identified genetic modifiers, such as Modifier of Min (Mom) loci, which influence tumor distribution and severity.[2] These findings have guided investigations into analogous pathways in humans, offering insights into genetic factors that may underlie CRC susceptibility in diverse populations. The translational relevance of these modifiers underscores the utility of Collaborative Cross (CC) models in uncovering genetic interactions that are challenging to observe in human studies due to population heterogeneity. Mom1 encodes secretory phospholipase A2, a protein implicated in inflammation and cancer biology in both mouse and human studies, emphasizing its translational relevance.[19] Similarly, modifiers like Mom2, which affect ATP synthase components, align with metabolic vulnerabilities observed in human CRC.[5] These parallels highlight the utility of the CC×Apc^{Min} hybrid model in identifying conserved pathways critical to CRC susceptibility.

Whereas the CC mouse population offers advantages for studying genetic diversity and complex traits, models like Diversity Outbred (DO) mice and recombinant inbred (RI) populations provide complementary benefits. DO mice, with extensive genetic variability and higher rates of recombination, are well suited for detailed genetic mapping, and F1 hybrids derived from DO mice offer valuable models for studying human-like genetic diversity and gene–environment interactions.[20] RI strains have also contributed to "experimental precision medicine," as described by Simon et al.[21] These models integrate genetic, phenotypic, and environmental data to tackle complex biological questions. Whereas this study emphasizes the unique ability of CC×Apc^{Min} hybrids to delay carcinogenesis and mimic human CRC progression, findings from DO and RI models could further enhance translational research in CRC and related conditions.[21]

2 | IDENTIFYING INTESTINAL CANCER–ASSOCIATED GENES IN HUMAN-ASSOCIATION STUDIES

Genetic research on human cancer risk aims to identify genes linked to occurrence or nonoccurrence of disease that largely begins with the identification of single-nucleotide polymorphisms (SNP) and the selection of candidate genes with the potential to explain modification of the rates, extent, and severity of carcinogenesis.[22] Indeed, "hypothesis-free" GWAS have expedited the investigation of human illness genetic risk components. Currently (data presented in Table 1 for cancer, Table 2 for aging, and Table 3 for periodontitis [PD]), a small number of GWASs employing SNP arrays with additional imputation have been carried out to find germline polymorphisms linked to intestinal cancer (IC) susceptibility.[36] Furthermore, a small number of IC cases have been used in large-scale genome sequencing investigations to identify somatic alterations that possibly connected to the emergence of IC.[37] At this time, the majority of the genetic risk associated with IC cannot be explained by the combined impact of these genes. Further, even though a traditional GWAS technique can find genetic variations linked to complex disorders, this method may ignore a large number of risk-modifying alleles because of the strict significance criteria required to reduce the false discovery rate.[38] Therefore, it is challenging to identify several genetic loci with

TABLE 1 This table presents the most significant genes associated with intestinal cancer (IC), identified using genome-wide association studies specific to IC.

Title of the study	Samples and size	Ancestry	Trait(s)	Mapped genes	Variant and risk allele	*p*	Chromosome position (bp)	Reference
Genome-wide study on 72298 individuals in Korean biobank data for 76 traits.	251000 East Asian ancestry individuals	East Asian (Japan)	Colon carcinoma	*RPL6P14, CXXC4-AS1*	chr1:222148971-?	4×10^{-11}	chr1:222148971-?	[23]
				NHLRC1	chr5:134457675-?	2×10^{-13}	chr5:134457675-?	
				CARMIL1, SCGN	chr8:117632965-?	3×10^{-9}	chr8:117632965-?	
				PPP1R17	chr8:117715457-?	8×10^{-9}	chr8:117715457-?	
				CYRIA, GACAT3	chr8:128421128-?	1×10^{-13}	chr8:128421128-?	
				SMARCC1	chr10:8734761-?	1×10^{-16}	chr10:8734761-?	
				TERT	chr10:104867686-?	4×10^{-8}	chr10:104867686-?	
				NHLRC1	chr10:114306441-?	9×10^{-10}	chr10:114306441-?	
				HLA-W, HLA-A	chr12:57540848-?	4×10^{-8}	chr12:57540848-?	
				P2RX3	chr12:112468206-?	6×10^{-10}	chr12:112468206-?	
				TMEM263-DT, RPL30P12	chr18:46453156-?	4×10^{-19}	chr18:46453156-?	
				ATP8B4	chr20:7784672-?	4×10^{-10}	chr20:7784672-?	
				ZFHX3	chr20:57432606-?	3×10^{-8}	chr20:57432606-?	
Genomic analysis of germline variation associated with survival of colorectal cancer patients treated with chemotherapy plus biologics in CALGB/SWOG 80405 (alliance).	613 European ancestry individuals	European (Nuclear response [NR])	Metastatic colorectal cancer survival in treatment with chemotherapy plus biologics	*MGST1*	rs916264-A	1×10^{-6}	12:16521286	[24]
				AXIN1	rs1327265-A	4×10^{-6}	16:309567	
				STMN2, HEY1	rs17014720-T	5×10^{-6}	8:79677902	
				KCNS3, RDH14	rs1571695-A	5×10^{-6}	2:18553898	
				ANO9	rs10051946-?	6×10^{-6}	11:436461	
				KCNS3, MSGN1	rs13253048-?	8×10^{-6}	2:17858241	
Performance of the use of genetic information to assess the risk of colorectal cancer in the Basque population.	624 European	235 Basque ancestry cases, 389 Basque ancestry controls	Rectum cancer, colonic neoplasm	*CNTNAP2*	rs73171906-T	5×10^{-6}	7:148289437	[25]
				XKR5	rs9773025-G	8×10^{-31}	8:6816936	
				DYRK1A	rs79619562-C	2×10^{-9}	21:37370120	
Genome-wide association study for predictors of progression-free survival in patients on capecitabine, oxaliplatin, bevacizumab and cetuximab in first-line therapy of metastatic colorectal cancer.	256 cases treated with CAPOX-B plus cetuximab, 264 cases treated with CAPOX-B	520 European (NR)	Progression-free survival, metastatic colorectal cancer, response to cetuximab, response to CAPOX-B	AGPAT5, XKR5	rs2936519-A	2×10^{-7}	8:6781719	[26]
				OARD1	rs2073016-T	4×10^{-6}	6:41053183	
				ARHGEF4	rs4377367-C	6×10^{-6}	2:130967332	
				MGAT4A	rs885036-A	2×10−8	2:98688331	

