Received: 16 May 2019 | Accepted: 23 May 2019

DOI: 10.1002/ame2.12074

REVIEW ARTICLE

Efficient protocols and methods for high-throughput utilization of the Collaborative Cross mouse model for dissecting the genetic basis of complex traits

Hanifa J. Abu-Toamih Atamni | **Fuad A. Iraqi**

Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Ramat Aviv, Israel

Correspondence
Fuad A. Iraqi, Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv 69978, Israel.
Email: fuadi@tauex.tau.ac.il

Funding information
Hendrech and Eiran Gotwert Fund; Wellcome, Grant/Award Number: 085906/Z/08/Z, 075491/Z/04 and 090532/Z/09/Z; Tel-Aviv University; Israeli Science foundation, Grant/Award Number: 429/09, 961/15 and 1085/18; Binational Science Foundation, Grant/Award Number: 2015077; German Israeli Science Foundation, Grant/Award Number: I-63-410.20-2017; Israeli Cancer Research Fund; Cancer Research Counsel-UK; Cancer Biology Research Center

Abstract

The Collaborative Cross (CC) mouse model is a next-generation mouse genetic reference population (GRP) designated for a high-resolution quantitative trait loci (QTL) mapping of complex traits during health and disease. The CC lines were generated from reciprocal crosses of eight divergent mouse founder strains composed of five classical and three wild-derived strains. Complex traits are defined to be controlled by variations within multiple genes and the gene/environment interactions. In this article, we introduce and present variety of protocols and results of studying the host response to infectious and chronic diseases, including type 2 diabetes and metabolic diseases, body composition, immune response, colorectal cancer, susceptibility to *Aspergillus fumigatus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, sepsis, and mixed infections of *Porphyromonas gingivalis* and *Fusobacterium nucleatum*, which were conducted at our laboratory using the CC mouse population. These traits are observed at multiple levels of the body systems, including metabolism, body weight, immune profile, susceptibility or resistance to the development and progress of infectious or chronic diseases. Herein, we present full protocols and step-by-step methods, implemented in our laboratory for the phenotypic and genotypic characterization of the different CC lines, mapping the gene underlying the host response to these infections and chronic diseases. The CC mouse model is a unique and powerful GRP for dissecting the host genetic architectures underlying complex traits, including chronic and infectious diseases.

KEYWORDS
Collaborative Cross (CC) mouse model, complex traits, genetic reference population (GRP), infectious and chronic diseases, quantitative trait loci (QTL)

1 | INTRODUCTION

The laboratory mouse is one of the most important tools in the scientific research to understand the mammalian gene function. The scientific community has taken advantage of this mouse because of its fundamental similarity to humans at the genetic level (>95% at the gene level), physiological and anatomical similarity, relatively low cost compared to other mammals, and nearly

100 years of genetic study. Janan Eppig of The Jackson Laboratory recently presented the results of a survey of the Mouse Genome Informatics (MGI) (http://www.informatics.jax.org) of published model organisms used in studies of human disease. The study demonstrates the rise of the mouse as the most common experimental organism to study human disease and gene function. In genetically defined strains of mice, chromosomal regions responsible for the genetic variance of complex traits can be mapped as quantitative trait loci (QTL) in experimental populations available for precise study under defined conditions.[1-3] Once QTL is identified, genetic analysis can be extended successfully to humans.[4,5] It has been suggested that the variation among strains of mice can be used for mapping QTL associated with susceptibility to chronic and infectious diseases.[6-10] Here, we propose to use advanced next-generation approach for determining the genes involved in the development of complex trait diseases and various comorbidities using the CC mouse genetic reference population (GRP). Once inbred lines of mice are crossed, the cross-progeny often exhibit transgressive variation or present novel traits that were not present in the parental strains. This indicates that the inbred strains of mice harbor a tremendous amount of natural genetic variation, above what is seen when the lines themselves are compared. This variation has been accessed in part by construction of recombinant inbred lines (RIL) derived from crosses between mouse pure lines.[11] However, the number of available RIL is too small to provide reliable statistical support to detect smaller genetic effects. With the realization that a new model population was needed to understand complex human diseases, the Collaborative Cross (CC) was designed to generate a large number of RIL.[11] The CC population is being created and developed by community efforts of the complex trait consortium (CTC, www.complextrait.org). This unique genetic resource eventually comprises a set of approximately 100 RIL that will be created by full reciprocal eight-way mating of eight divergent strains of mice: A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HiLtJ, CAST/Ei, PWK/PhJ, and WSB/EiJ. Controlled randomization and minimization of selection during the breeding process recombined the natural genetic variation presented in these inbred strains (Figure 1). The result is a unique collection of RIL exhibiting a large phenotypic and genetic diversity, and bringing the tremendous genetic variation potential of the mouse inbred lines to phenotypic expression.[2,11] Full details of CC lines and their power of mapping QTL with host susceptibility to complex traits are presented in various publications.[12-24] The CC mouse model is the focal point in our research, having exclusive access to the CC lines produced at our laboratory at Tel-Aviv University (TAU). The CC lines were three times genotyped, once using 620 000 single nucleotide polymorphisms (SNPs) markers of mouse diversity array,[25] once with mouse universal genotype array (MUGA), and later on, with the MegaMUGA genotype array. The updated genotype status of the population was presented recently[19] showing the diversity of the phenotypic response of the CC lines with a complex disease and their power for mapping QTL associated with this trait to a small genomic interval less than 0.5 cm, which consists of few genes. Herein, we present our various protocols and methods implemented while using the CC mouse population for dissecting the genetic architecture underlying the phenotypic variations (complex traits) observed among certain populations. Order of protocols' presentation starts with: (a) phenotyping protocols, that is, traits at baseline/ chronic diseases/ colorectal cancer and infectious diseases, and then (b) genotyping and bioinformatics protocols, that is, genotyping methods, QTL analysis using HAPPY software, merge analysis, and founder effect.

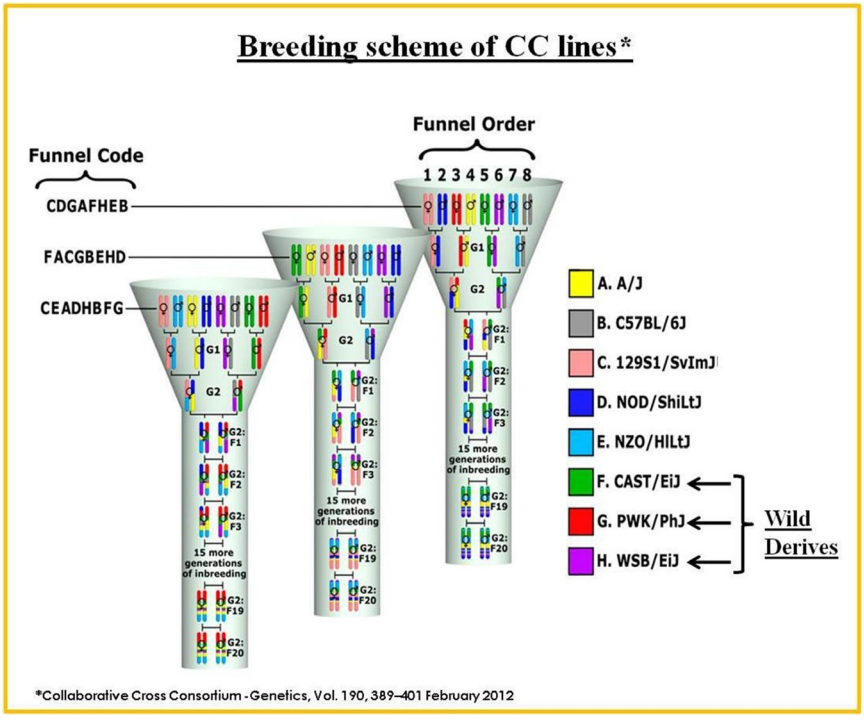

FIGURE 1 Funnel breeding schemes of the Collaborative Cross (CC) lines by eight founder strains. Each CC line originates from an independently breeding funnel of the eight CC founders so that every recombination site in the CC population is uniquely generated

2 | THE USAGE OF THE CC MOUSE POPULATION

All the conducted projects in our laboratory were based on the idea of using mice of different CC lines as the genetic reference population, and determine their response toward an environmental challenge that affect the phenotype of interest. Depending on the nature of the environmental challenge and pathogenesis of each disease, the experiment period varies between few days to months, while phenotypic data are recorded at different time points starting at the initial time point of the experiment (time zero) up to the terminal time point. Subsequently, data analysis is performed using the IBM SPSS software platform for one-way and two-way analysis of variance (ANOVA) tests, which determines the significance level of the observed phenotypic variance between the CC lines and their cross-interactions. Next, linkage analysis between phenotypic and genotypic data is performed using the HAPPY software for mapping the QTL and reveal the candidate genes related to the measured phenotype (trait). Figure 2 summarizes the overall frame and steps for implementing an experiment using the CC mouse models.

Based on our experience and obtained and published results from the different projects, we found that assessing a minimum of 30 CC lines in each experiment may provide a sufficient statistical power for the QTL mapping and data analysis. The mice study cohort should include the representation of both sexes from each CC line, with the number of animals range from 3 to 5 from each sex (ie, 6-10 mice in total from each CC line). It is noteworthy that the limiting factor of the cohort selection is the breeding rate of each individual CC line, which varies between the CC lines due to their genetic background structure. Therefore, cohort selection during the experiment period was performed in batches and not at once, where at the end of each breeding cycle (3 weeks period) a batch (group) of mice from different available CC lines is selected. In general, completing the assessment of entire experimental cohort for covering both sexes of 30 and more CC lines probably requires at least 10-12 breeding cycles (batches). Mapping QTL and identifying the founders at each interval were calculated using the HAPPY HMM.[26]

Basic required equipment for conducting a given phenotype assessment include the following:

1. Appropriate small animal unit (SAU) for mouse housing.
2. Rooms and SAU held at 22°C and 12 hours of light/dark cycle.
3. Open environment (OE) or individual ventilation (IV) cages containing appropriate rodent diet and water ad libitum.

2.1 | Basic Protocol 1

2.1.1 | CC lines for fundamental body traits

This protocol focus on the assessment of mouse whole body composition of mice from the different CC lines, in order to determine the genetic factors underlying these fundamental body traits using the

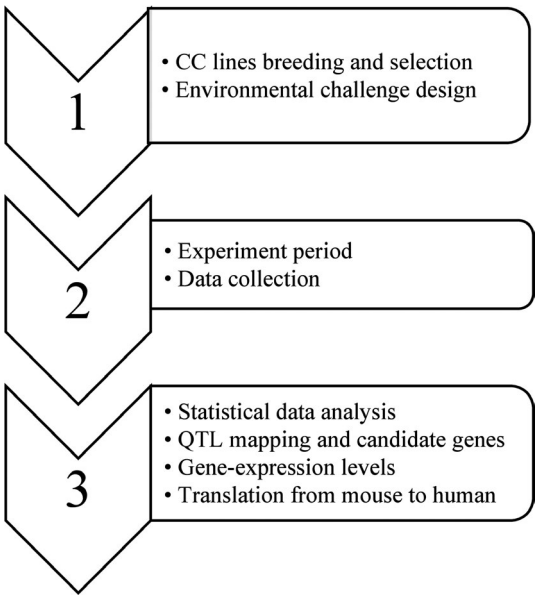

FIGURE 2 General steps by sequential order for implementing an experiment using the CC mouse model. Initial step is the selection of the CC lines mice cohort and design the environmental challenge, then start the experiment period and data collection until terminal time point, and final step focusing on statistical data analysis, QTL mapping, gene expression, and eventually translation from mouse to human

CC population and the power of the dual-energy X-ray absorptiometry (DEXA) scan technique (Figure 3A left). The DEXA scan duration for each mouse is short (<5 minutes) and eventually an output (Figure 3A right) of whole body composition is obtained, including the following phenotypes: (a) bone tissue features: bone area (cm^2), bone mineral content (BMC), bone mineral density (BMD), and (b) soft tissues: fat (grams), lean (grams), percentage fat (%fat). Using the phenotypic and genotypic data of the CC lines will enable genome-wide scan analysis for identifying QTL associated with these traits. Figure 3B-D show the profiles of the different tested traits of different CC lines using DEXA scan. The statistical analysis of the phenotypic results has shown significant ($P < 0.05$) variations between the different CC lines.

2.1.2 | Protocol steps

1. Turn on the DEXA scanner and then the computer.
2. Login to the Piximus software ("Lunar PIXImus").
3. Start system calibration ("Calibration Main") using the DEXA phantom and following the machine manual instructions. Only if the quality control passes, move to next step. If not, then follow the manual instructions for trouble shooting.
4. Create a folder to store your project scans.
5. Animal preparation for the scan: The mouse should be motionless during the DEXA scan by using anesthetics or sacrifice the animal prior the scan if it is terminal point of the experiment.
6. Animal scan: Place the mouse on the on the dedicated plastic tray of the scanner. Press "F3" (measure subject) then fill in the animal

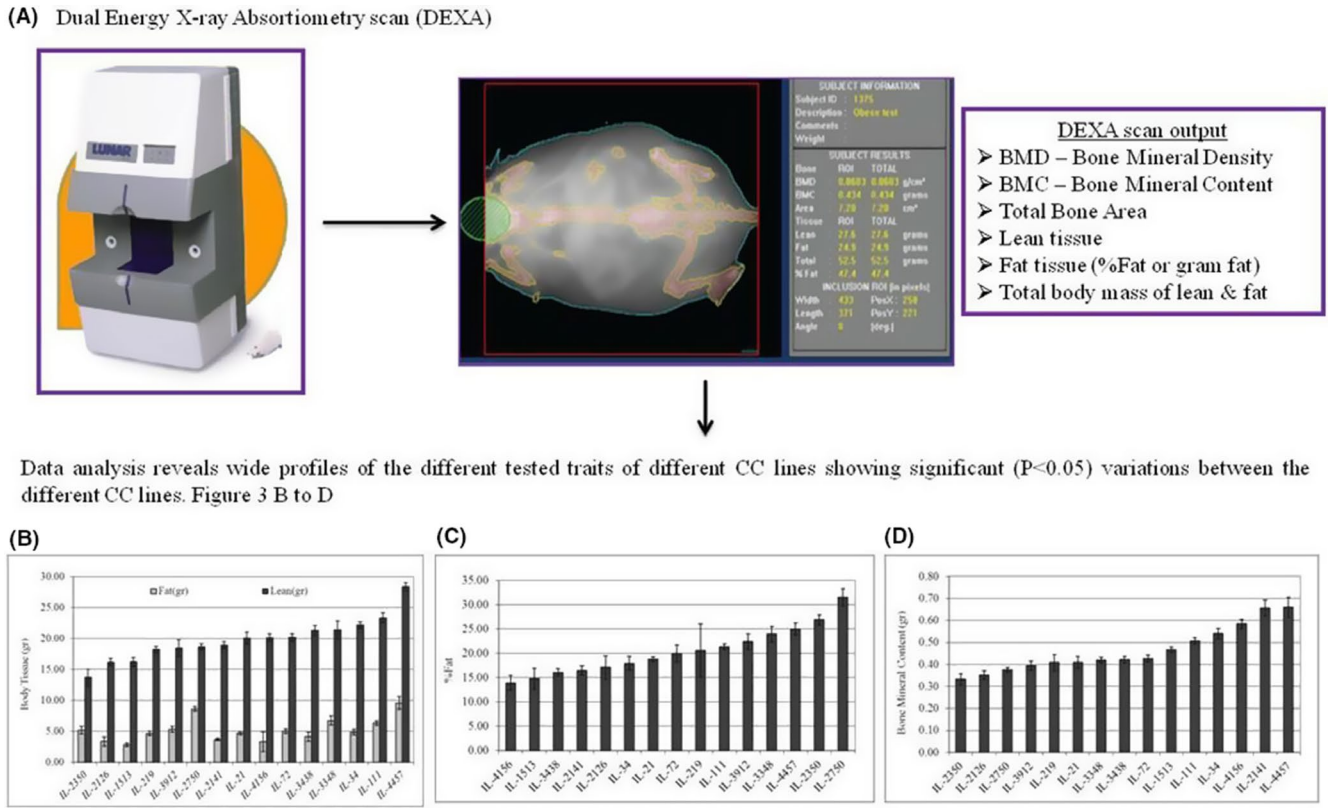

FIGURE 3 Whole body compositions assessment using the dual-energy X-ray absortiometry (DEXA) scanner. (A) DEXA scanner device (left) and screenshot of scan output for example (right). (B-D) Example of the statistic data analysis outcome revealing a wide phenotypic profile of the different CC lines for each trait, including the phenotypes for fat and lean tissues body composition (B), body percentage (%) fat (C), and bone mineral density (D) of 20-wk-old mice of 15 CC lines. The x-axis of B-D represents the different CC lines while y-axis represents the mean of the measured trait

details (ID/Code), which be used as the scan name. Click "OK" to start the scanning. Watch the system signs for radiation (green/yellow icon) during the scanning process and keep away until the icons disappear and the mouse image appears on the computer screen.