TABLE 1 (Continued)

Title of the study	Samples and size	Ancestry	Trait(s)	Mapped genes	Variant and risk allele	*p*	Chromosome position (bp)	Reference
Predictive polygenic score for outcome after first-line oxaliplatin-based chemotherapy in colorectal cancer patients using supervised principal component analysis.	338 European ancestry cases with oxaliplatin treatment, 96 European ancestry cases with other treatment, 8 Asian ancestry cases with oxaliplatin treatment, 26 African American cases with oxaliplatin treatment, 10 African American cases with other treatment, 26 cases with oxaliplatin treatment, 1 case with other treatment	434 European (NR), 8 Asian unspecified (NR), 36 African American or Afro-Caribbean (NR), 27 NR (NR)	Overall survival in metastatic stage IV colorectal cancer×oxaliplatin treatment interaction	PLPP3, LINC01767	rs56027745-?	5×10^{-8}	1:56456747	[27]
				MYT1L	rs115226504-?	5×10^{-8}	2:2178514	
				HMGN1P19, EPS15P1	rs11767153-?	5×10^{-8}	7:46735188	
GWAS identifies two novel colorectal cancer loci at 16q24.1 and 20q13.12.	6692 Japanese ancestry cases, 27 178 Japanese ancestry controls	33 870 East Asian (Japan)	Colorectal cancer	CASC20, LINC01713	rs4813802-?	6×10^{-6}	20:6718948	[28]
				MYRF, TMEM258	rs174537-G	2×10^{-7}	11:61785208	
				CABLES2	rs2427308-C	2×10^{-6}	20:62394395	
				LINC01705, LINC02474	rs6687758-?	2×10^{-8}	1:221991606	
				LINC00536, EIF3H	rs2450115-T	5×10^{-7}	8:116611854	
				ZMIZ1-AS1	rs704017-?	7×10^{-6}	10:79059375	
				RNA5SP299, LINC02676	rs11255841-T	2×10^{-14}	10:8697617	
				CCAT2, PCAT1, CASC8, POU5F1B	rs6983267-?	1×10^{-19}	8:127401060	
				SMAD7	rs7229639-?	3×10^{-12}	18:48924606	
				TCF7L2	rs11196172-A	4×10^{-10}	10:112967084	
				PLCH1	rs114436839-A	1×10^{-6}	3:155691561	
				LINC02917, MBNL1	rs75853696-G	3×10^{-6}	3:152228136	
				PLA2G10FP, SPRING1P3	rs1453514-G	1×10^{-6}	16:16899146	
				PCAT1, CASC8, POU5F1B	rs12682374-C	7×10^{-29}	8:127398703	
				SMAD7	rs4939567-G	1×10^{-22}	18:48925503	
				VTI1A	rs12241008-C	3×10^{-12}	10:112520943	
				CCND2-AS1	rs4572213-T	2×10^{-9}	12:4256383	
				LINC02474, LINC01705	rs6691195-A	3×10^{-10}	1:221989031	
				PITX1-AS1	rs254563-A	1×10^{-9}	5:135104736	
				LINC01728, TOX2	rs6065668-T	4×10^{-11}	20:43904181	
				LINC01228, DYNLRB2-AS1	rs4407073-T	7×10^{-8}	16:80022910	

(Continues)

TABLE 1 (Continued)