7. Save file for the completed scan.
8. Image processing: It is possible to define a region of interest (ROI) labeled in a green square while in a red circle is the excluded region of the image.
9. To shut down the machine: Press "ESC" and exit the animal scan, then turn off the DEXA scanner and computer.

Additionally to the DEXA scan, manually measurements were performed for each mouse in order to define further body size phenotypes for determining the variation between the different CC lines.

2.1.3 | Protocol steps

1. Body length (BL)—distance between nose and anus (in cm).
2. Waist Circumference (WC)—the circumference of the mouse abdomen using a measure tape (in cm).
3. Body weight (BW)—measured using electronic weight.
4. Calculation of the body mass index (BMI) using the formula: $BMI = BW/BL^2$.

2.2 | Basic Protocol 2

2.2.1 | Genetic background of Immune response

This protocol focus on the assessment of immune response cells lineages in the peripheral blood for mapping QTLs in linkage with these phenotypes and subsequently identifying the genes underlying these QTL, which can lead to better understanding of the host immune responses. For this purpose, we initiated a study aimed of mapping the genes, which control the production of various subsets of cells that are participating in the host defense by quantifying the amount of peripheral blood T, B, and macrophages cells of mature mice (3-5 mice per CC line at the age of 10 weeks old). The immunophenotyping was performed using the flow cytometry analysis based on fluorescence-activated cell staining (FACS) technology. Blood samples were collected from the orbital venous plexus of mice. Blood samples from individual mice were incubated with specific antibodies including anti-CD3e (T cells), anti-CD19 (B cells), and anti-CD11b (macrophages). Our study revealed marked differences in the relative proportions of different cells in the different CC mouse lines (Figure 4). The relative proportions varied between 13.5% and 43% for B lymphocytes and between 6.5% and 36% for T lymphocytes. As for macrophages, their proportion value range was 10.6%-39% in the different CC lines. Our

results are compatible with the results, which already published for different commercially available inbred mouse strains.

2.2.2 | Protocol steps

1. Peripheral blood (~100 μL) was collected from the orbital venous plexus of each immune-competent mouse using heparin-washed glass Pasteur pipettes.
2. Mice were anesthetized using ketamine and xylazine (120 and 8 mg/kg body weight, respectively) before blood sampling.
3. The blood samples (100 μL) from individual mice were incubated at room temperature with 3 μL mixture (1:1:1) of specific antibodies including 0.5 μg anti-mouse CD3e (expressed on T cells, NK-T cells, thymocytes during T-cell differentiation), 0.1 μg anti-mouse CD19 (expressed on pro-B to mature B cells), 0.1 μg anti-mouse CD11b (expressed on granulocytes, monocytes/macrophages, and some T and B cells) labeled with FITC, PE/Cy5, and PE, respectively, for 30 minutes. The antibodies were purchased from BioLegend (San Diego, CA).
4. Red blood cells were subjected twice to lysis procedure for 30 minutes at 4°C using 400 μL of lysing buffer (Quality Biological Inc Gaithersburg, MD). After full lysis, the labeled mixture was washed with phosphate-buffered saline (PBS; 1200 rpm 7 minutes) and suspended with 300 μL 0.5% paraformaldehyde PBS buffer.
5. Flow cytometry: The cells were kept at 4°C until use. Samples were run on FACS scan. At least 10 000 and up to 50 000 events were acquired per sample.

2.3 | Basic protocol 3

2.3.1 | Type 2 diabetes and metabolic syndrome assessment

This protocol focus on the assessment of the glucose tolerance ability of the mouse as an indicator for the development of impaired glucose tolerance (type 2 diabetes) in response to different diets, standard rodents diet vs high-fat diet (HFD; 42% fat). The dietary challenge starts when the mouse is at age of 8 weeks old and last for 3 months, that is, until age of 20 weeks old. At the terminal time point of the dietary challenge, an intraperitoneal glucose tolerance test (IPGTT) is performed to evaluate the diabetogenic status of the mice. Published findings present an extensive variations of all the measured phenotypes between the CC lines, importantly body diabetogenic and weight response to the high-fat dietary challenge significantly influenced by the host genetic background with strong sex effect in each CC line.[12] The CC lines showed multiple patterns for glucose clearance (Figure 5), including resistance, susceptibility, and moderate intermediate responses. QTL that determines susceptibility to HFD challenge, as expressed in total area under curve of overall IPGTT phenotypes were identified and published.[14]

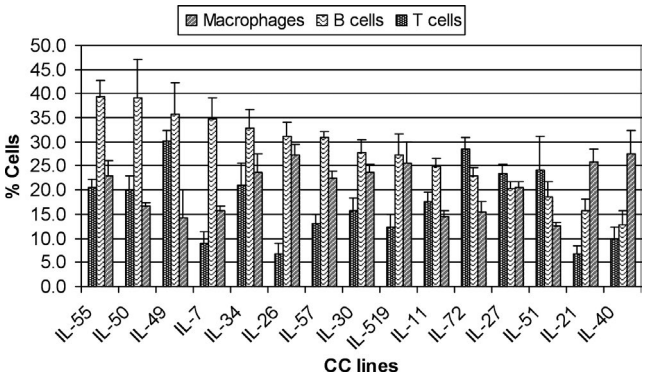

FIGURE 4 Percentage of T, B, and macrophage immune cells in 15 naïve CC mouse lines. The x-axis represents the different CC lines while the y-axis represents the percentage, including standard errors of the three tested immune cells in CC lines at age of 10-wk-old mice. The percentage of T, B, and macrophage immune cells in peripheral blood was assessed by fluorescence-activated cell staining (FACS) analysis

2.3.2 | Protocol steps

1. Animal 6 hours fasting: In the morning of the IPGTT, move the animals to new cage without food, but with free access to water. It is highly recommended to make sure using new bedding and cover in order to avoid cases of interrupted fasting due to food leftovers hidden in the old bedding or attached to the cover. Wait for 6 hours fasting.
2. Glucose solution preparation: Weigh 15 g glucose (Sigma #G8270) to dissolve in 60 mL of distilled water (DDW) using a magnetic stirrer and heating on low temperature, until the glucose totally dissolved. It is possible to prepare glucose solution stock, divide into tubes (to avoid stock contamination), and keep in the refrigerator (4°C) to use for several IPGTT.
3. At the end of 6 hours fasting: Weigh the animals using electronic scale and label the tails of the mice using marker for easy identification (saves time during the IPGTT measures).
4. Time zero glucose levels measure: Cut gently the edge of the tail and collect the blood to the glucometer device (AC PERFORMA KIT 53597) and record the result.
5. Calculation of glucose load: The volume of the injected glucose solution is adjusted to the mouse body weight (2.5 gram glucose per each kilogram of mouse body weight). Hence, to figure out the total volume (μL) of glucose injection, multiply the mouse body weight (g) × 10.
6. Intraperitoneal (IP) glucose injection: Set up the timer for 15 minutes, then inject (IP) the first mouse with glucose solution and start the timer. Continue the IP injections for all the mice, to complete the injection prior to the end of the 15 minutes (time 15). Usually, for beginners, it is recommended to start with small group of mice (10-15 mice), so you ensure accurate work and avoid glucose reads missing due to time trouble.
7. Time 15 minutes glucose measure: After 15 minutes, put the timer for the next read (15 minutes), and initiate the tail blood collection and recording of the glucose reads of 15 minutes.
8. Repeat step (7) at 30, 60, 120 minutes, and finally, at 180 minutes, with attention to setting up the timer in between. No need to cut

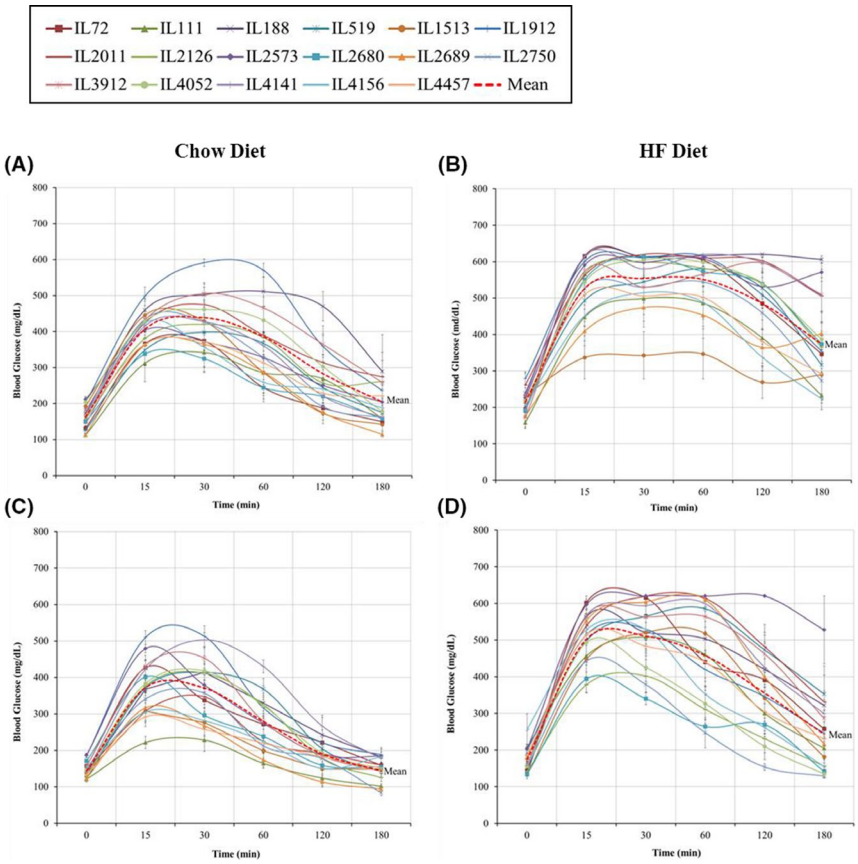

FIGURE 5 Kinetics of glucose clearance for 17 CC lines (split by sex) as assessed during the 180-min intraperitoneal glucose tolerance test (IPGTT) after 12 wk on chow diet (CH, 18% fat) and on high-fat diet (HFD, 42% fat). The dark central line shows average glucose levels (mg/dL) across lines. Chart A, males—Chow; B, males–HFD; C, females–Chow; D, females–HFD[12]

the tail again for each measure, try to remove the scab gently and the tail will bleed.

9. At the end of glucose reads after 180 minutes, the IPGTT is completed and mice should be returned to their home cages for recovery with free access to food and water. Next day, check on the mice to make sure that they are vital and recovered well.

2.4 | Basic protocol 4

2.4.1 | CC lines for studying of colorectal cancer

One form of the inherited colorectal cancer is *familial adenomatous polyposis* (FAP) syndrome, a rare dominant genetic disease, which is characterized by multiple intestinal polyps.[27-31] It is caused by a functional mutation in the *adenomatous polyposis coli* (*Apc*) gene. In order to investigate the syndrome in vivo, *Apc*$^{Min+/-}$ mouse was developed, imitating the polyp phenomenon in humans.[32-36] In previous studies, Min$^{+/-}$ mouse was used for identifying the genes (modifiers) affecting the function of *Apc* gene. The CC mice are a powerful tool for high-resolution mapping of loci associated with complex traits, and can lead to suggest strong candidate genes underlying these loci. As proof of concept about the use of CC mouse population for mapping modifiers of *Apc*, we have generated a (*CC-C57BL/6 lines carrying the Apc* $^{Min/+}$*mutation*) F1 mouse population, by crossing male mice of C57BL/6J carrying *Apc*$^{Min+/-}$ with females of advanced inbreeding generations of CC lines. The study cohort consisted of 27 F1 CC-C57BL/6 lines carrying the *Apc* $^{Min/+}$mutation. The litters from these mating were tested by polymerase chain reaction (PCR) in order to distinguish the litter carrying *Apc* mutant genes from the wild type. F1 heterozygous mouse carriers for the mutated *Apc* gene were housed and kept for a 5-month period in a 12-hour light/dark cycle. During this period, body weight (grams) was monitored biweekly. At the terminal time point, mice were sacrificed, packed cell volume was measured (anemia indicator) and the spleen and intestine were removed. The number and sizes of polyps in the small intestine and colon were recorded after fixation in 10% neutral buffered formalin (NBF) overnight and stained with methylene blue. The findings of the study were recently published[16] revealing a significantly wide variation in all the phenotypic parameters including total number of polyps (Figure 6) among the CC-C57BL/6 lines carrying the *Apc*$^{Min/+}$mutation, despite the constant environmental conditions for the overall population.

2.4.2 | Protocol steps

1. F1 (CC-C57BL/6*Apc*$^{Min+/-}$) mice were produced by crossing females of the different CC lines with males of C57BL/6*Apc*$^{Min+/-}$.
2. Genotypes of the F1 for C57BL/6*Apc*$^{Min+/-}$ was determined by PCR.
3. F1 (CC-C57BL/6*Apc*$^{Min+/-}$) were kept for 5 months on a standard mouse chow diet and water ad libitum.

4. Mice were terminated, and their small intestine and colon were extracted and washed with PBS.
5. The small intestine were divided into three segments (SB1—proximal, SB2—middle, and SB3—distal), and the colon was kept as whole and spread over a 3-mm paper.
6. The intestines were fixed in 10% NBF overnight and stained with 0.02% methylene blue. The samples were then examined by binuclear.
7. The number and sizes of polyps in each of the four intestinal subregions were recorded as described.[37]

2.5 | Basic protocol 5

2.5.1 | Map host susceptibility to *Aspergillus fumigatus* infection

Invasive disseminated aspergillosis is a serious disease in humans, inflicting severe damage to the kidneys, liver, spleen, brain, heart, and other organs.[38,39] The risk of acquiring invasive pulmonary aspergillosis is also higher in individuals with defective immune systems, such as those suffering from neutropenia. Users of corticosteroids or other immunosuppressive therapies, such as those used to prevent rejection following organ transplantation, and late-stage human immunodeficiency virus infection are also at higher risk. Toward mapping of host susceptibility to *A fumigatus* (AF), in previous study, we phenotyped 371 mice from 66 CC lines for susceptibility to AF infection. The survival time after infection ranged from 4 to 28 days and varied significantly between CC lines ($P < 0.05$). Figure 7 shows the mean survival profile of the CC lines after infection with AF. Broad sense heritability was 0.18. QTL mapping based on survival analysis and ancestral haplotype reconstruction of the CC genomes identified genome-wide significant QTLs on chromosomes 2, 3, 8, 10 (two QTL), 15, and 18. QTL mapping resolution varied between 2 and 11.6 Mb. Use of variation data from the genomes of the CC founder strains refine these QTLs further and suggest several candidate genes.[17] To our knowledge, this was the first report mapping susceptibility loci for invasive aspergillosis in immune-competent mice.