Title of the study	Samples and size	Ancestry	Trait(s)	Mapped genes	Variant and risk allele	p	Chromosome position (bp)	Reference
				AADACL2-AS1, LINC02917	rs2723362-C	7×10^{-6}	3:151958534	
				LINC00536, EIF3H	rs2450115-T	9×10^{-7}	8:116611854	
				SLCO2A1	rs61510274-G	1×10^{-6}	3:134030671	
				CALB1, LINC00534	rs2189261-G	1×10^{-6}	8:90159507	
				LINC01082, LINC01081	rs847208-A	3×10^{-9}	16:86220445	
				GOLGA8B	rs4924045-C	3×10^{-6}	15:34585308	
				TCF7L2	rs11196172-A	1×10^{-9}	10:112967084	
				FADS2	rs174594-A	2×10^{-13}	11:61852357	
				RNA5SP299, LINC02676	rs9733843-A	1×10^{-14}	10:8676041	

significant synergistic, additive, or epistatic effects through current GWASs. This limitation arises because GWASs primarily detect additive effects due to its reliance on statistical models designed to associate SNPs with traits independently. In contrast, synergistic and epistatic effects result from complex interactions between multiple loci, which are difficult to detect without extremely large sample sizes and specialized models. An alternate approach involves identifying these undiscovered relationships between genes or between genes and the environment in laboratory animals during various stages of the emergence of IC. The loci/genes corresponding to these interactions could then be studied in humans to validate their relevance.

3 | DOES MoM HOLD THE ANSWER TO THIS PROBLEM?

There has been a three-decade genetic approach to identify mouse loci that alter either the anatomical location or the frequency of polyposis in the $Apc^{Min/+}$ background. The first-identified *Mom* gene, Modifier of Min 1 (*Mom1*) expresses secretory phospholipase A2, an enzyme unique to Paneth cells whose loss of function increases tumorigenesis in the intestinal epithelia.[10,39,40] Thus, *Mom1* has shown that non-cell-autonomous mechanisms can alter *Apc* carcinogenesis. The second identified modifier, *Mom2*, inactivates ATP5a1, an essential component of ATP synthase. Because *Atp5a1* loss-of-function mutations fall on the same chromosome arm as *Apc*, the incidence of polyposis is suppressed because intestinal cells that undergo homozygous loss-of-function mutation in an essential gene are inviable.[5,41,42] Additional *Mom* loci have been identified, though they have yet to be cloned.[43] Because the genetic backgrounds of inbred strains profoundly affect polyp counts,[6,43] it is highly likely that additional modifiers are present. These modifiers may influence a range of phenotypic traits, including not only the total number of polyps but also additional characteristics such as the rate of progression to malignancy and the specific locations within the large intestine where polyps tend to develop.[43] A recent study experimentally accelerates alveolar bone loss in PD in obese mice and affects the inflammatory response to the oral pathogen *Porphyromonas gingivalis* infection.[44] Another study showed that alveolar bone loss was significantly increased in mice on a high-fat-diet (HFD) alone compared to normal chow diet, independent of infection with periodontal pathogens.[45,46] In addition, various studies suggested that the incidence of metabolic diseases could be associated with a dysbiosis of periodontal microbiota, which results in an inflammatory response that eventually causes the development of PD.[47,48]

4 | CC MOUSE POPULATION: A PRECISION TOOL FOR IN-DEPTH GENETIC ANALYSIS OF AGING, TYPE 2 DIABETES, CANCER, AND PD

The CC is a large, genetically diverse panel of RI mouse strains designed to analyze complex traits effectively. It is derived from eight

TABLE 2 A list of the most significant genes associated with aging, based on published genome-wide association studies data.