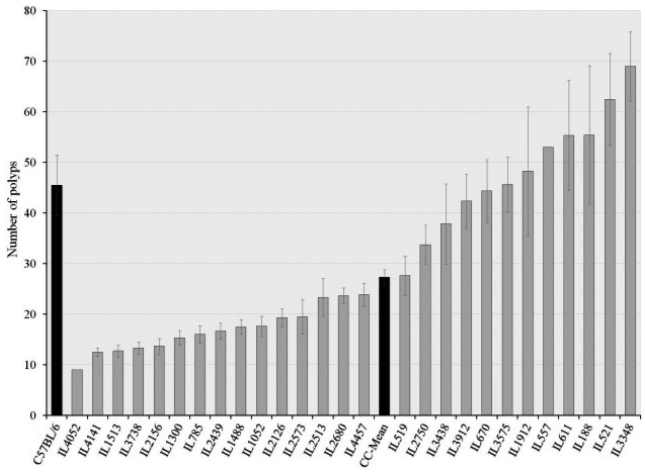

FIGURE 6 Average number of polyps (±SEM) in the 27 F1 CC-C57BL/6-*Min* lines (n = 1-16 mice/line). The x-axis represents CC lines and the C57BL/6 strain which is *Apc* $^{Min/+}$ mutation carrier (first black column from the left) while the y-axis represents number of polyps[16]

2.5.2 | Protocol steps

1. Immune-competent mice of both sexes were challenged with 10^7 spores by intravenous (IV) in the tail.
2. Mice were on a standard mouse chow diet and water ad libitum.
3. Surviving mice were censored at 28 days.
4. The response was measured as survival time (days).

2.6 | Basic Protocol 6

2.6.1 | Mapping host susceptibility to *Klebsiella pneumoniae* infection

Klebsiella pneumoniae (Kp) is one of the major pulmonary pathogens causing severe pneumonia and sepsis mainly in immunocompromised patients. Using the CC population as a high genetically diverse reference and IP injection of Kp as infectious challenge, enabled us dissecting the genetic architecture contributing to Kp host susceptibility at different time points during the challenge. For this purpose, mice generated from 73 CC lines were challenged with K-2, strain of Kp, at concentration of 10^4 colony forming units per milliliters (CFU/mL) injected IP. Mice response toward the infection was monitored daily following the challenge for 15 days, in terms of body weight and survival.[23] The survival time following the Kp infection varied among the CC lines to result in survival between 2 and 15 days post infection; importantly, we noticed that mice that survived more than 7 days could survive the complete challenge period, which might be critical time point for the disease. The wide variation observed in response to infection proves that the host response to infection is highly heritable, controlled by genes. Genetic regions underlying the significant variances of the mean survival time at different time points between days 1 and 2 were mapped. Using only 48 CC lines of the unique mouse model of CC population enables successful mapping of at least three QTLs associated with host susceptibility to Kp (Figure 8). Linkage analysis has confirmed the mapping of three significant QTLs, named Kp-*r*esistant *l*ocus 1, 2, and 3 (*Kprl1*, *Kprl2*, and *Kprl3)*, located on chromosomes 4, 8, and 18, respectively. The mapped QTLs were specific to certain time points during the infection, suggesting different "players" (genes) changing during response progress (Chr. 4—day 2 and Chr. 8 and 18—day 8).

2.6.2 | Protocol steps

1. Immune-competent mice of both sexes were subjected to IP challenge with 10^4 CFU of Kp-2 serotype.
2. Mice were on a standard mouse chow diet and water ad libitum.
3. Surviving mice censored at 15 days.
4. The response was measured as survival time (days).

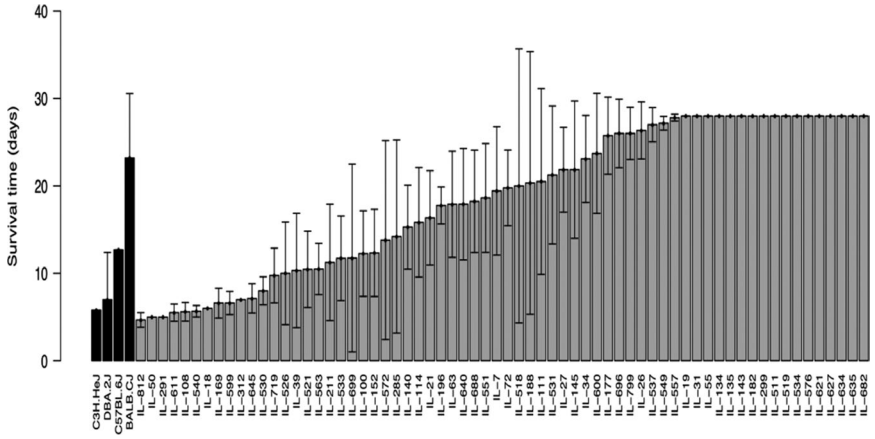

FIGURE 7 Mean survival time (days) of different CC lines in response to *Aspergillus fumigatus* infection. The x-axis represents the different CC lines while the y-axis represents the mean survival days (±SEM). Full details of the analysis are presented earlier[17]

2.7 | Basic protocol 7

2.7.1 | Mapping host susceptibility to oral mixed infection (*Porphyromonas gingivalis* and *Fusobacterium nucleatum*)

Periodontal infection (periodontitis) is the most common chronic inflammatory disease in humans, which results in destruction of tooth-supporting tissues and eventually leading to tooth loss. This process is characterized by destruction of the periodontal ligament, formation of periodontal pockets, and alveolar bone resorption.[40] The disease is initiated by periodontal pathogenic bacteria, which accumulate as subgingival biofilm and stimulate an inflammatory response in the host gingiva.[41] An excessive or sustained response leads to chronic inflammation, which is a potent amplification system for recruiting humoral and cellular components of the immune system. Recently, several lines of evidence suggest that there is a significant genetic component associated with the susceptibility to chronic periodontitis.[42,43] We use the oral mixed infection system (of the two anaerobic gram negative bacteria *P gingivalis* and *F*

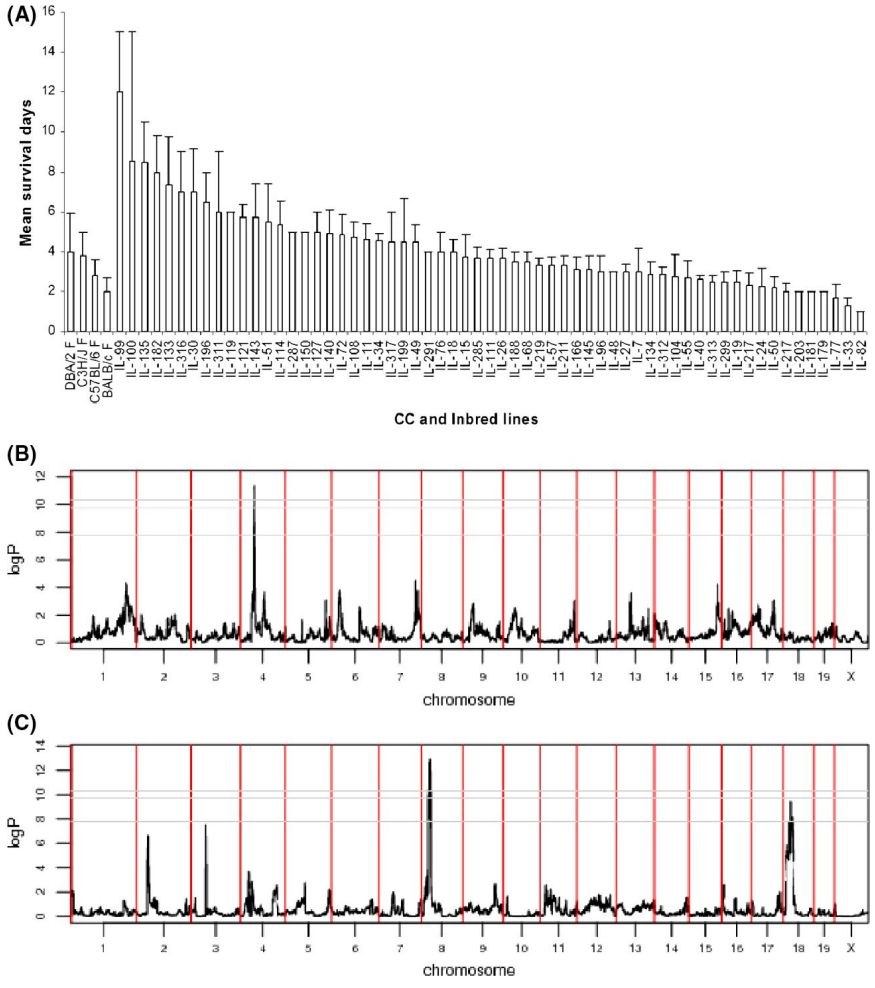

FIGURE 8 Genetic dissection of the host response to *Klebsiella pneumonia* infection. (A) Phenotypic profile of the mean survival time (days) of the different CC lines and four inbred strains after infection with *Klebsiella pneumonia*. The x-axis represents the CC lines, while the y-axis represents the time in days (±SEM). (B and C) QTL mapping results using the phenotypic and genotypic data of the CC lines. In total, results revealed three significant QTLs associated with survival time after infection with Kp, on day 2 a QTL on chromosome 4 named *Kprl*1 (B) and on day 8 two QTLs named *Kprl*2 and *Kprl*3 on chromosomes 8 and 18, respectively (C). The x-axis shows the 19 chromosomes while the y-axis logP of the linkage analysis[23]

nucleatum) as was previously described.[44] The phenotype is measured as the residual alveolar bone volume (in mm^3) after infection using the micro-CT scan, which provides an accurate measurement of the attachment loss around the animal teeth.[45] As a step toward identifying and subsequently cloning these genetic factors, we have assessed a total of 272 mice (103 females and 169 males) generated from randomly selected 23 CC lines used as genetic referencea (genotype) and infection with *P gingivalis* and *F nucleatum* as environmental challenge for bone loss evaluation (phenotype). The bone loss phenotype in response to infection was calculated by differences between the bone volumes among these two groups for each CC line. The study findings reveals highly significant variation ($P < 0.05$) in bone loss phenotype among the CC lines in response to infectious challenge (Figure 9). The CC lines were clustered by subgroups based on their response to the infection in terms of bone volume, susceptible, resistant, and intermediate. Performing QTL mapping revealed significant QTL located on chromosomes 1 and 14.[21]

2.7.2 | Protocol steps

1. Immune-competent mice of both sexes were selected for the experiment.
2. Mice were on a standard mouse chow diet and water ad libitum.
3. *P gingivalis* 381 and *F nucleatum* strain PK 1594 were grown in peptone yeast extract containing hemin and Vitamin K (Wilkins Chalgren broth, Oxoid Ltd, UK), in an anaerobic chamber with 85% N_2, 5% H_2, and 10% CO_2 followed by three washes in PBS.
4. Bacterial concentrations were measured spectrophotometrically standardized to OD_{650}nm = 0.1 for *P gingivalis*, corresponding to 10^{10} bacteria/mL, and OD_{660}nm = 0.26 for *F nucleatum*, corresponding to 10^9 bacteria/mL.
5. The mice were treated with sulfamethoxazole (0.8 mg/mL) in drinking water for a continuous period of 10 days, followed by an antibiotic-free period of 3 days, before oral application of mixed culture of *P gingivalis* and *F nucleatum* (400 μL of 10^9 bacteria/mL for each mice) at days 0, 2, and 4 (control groups were treated with PBS and 2% carboxymethycellulose instead).
6. At 42 days post infection, the mice were sacrificed after complete anesthesia, using two anesthetic materials (xylisine and ketamine) and maxillary jaws were harvested for micro-CT analysis.
7. A compact fan-beam-type CT system (MicroCT40, Scanco Medical, Bassersdorf, Switzerland) was used for quantitative three-dimensional analysis as described previously.
8. Because the micro-CT measurement is a destructive procedure, control bone volume (CBV) and residual bone volume (RBV) due to infection cannot be measured in the same mouse. Consequently, it was not possible to obtain estimates of bone loss due to infection for individual animals.
9. Since the CC mice are inbred lines, genetic differences would not present among the mice in a given line, as discussed previously. Therefore, the percentage of alveolar bone loss (PBL) for each infected mouse was calculated relatively to a control group from the same line.

2.8 | Basic protocol 8

2.8.1 | Host susceptibility to *Pseudomonas aeruginosa*

The World Health Organization (WHO) listed *P aeruginosa* as one of the leading resistant pathogens with greatest public health concern, and is one of the major bacterial infections (10%-15% of cases) in hospitals and health care systems worldwide. Severe infection with *P aeruginosa* is typical among intensive care unit (ICU) patients with high risk for development of ventilator-associated pneumonia (VAP) and sepsis.[46] Wide variations are observed in the host response toward *P aeruginosa* infection, mainly in individuals at risk and patients with cystic fibrosis. So far, many studies investigated on dissecting the *P aeruginosa* genotypic features that might be underlying the variations in the host response leading to differential disease phenomenon and pathogenesis.[47-51] Yet, recent studies focused on dissection the host genetic background that might be playing crucial role controlling the variations in response severity.[52] To address this approach, various studies in murine models assessed multiple

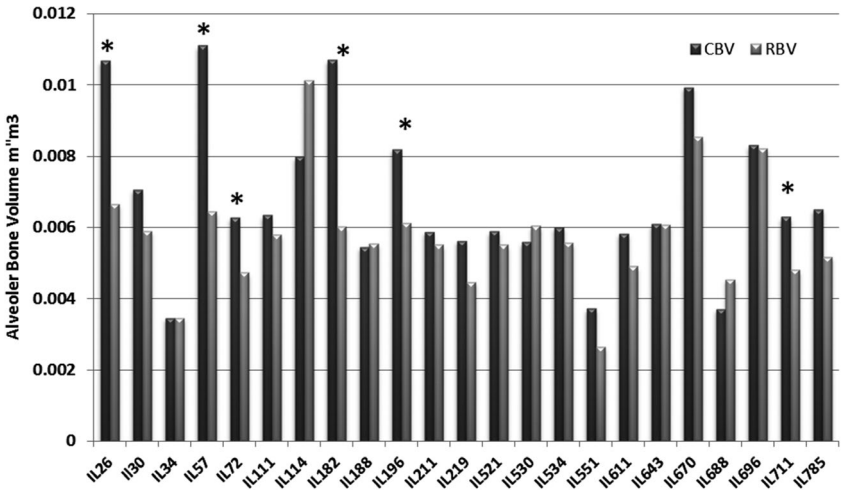

FIGURE 9 Collaborative Cross (CC) lines alveolar bone volume changes in response to oral mixed infection. A dark gray column presents control bone volume (CBV) and a light gray column presents residual bone volume (RBV). Labeled with asterisk (*) are 6 CC lines of total 23 CC lines (eg, Il26, IL57, IL72, IL182, IL196, and IL711) showing a significant bone loss ($P < 0.05$) and considered to be susceptible to the infection. The x-axis represents the CC lines while the y-axis represents the alveolar bone volume (mm^3).[60]

mice strains infected by *P aeruginosa* and repeatedly the results confirmed significant variation in infection response between the inbred strains.[48,53] As step forwards fine mapping of these regions in the genome using the CC mouse population, Lore' et al[54] assessed a total of 92 mice (50 males and 42 females) generated from 17 CC lines and challenged by *P aeruginosa* infection (intratracheal injection), the host response was determined for 7 days in terms of survival time (days) and body weight (grams) changes. As reported, the CC lines varied significantly in their survival time (Figure 10A) ranging between lethal response (survival 1.5 days) and complete resistance (100% survival).[54] As well, the CC lines varied in their body weight changes following the challenge, where few CC lines showed significant decrease in body weight and others showed recovery of BW after day 5. Figure 10B summarizes the mean survival time (MST) and percentage change in body weight at day 1 (CBW1). Hereby, using few lines of the CC population, we demonstrate the role of strong genetic components controlling the host response despite fixed and strictly controlled environmental conditions.[54]

2.8.2 | Protocol steps

1. Immune-competent mice of both sexes were selected for the experiment.
2. Mice were on a standard mouse chow diet and water ad libitum.
3. Prior to the animal experiments, *P aeruginosa* AA2 clinical strain was grown in trypticase soy broth (TSB) to reach the exponential phase at 37°C.
4. The mice were anesthetized and infected by intratracheal injection with a 10^6 CFU implanted into the lung via the cannula, with all lobes inoculated as described.
5. The survival time and body weight of mice were monitored daily for 1 week; then, the surviving mice were euthanized and tested for CFU in the blood, liver, and lung.