Title of the study	Samples and size	Ancestry	Trait(s)	Mapped genes	Variant and risk allele	*p*	Chromosome position in base pair	Reference
Genome-wide association studies identify 137 genetic loci for DNA methylation biomarkers of aging.	34461 European ancestry	European (Denmark, Estonia, Finland, Germany, Italy, Netherlands, Sweden, Switzerland, United Kingdom, United States, Australia)	Intrinsic epigenetic age acceleration	*RPL6P14, CXXC4-AS1*	rs144317085-T	6×10^{-10}	4:104884951	[29]
				NHLRC1	rs10949481-T	4×10^{-54}	6:18120798	
				CARMIL1, SCGN	rs10447389-A	4×10^{-16}	6:25642349	
				PPP1R17	rs12666349-C	5×10^{-9}	7:31688566	
				CYRIA, GACAT3	rs4832646-C	1×10^{-12}	2:16521131	
				SMARCC1	rs79111787-C	1×10^{-10}	3:47674055	
				TERT	rs2736100-A	3×10^{-11}	5:1286401	
				NHLRC1	rs10949483-A	5×10^{-32}	6:18122275	
				HLA-W, HLA-A	rs28780071-T	4×10^{-9}	6:29954890	
				P2RX3	rs55637147-C	4×10^{-14}	11:57343922	
				TMEM263-DT, RPL30P12	rs10735418-C	1×10^{-9}	12:106949598	
				ATP8B4	rs12903325-G	5×10^{-10}	15:50061080	
				ZFHX3	rs34003787-T	2×10^{-8}	16:73037482	
				LINC01478	rs954794-A	2×10^{-9}	18:44501960	
				RIPPLY3, MRPL20P1	rs57941717-T	4×10^{-16}	21:37001879	
				TMEM121B	rs75243280-C	7×10^{-12}	22:17120576	
				RERE, ENO1	rs2038903-T	3×10^{-10}	1:8856458	
				AKIRIN1	rs12043492-T	5×10^{-12}	1:38991334	
				PBX1	rs2275558-A	6×10^{-9}	1:164559883	
				SELP, FIRRM	rs6687517-C	6×10^{-18}	1:169652823	
				MIR29B2CHG	rs7550821-T	3×10^{-13}	1:207856602	
				EDARADD	rs1726672-T	6×10^{-10}	1:236356202	
				LINC01565, RPN1	rs2492286-T	5×10^{-11}	3:128617455	
				TRIM59, TRIM59-IFT80	rs1047210-C	5×10^{-10}	3:160436470	
				RPL6P14, CXXC4-AS1	rs144317085-T	6×10^{-10}	4:104884951	
Epigenetic aging is accelerated in alcohol use disorder and regulated by genetic variation in APOL2.	154 European ancestry individuals, 156 African American individuals	154 European ancestry individuals, 156 African American individuals	Physiological aging rate	*APOL2*	rs916264-A	5×10^{-8}	22:36237790	[30]
				FTH1P5, PKHD1	rs1327265-A	6×10^{-7}	6:51306610	
				LINC01320	rs17014720-T	1×10^{-6}	2:34103624	
				CLIC6, RCAN1	rs1571695-A	8×10^{-6}	21:34651056	
				TUBAP15, RNU6-718P	rs10051946-?	2×10^{-7}	5:120238715	
				LINC00251, PPIAP86	rs13253048-?	9×10^{-7}	8:65190078	
				APOL2	rs2157250-?	4×10^{-7}	22:36235645	
				ACOT11	rs12059231-?	3×10^{-6}	1:54580003	
				CRYZL2P-SEC16B, SEC16B	rs10913472-?	9×10^{-6}	1:177960419	
				LINC01515	rs10996604-?	9×10^{-6}	10:65706190	
				OR7A1P, OR7A18P	rs11085919-?	9×10^{-7}	19:14890285	
Genome-wide association study for four measures of epigenetic age acceleration and two epigenetic surrogate markers using DNA methylation data from Taiwan biobank	2309	East Asian ancestry individuals (Taiwan)	DNA methylation-estimated granulocyte proportions	*CSF3, PSMD3*	rs8070454-T	5×10^{-6}	17:40004501	[31]
				RFX7	rs8030605-A	8×10^{-31}	*15:56212400.*	
				IBA57	rs117530284-A	2×10^{-9}	1:228176631	
				TERT	rs2736100-C	2×10^{-6}	5:1286401	
				PTPRT, RNU6-1018P	rs189669793-A	2×10^{-8}	20:41718727	
				P2RX6	rs3893495-C	2×10^{-9}	22:21015387	

founder strains through a strategic mating process, incorporating over 36 million SNPs and a high degree of recombination.[2,49–59] Notably, three CC founders (CAST/EiJ, PWK/PhJ, and WSB/EiJ) are wild-derived strains representing distinct subspecies, introducing unique genetic variants absent in classical strains.[60,61] Consequently, quantitative trait locus (QTL) mapping in CC mice often reveals QTL emphasizing differences among these strains, as seen in early experiments.[52–54,57,62,63] Using genome sequence data from the Sanger Mouse Genomes Project 54, researchers can refine QTLs by focusing on variants aligned with QTL candidate genes.[52–54,57,62,64]

TABLE 3 This table presents the most significant genes linked to periodontitis, as determined by published genome-wide association studies.

Title of the study	Samples and size	Ancestry	Trait(s)	Mapped genes	Variant and risk allele	*p*	Chromosome: position in bp	Reference
A genome-wide association study identifies nucleotide variants at SIGLEC5 and DEFA1A3 as risk loci for periodontitis.	European	* 7687 Europeans * 787 others	Aggressive periodontitis	*OSTCP2*	rs4970469-G	1×10^{-6}	1:26986325	32
				SLC1A3-AS1	rs1122900-A	8×10^{-7}	5:36689079	
				FCER1G	rs2070901-T	4×10^{-6}	1:161215268	
				DEFA9P, DEFA10P	rs2978951-A	2×10^{-7}	8:6965773	
				SIGLEC5	rs4284742-G	1×10^{-6}	19:51628480	
Genome-wide exploration identifies sex-specific genetic effects of alleles upstream NPY to increase the risk of severe periodontitis in men.	329 European ancestry cases, 983 European ancestry controls	1312 European (Germany)	Periodontitis	*RNA5SP228, NPY*	rs198712-?	4×10^{-6}	7:24141436	33
Smoking modifies the genetic risk for early-onset periodontitis.	741 European (Australia, Germany, Netherlands, NR)	499 European ancestry ever-smoker cases, 242 European ancestry never-smoker cases	Early-onset periodontitis × smoking status interaction	*CRYBB2P1*	rs56217753-T	6×10^{-7}	22:25517801	34
				ABLIM2	rs10029338-A	4×10^{-6}	4:8048616	
				GRK5	rs11198898-A	6×10^{-7}	10:119358980	
Sex-specific genetic factors affect the risk of early-onset periodontitis in Europeans.	896 European (Austria, Germany, Netherlands)	545 Dutch ancestry women, 351 Dutch ancestry men	Early-onset periodontitis × sex interaction	*DELEC1*	rs10982617-?	6×10^{-7}	9:115219960	35
				GFI1B	rs11243957-?	6×10^{-6}	9:132968138	
				UBIAD1, MTCYBP45	rs7552089-?	8×10^{-7}	1:11398960	
				VIT	rs11685689-?	7×10^{-6}	2:36809984	