2.9 | Basic Protocol 9

2.9.1 | Mapping host susceptibility to sepsis

Sepsis is a severe health complication, usually triggered by the immune response of the body against infectious disease.[55] Usually, sepsis is treated with antibiotic therapies together with aggressive operative intervention and supportive treatments for the body systems in ICU. Despite continues advances in antibiotic therapies and medical support, high morbidity and mortality of sepsis are reported.[56] Sepsis pathogenesis is studied from multiple directions, one is based on dissecting the microbial factors contributing to the disease and another is host factors contributing to the sepsis development and progress. Focusing on the host response, so far studies show that the septic response of the host is triggered mainly by microbial toxins, primarily lipopolysccharide (LPS) components in gram-negative bacteria and lipoteichoic acid (LTA) in gram-positive bacteria.[57,58] Due to the variations in sepsis incidence and severity among populations, it is evident that strong genetic factors might be controlling the development of sepsis in response to interaction with microbial toxins during infectious disease. Dissecting the genetic architecture underlying the sepsis development and severity is necessary for prevention and development of personalized implementations to combat sepsis deterioration. Hence, the ongoing study in our laboratory using the CC lines population as different host genetic references and LPS injections as environmental challenge for host exposure to microbial toxins. Hitherto, 296 mice generated from 16 CC lines were given an IP injection of LPS (dissolved in PBS), thereafter the host response was monitored for 72 hours in terms of survival time (hours), body temperature, and weight (grams) changes. Preliminary data analysis showed (Figure 11A) a significant variation in survival time, where some CC lines exhibited increased LPS susceptibility and died after an average of 30 hours in contrast with the LPS-resistant CC lines that survived the 72 hours challenge. More importantly, two-way ANOVA of sex effect cross CC lines revealed a significant sex effect ($P < 0.05$), in which for most of the scanned CC lines, males were more prone to septic shock than females of the same CC line (Figure 11B). At the level of thermal response, the main response during the first 30 hours following the LPS injection was characterized by hypothermia for most of the CC lines; afterwards the CC lines varied in their response toward either hyperthermia or hypothermia (Figure 11C). Similar variations were observed in body weight changes after the challenge (Figure 11D).

2.9.2 | Protocol steps

1. Immune-competent mice of both sexes were selected for the experiment.
2. Mice were on a standard mouse chow diet and water ad libitum.

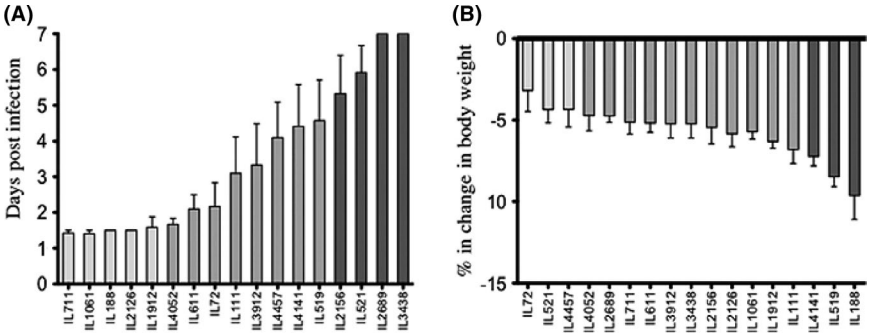

FIGURE 10 CC lines response to *Pseudomonas aeruginosa* airway infection. (A) Mean survival time (MST) in days (±SEM). (B) Percentage changes in body weight (CBW1) following the infection. The x-axis represents the CC lines while the y-axis represents the mean survival time in days (±SEM) in (A) and % change in body weight in (B)[54]

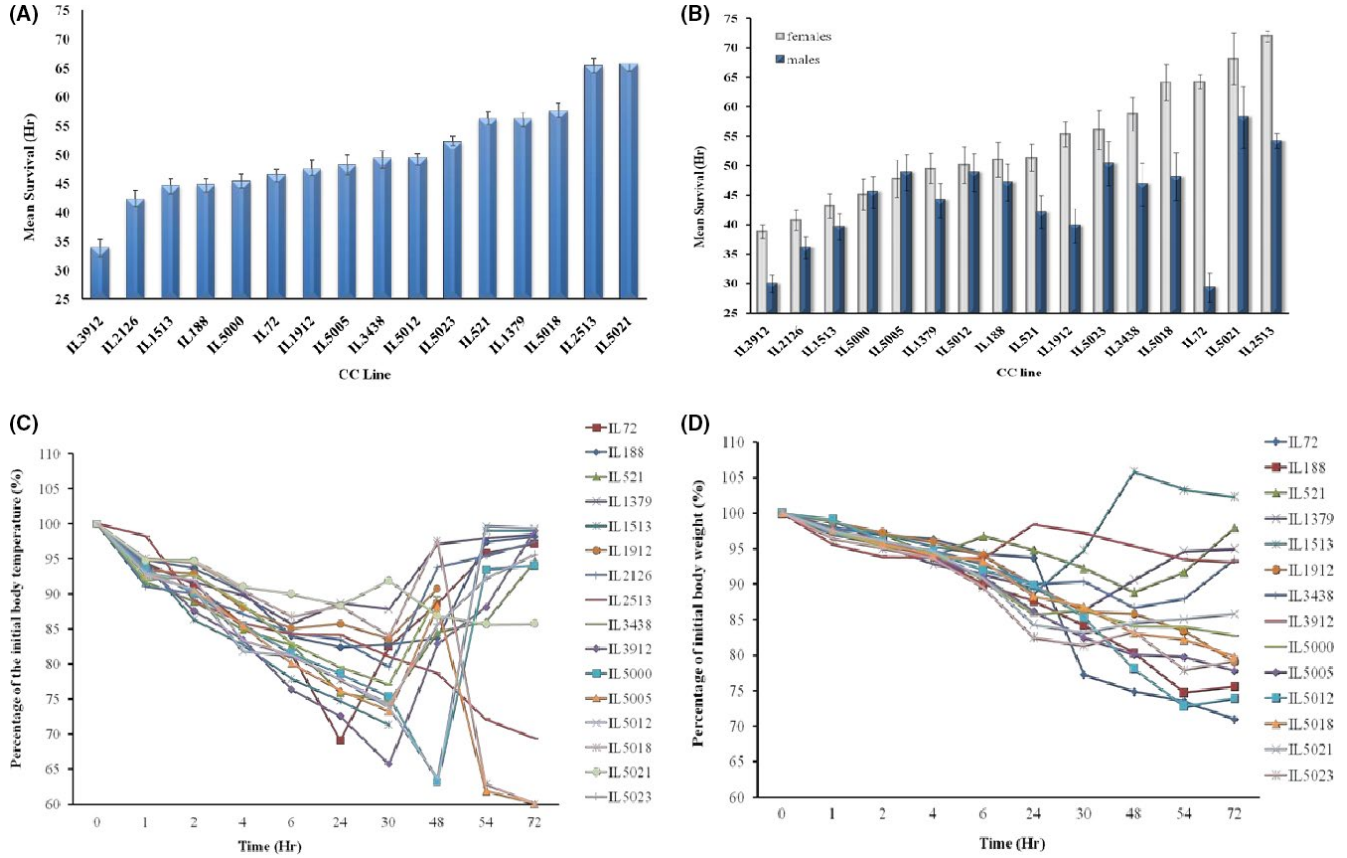

FIGURE 11 CC lines septic response toward the microbial toxic component lipopolysccharide (LPS). (A) CC lines mean (±SEM) survival time (Hr) profile during the 72 hours after the infection. (B) Data split according to sex in each CC line due to significant sex effect; the x-axis represents the CC lines while the y-axis represents the mean survival time in hours (±SEM). (B) White column for females, blue column for males. (C and D) represent multiple patterns of temperature (C) and body weight (D) changes during the 72 h post infection. Changes in temperature and body weight were calculated as percentage of initial value. The x-axis represents time after LPS injection (hr) while the y-axis represents percentage of the initial body temperature (C)/ weight (D)

3. Mice were injected IP with LPS (15 mg/1 kg mouse) dissolved in PBS.
4. The volume of the injection was set at 100 µL using 1-mL 25 G Syringes.
5. LPS from *Escherichia coli* (*E coli* 0111:B4) was ordered from Sigma Aldrich.
6. The host response was monitored for 72 hours in terms of survival time (hours), body temperature and weight (grams) changes.