Studies indicate that ~100 well-replicated CC lines can achieve a mapping resolution of ~1 Mb.[55,57,62,65]

Mice can help overcome limitations in human studies given their similar biological responses. Genes identified in mice can be explored in humans through comparative analysis.[66] Yang et al., for example, used the CC population to study cancer risks linked to thirdhand smoke exposure, reporting reduced tumor-free survival in certain strains.[67] Another study of 293 mice from 18 CC strains documented various spontaneous tumor types and frequencies, identifying Nfκb1 as a candidate gene linked to increased inflammatory responses.[68] Additionally, cancer incidence in CC mice correlates with metabolic conditions like type 2 diabetes (T2D), where higher glucose levels and intestinal measurements (e.g., polyp counts) are observed.[69] A HFD also affects body weight differently across CC lines, with female liver weight more impacted than males.[70]

The CC model has furthered research on microbiome changes during periodontal infections, revealing significant composition shifts and genetic variations influencing resistance or susceptibility to PD.[71] Phenotypic responses to bacterial challenges indicated that some CC lines were susceptible to bone loss, confirming genetic factors' roles in disease susceptibility.[72] Functional gene knockouts and RNA sequencing can validate candidate genes' roles and identify relevant pathways. Overall, the CC mouse model serves as a crucial tool for exploring the genetic basis of interconnected conditions like T2D, cancer, and PD, especially in the context of aging and diet-related changes.[73–75] The CC × *Apc*^{Min} hybrid model exemplifies how hybrid vigor can slow carcinogenesis, allowing for a more human-like progression of CRC. This delay is critical for studying how genetic and environmental factors contribute to CRC onset and progression in aging populations.

5 | CANCER EPIGENETICS, AGING, AND THEIR ASSOCIATION WITH COMORBIDITIES

Epigenetic mechanisms play complex roles in cancer progression, offering potential avenues for chemoprevention or treatment through epigenetic modification.[76–82] Histone modifications within chromatin are key contributors to diabetes pathophysiology and its complications. Notably, diabetic nephropathy and "metabolic memory"—the lasting impact of prior hyperglycemia—reflect the importance of early glycemic control in reducing microvascular complications, as shown in the Diabetes Control and Complications Trial (DCCT) and Epidemiology of Diabetes Interventions and Complications (EDIC) trials.[83] Research into diabetes-related epigenetic changes may further determine the interaction between environmental factors and gene regulation, with promising implications for new therapies to mitigate diabetes complications.[83]

In diabetes, DNA methylation, imprinting, histone modification, and microRNAs (e.g., miR-29a and miR-29c) have been linked to disease susceptibility and progression.[84] Histone modifications, like acetylation and methylation, are particularly impactful in regulating diabetes-related genes, with current research focusing on T2D susceptibility genes.[85]

Genetic and epigenetic factors are similarly vital in periodontal diseases, where DNA methylation and histone modifications are responsive to bacterial presence and oral inflammation.[86] Studying these interactions offers potential for innovative treatments and biomarker development, improving the prevention and management of PD. Specific epigenetic changes, including those initiated by periodontal pathogens, are key to understanding chronic inflammation in gingivitis and PD.[87–89] DNA methylation, regulated by DNA methyltransferases, selectively activates or silences genes, which may aid in developing diagnostic tools or treatments targeting gene transcription in PD.[90]

Aging and cancer are connected via epigenetic changes, where shifts in histone modifications and chromatin accessibility can promote cancer.[91] Such modifications can deregulate gene expression, impacting pathways for cell proliferation, differentiation, and apoptosis, thereby increasing cancer risk. Understanding aging-related epigenetic alterations in cancer is essential for identifying therapeutic targets and advancing prevention strategies.[92]

6 | PROSPECTS FOR DEVELOPING A NOVEL PARADIGM FOR IDENTIFYING *Apc*^{Min} MODIFIER GENES IN CC MICE FOR RESEARCH ON DIABETES, AGING, IC, AND PD

Developing a model that reflects human genetic diversity is essential for understanding genetic contributors to IC. By crossing an IC-prone mouse strain (*Apc*$^{Min+/-}$) with CC lines, researchers can explore the genetic factors influencing IC and PD. This method creates F1 hybrids (*Apc*$^{Min+/-}$×CC) with diverse genetic backgrounds that exhibit variable susceptibility to IC and PD. Figure 1 shows the proposed approach to obtain systems genetics data and phenotypic profiles from this population, which is expected to show substantial variation in IC and PD susceptibility.

In previous research, crossing *Apc*$^{Min+/-}$ mice with CC lines identified genetic modifiers related to CRC. Notably, hybrids carrying 50% CC-derived genetic information exhibited resilience to cachexia at 16 weeks, allowing phenotypic analysis at 26 weeks. The proposed research will measure polyp counts in the intestines and colon, PD development, and gene expression responses in susceptible and resistant F1 hybrids (*Apc*$^{Min+/-}$×CC) under two conditions: a 52-week western diet (42% high fat) and a standard chow diet. This study aims to identify modifiers of the *Apc* gene and host susceptibility genes related to IC and PD under HFD and aging conditions.

High-resolution QTL mapping will investigate genomic regions associated with IC and PD susceptibility, comparing findings with earlier results at 23 weeks (IC) and 12 weeks (PD). The approach involves generating F1 crosses from 70 different CC female lines with *Apc*$^{Min+/-}$ males, producing 420 F1 hybrids for each diet condition (HFD and standard chow). Each cohort will assess the effects of diet and age on IC and PD development, using metrics like polyp count, jawbone absorption (PD indicator), body weight, and the intraperetonial glucose tolerance test (IPGTT) at multiple intervals up to 52 weeks. A comprehensive genome-wide scan will identify QTLs linked to IC and PD susceptibility. RNA sequencing of polyps, liver, and hemi-maxilla will analyze gene expression differences in the phenotypic extremes of susceptible and resistant F1 lines under HFD and aging conditions. This research represents the first approach of its kind to address complex metabolic disorders such as obesity, HFD-induced T2D, IC, and PD in genetically varied mice at 52 weeks. The study aims to identify genes contributing to resistance and progression of IC and PD related to HFD-induced T2D and obesity in aging mice. This data could aid in predicting individual disease risk and support the development of genetically informed prevention and treatment strategies. The delayed progression of carcinogenesis in CC×*Apc*^{Min} hybrids represents a critical advancement in modeling CRC as a disease of adulthood. Unlike the rapid tumor onset observed in standard *Apc*^{Min} mice, the slowed progression in CC hybrids allows researchers to examine the interplay between aging, metabolic dysfunction, and cancer development. This transformative approach provides new opportunities to identify genetic modifiers and develop age-specific interventions.

The identification of genetic modifiers, including Mom1 and Mom2, in CC×*Apc*^{Min} hybrids has advanced our understanding of tumorigenesis. Although direct validation of these modifiers in human populations remains challenging, parallels can be drawn using human GWASs, which have linked homologous pathways, such as Wnt signaling and inflammatory responses, to CRC susceptibility. This highlights the translational potential of CC mice in identifying human-relevant genetic pathways.

The translational potential of these findings is underscored by overlaps between genetic modifiers in CC×*Apc*^{Min} mice and pathways implicated in human CRC through GWASs. For example, inflammation-related genes corresponding to Mom1 and metabolic regulators akin to Mom2 have emerged as key players in human CRC susceptibility. Integrating comparative genomic studies and human data could further validate these findings, paving the way for precision interventions.

7 | STRATEGIES FOR THE DESIGN AND POWER OF QTL MAPPING EXPERIMENTS USING THE CC-F1 CROSS APPROACH

The success of an experiment utilizing the CC resource in detecting quantitative trait loci influencing a specific trait—essentially the experiment's statistical power—relies on three key factors: the effect size of the QTL on the trait value (historically represented as h^2QTL), the number of CC strains included (n), and the number of replicates tested per strain (r). The overall power of a given experimental setup is dictated by the interaction between these three elements. Recently, we published a simulation report for designing QTL mapping experiments using a CC mouse population.[93]

Table 4 in section 7 as it shows, presents the power of the established CC reference population based on the three factors discussed earlier in the simplest scenario, where all genetic variation in the target trait across CC strains stems from a single diallelic QTL with

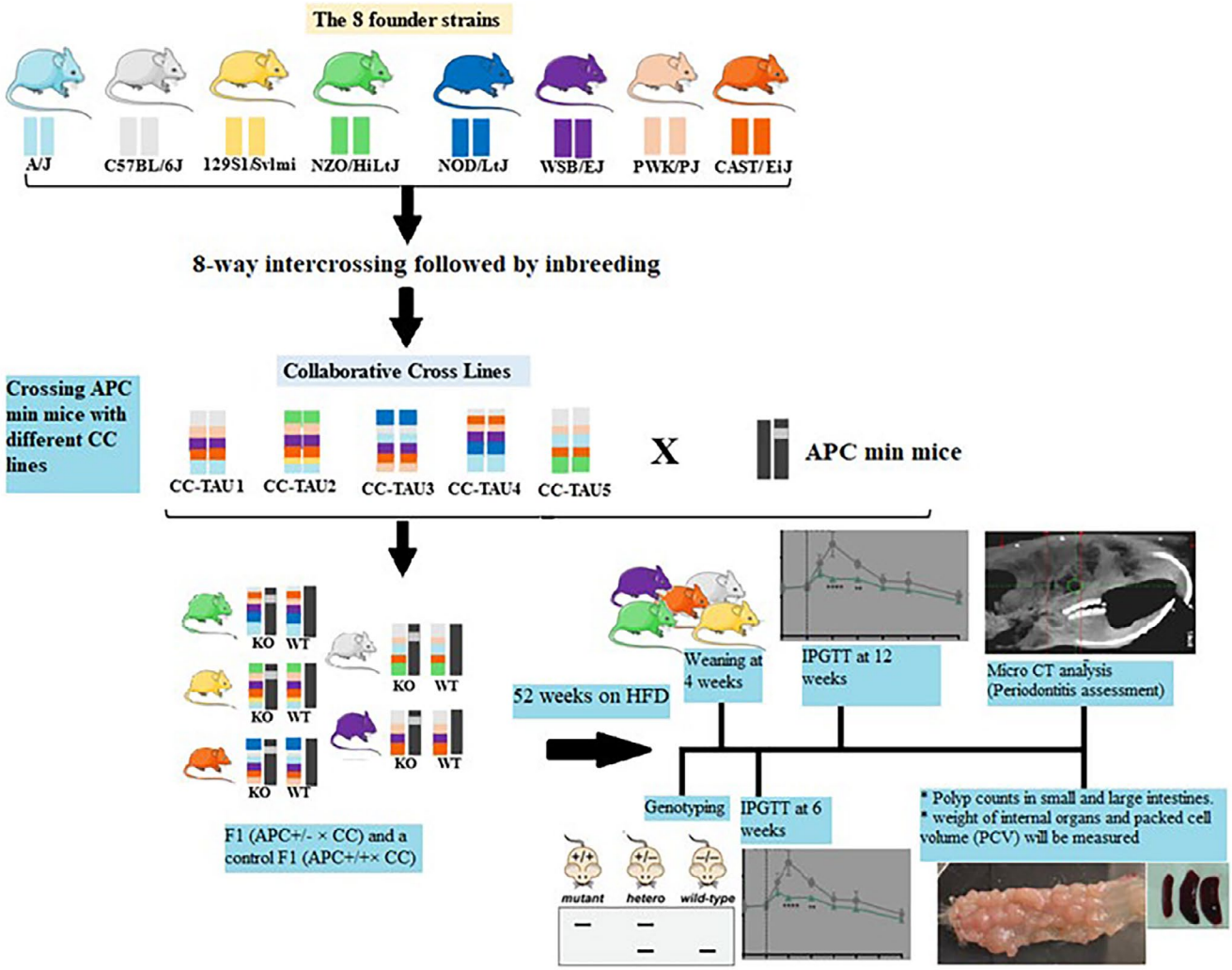

FIGURE 1 This diagram shows the original breeding program that led to the Collaborative Cross (CC) mouse model, with the aim of producing genetic diversity by making each inbred line's genome composition unpredictable. Each recombinant inbred (RI) line (representing genomes from the eight CC founder strains) is produced using a unique breeding procedure. The founders (labeled 1–8) are positioned in randomized sequences for each line, ensuring no repeated arrangements. Through a funnel-breeding design, the genetic material from all eight founders is combined by the G2 generation. The RI line undergoes 20 generations of inbreeding to ensure stability. This flowchart also outlines the process of generating a systems genetics dataset from the high-diversity F1 ($Apc^{Min\pm}$×CC) population, which exhibits substantial variability in intestinal cancer (IC) susceptibility. Mice are evaluated for clinical and histological IC-related traits. Primary cell cultures from the F1 ($Apc^{Min\pm}$×CC) population undergo cellular property assessments, and data on cellular, molecular, and clinical traits are integrated to explore various IC phenotypic relationships. By combining SNP (single-nucleotide polymorphism) genotype data across CC lines, QTL (quantitative trait locus) mapping identifies genomic regions regulating phenotypic variation in both in vitro and in vivo traits. Integrating this data with human candidate-gene association studies may reveal susceptibility genes linked to human IC development.

an allele frequency of 0.5 among the eight CC founder strains (i.e., a positive allele is present in four of the founder strains, whereas the remaining four carry the negative allele). More complex cases—such as multiple alleles, uneven allele distribution, the influence of multiple QTLs, and/or polygenic background genetic variation—are analyzed in greater depth in subsequent sections.[94]

Previously, we estimated the heritability (H2) of the IC development in the F1 (APC-CC) cross and found it to range between 0.305 and 0.581.[6] According to Table 4, we expect that for using 70 CC lines and considering the estimated heritability of the different traits to be assessed between 0.305 and 0.581, using three biological replicas per F1 cross line, we should have >0.981 power for mapping QTL associated with these traits. Furthermore, in our previous study by Shusterman et al. the estimated heritability for PD development was 0.20.[95] It is expected that three biological replicas per F1 cross line should have >0.820 power for mapping QTL associated with these traits.

8 | KEY STRATEGIES AND TECHNICAL METHODS FOR RESEARCH USING CC MICE

CC mice present unique opportunities and challenges for studying complex traits like CRC. We outline the key strategies and technical

TABLE 4 This table presents the statistical power of detecting QTLs as a function of QTL effect size, the number of strains (*n*), and the number of replicates per strain in the experiment.

	Number of strains/number of replicates								
QTL effect	30			50			72		
	1	3	5	1	3	5	1	3	5
0.01	0	0.001	0.009	0.001	0.001	0.002	0.000	0.001	0.003
0.05	0.001	0.002	0.043	0.002	0.009	0.036	0.003	0.035	0.125
0.1	0.001	0.009	0.102	0.004	0.068	0.238	0.013	0.230	0.605
0.15	0.002	0.024	0.274	0.011	0.213	0.550	0.043	0.568	0.902
0.2	0.006	0.054	0.419	0.029	0.424	0.823	0.104	0.816	0.978
0.25	0.009	0.105	0.595	0.068	0.654	0.923	0.230	0.951	
0.3	0.012	0.205	0.776	0.113	0.837	0.964	0.392	0.981	
0.35	0.025	0.330	0.886	0.221	0.921		0.583		
0.4	0.043	0.488	0.947	0.339	0.948		0.740		
0.45	0.063	0.644	0.946	0.487	0.983		0.873		
0.5	0.105	0.781	0.973	0.654			0.951		
0.55	0.181	0.857		0.811			0.977		
0.6	0.276	0.909		0.915					
0.65	0.428	0.944		0.931					
0.7	0.607	0.960		0.981					
0.75	0.781								
0.8	0.888								
0.85	0.947								
0.9	0.973								

Abbreviation: QTL, quantitative trait locus.

methods for conducting research using CC mice, along with critical considerations.

Experimental design: CC mice are bred from eight founder strains, resulting in high genetic diversity and recombination. For studies like ours, F1 hybrids (e.g., $Apc^{Min/+}\times$CC) are produced to capture hybrid vigor and the genetic variability needed for QTL mapping. It is essential to include sufficient replicates and strain diversity to ensure statistical power in detecting QTLs.

Phenotyping: detailed phenotyping is critical for CRC studies, including measuring polyp counts, tumor sizes, and organ-specific features. Other phenotypic measures, such as jawbone absorption for PD, body weight, and metabolic markers like glucose tolerance, are integrated to explore comorbidities.

QTL mapping: genome-wide scans are performed to link phenotypes to genetic loci. Researchers must consider the genetic architecture of CC mice, including allele frequencies and linkage disequilibrium, to optimize mapping precision.

Diet and aging protocols: diet and aging protocols must be carefully standardized to model human CRC progression. For instance, HFDs are used to mimic diabesity, and long-term studies are designed to capture the gradual effects of aging on CRC.

Key considerations: the genetic diversity in CC mice, although advantageous, poses challenges in reproducibility and phenotyping consistency. Comprehensive genotyping and careful selection of founder strains for experiments are necessary to mitigate variability. Additionally, environmental factors such as housing and microbiome differences must be controlled to avoid confounding results.

9 | CONCLUSION

Research suggests that identifying the primary effects of particular human alleles alone will not be sufficient to fully understand the genetic influence on IC vulnerability. To further study the diversity and tumor heterogeneity observed in human IC, new IC mouse models must be developed. These models will serve as an excellent model platform and resource. A viable approach is to utilize CC genetic reference population (GRP) mice to conduct systems genetics studies to characterize the impact of genetic variation on IC signaling networks and phenotypic variety. By addressing the limitations of current models, the CC$\times Apc^{Min}$ hybrid enables an in-depth understanding of CRC as an age-dependent disease. Its human-like progression provides a robust platform for studying genetic and environmental modifiers, ultimately advancing strategies for prevention and treatment.

Future research should prioritize functional studies and comparative genomic approaches to validate the role of identified genetic modifiers in human CRC. By combining CC$\times Apc^{Min}$ models with human datasets, researchers can map conserved pathways, unravel

complex gene–environment interactions, and develop more precise CRC prevention and management strategies.

AUTHOR CONTRIBUTIONS

Iqbal M. Lone: Formal analysis; methodology. **Osayd Zohud:** Data curation; formal analysis; investigation. **Kareem Midlej:** Data curation; formal analysis; methodology. **Charles Brenner:** Funding acquisition; investigation; methodology; project administration; writing – review and editing. **Fuad A. Iraqi:** Conceptualization; data curation; formal analysis; funding acquisition; investigation; methodology; project administration; resources; supervision; validation; writing – review and editing.

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CONFLICT OF INTEREST STATEMENT

Charles Brenner is a cofounder of Alphina Therapeutics. Fuad A. Iraqi is an editorial board member of Animal Models and Experimental Medicine (AMEM) and a corresponding author of this article. To minimize bias, he was excluded from all editorial decision making related to the acceptance of this article for publication.

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Not Applicable.

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