3 | COMMENTARY

The laboratory mouse is one of the most important tools in that the scientific community possesses to further understand mammalian gene function. The scientific community has taken advantage of this because of its fundamental similarity to humans (>95% at the gene level), physiological and anatomical similarity, relatively low cost compared to other mammals, and nearly 100 years of genetic study. In genetically defined strains of mice, chromosomal regions responsible for the genetic variance of complex traits can be mapped as QTL in experimental populations available for precise study under defined conditions.[1,23,51] Once QTL is identified, genetic analysis can be extended successfully to humans.[4,5] It has been suggested that the variation among strains of mice can be used for mapping QTL associated with susceptibility to chronic and infectious diseases.[6-10] Here, we propose to use advanced next-generation approach for determining the genes involved in the development of complex trait diseases and various comorbidities using the CC mouse GRP. Once inbred lines of mice are crossed, the cross-progeny often exhibit transgressive variation or present novel traits that were not present in the parental strains. This indicates that the inbred strains of mice harbor a tremendous amount of natural genetic variation, above what is seen when the lines themselves are compared. This variation has been accessed in part by construction of RIL derived from crosses between mouse pure lines.[11] However, the number of available RIL is too small to provide reliable statistical support to detect smaller genetic effects. Therefore, the CC mouse model was suggested as a unique collection of RIL exhibiting a large phenotypic and genetic diversity, and bringing the tremendous genetic variation potential of the mouse inbred lines to phenotypic expression.[2,11] Full details of CC lines and their power of mapping QTL with host susceptibility to complex traits are presented in various

publications.[8,12-15,18-24,59] The CC mouse model is the focal point in our research, having exclusive access to the CC lines produced at our laboratory at Tel-Aviv University (TAU). The CC lines were three times genotyped, once using 620 000 SNPs markers of mouse diversity array,[25] once with MUGA, and later on, with MegaMUGA and GegaMUGA genotype arrays. The recent genotype status of the population was presented recently[19] showing the diversity of the phenotypic response of the CC lines with a complex disease and their power for mapping QTL associated with this trait to a small genomic interval less than 0.5 cm, which consists of few genes.

All the conducted projects in our laboratory were based on the idea of using mice of different CC lines as the genetic reference population, and determine their response toward an environmental challenge that affect the phenotype of interest. Depending on the nature of the environmental challenge and pathogenesis of each disease, the experiment period varies between few days to months, while phenotypic data are recorded at different time points starting at the initial time point of the experiment (time zero) up to the terminal time point. Subsequently, data analysis is performed using the IBM SPSS software platform for one-way and two-way ANOVA tests, which determines the significance level of the observed phenotypic variance between the CC lines and their cross interactions. Next, linkage analysis between phenotypic to genotypic data is performed using the HAPPY software for mapping the QTL and reveal the candidate genes related to the measured phenotype (trait). Figure 2 summarizes the overall frame and steps for implementing an experiment using the CC mouse models.

Based on our experience and obtained and published results from the different projects, we found that assessing a minimum of 30 CC lines in each experiment, may provide a sufficient statistical power for the QTL mapping and data analysis.

Currently, CC lines are available at four different locations: (a) The Jackson laboratory (JAX) in USA (https://www.jax.org/mouse-search?searchTerm=collaborative+cross+mice); (b) the University of North Carolina (UNC) in USA (http://csbio.unc.edu/CCstatus/index.py); (c) Tel-Aviv University in Israel (https://en-med.tau.ac.il/profile/fuadi); and (d) the Institute of Laboratory Animal Science (ILAS) in China (http://www.cnilas.org/html/en/).

ACKNOWLEDGMENTS

This work was supported by the Hendrech and Eiran Gotwert Fund for studying diabetes, Wellcome Trust grants 085906/Z/08/Z, 075491/Z/04 and 090532/Z/09/Z, core funding by Tel-Aviv University (TAU), Israeli Science foundation (ISF) grants 429/09, 961/15 and 1085/18, Binational Science Foundation (BSF) grant 2015077, German Israeli Science Foundation (GIF) grant I-63-410.20-2017, Israeli Cancer Research Fund (ICRF), Cancer Research Counsel-UK, and the Cancer Biology Research Center (CBRC) of Tel Aviv University.

CONFLICT OF INTEREST

None.

ORCID

Fuad A. Iraqi https://orcid.org/0000-0001-5525-206X

REFERENCES

1. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science*. 1994;265:2037-2048.
2. Paterson AH. Molecular dissection of quantitative traits: progress and prospects. *Genome Res*. 1995;5:321-333.
3. Vidal MS, Malo D, Vogan K, Skamene E, Gros P. Natural resistance to infection with intracellular parasites: isolation of candidate for Bcg. *Cell*. 1993;73:469-485.
4. Blackwell JM, Barton CH, White JK, et al. Genomic organization and sequence of human NRAMP gene: identification and mapping of a promoter region polymorphism. *Mol Med*. 1995;1:194-205.
5. Flint J, Valdar W, Shifman S, Mott R. Strategies for mapping and cloning quantitative trait genes in rodents. *Nat Rev Genet*. 2005;6:271-286.
6. Ishimori N, Li R, Kelmenson PM, et al. Quantitative trait loci analysis for plasma HDL-cholesterol concentrations and atherosclerosis susceptibility between inbred mouse strains C57BL/6J and 129S1/SvImJ. *Arterioscler Thromb Vasc Biol*. 2004;24:161-166.
7. Korstanje R, Li R, Howard T, et al. Influence of sex and diet on quantitative trait loci for HDL cholesterol levels in an SM/J by NZB/BINJ intercross population. *J Lipid Res*. 2004;45:881-888.
8. Machleder D, Ivandic B, Welch C, et al. Complex genetic control of HDL levels in mice in response to an atherogenic diet. Coordinate regulation of HDL levels and bile acid metabolism. *J Clin Invest*. 1997;15:1406-1419.
9. Wang X, Paigen B. Quantitative trait loci and candidate genes regulating HDL cholesterol: a murine chromosome map. *Arterioscler Thromb Vasc Biol*. 2002;22:1390-1401.
10. Wang X, Ishimori N, Korstanje R, Rollins J, Paigen B. Identifying novel genes for atherosclerosis through mouse-human comparative genetics. *Am J Hum Genet*. 2005;77:1-15.
11. Churchill GA, Airey DC, Allayee H, et al; Complex Trait Consortium. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat Genet*. 2004;36:1133-1137.
12. Abu-Toamih Atamni HJ, Mott R, Soller M, Iraqi FA. High-fat-diet induced development of increased fasting glucose levels and impaired response to intraperitoneal glucose challenge in the collaborative cross mouse genetic reference population. *BMC Genet*. 2016;17:10.
13. Abu-Toamih Atamni HJ, Botzman M, Mott R, Gat-Viks I, Iraqi FA. Mapping liver fat female-dependent quantitative trait loci in collaborative cross mice. *Mamm Genome*. 2016;27:565-573.
14. Abu-Toamih Atamni HJ, Ziner Y, Mott R, Wolf L, Iraqi FA. Glucose tolerance female-specific QTL mapped in collaborative cross mice. *Mamm Genome*. 2017;28:20-30.
15. Aylor DL, Valdar W, Foulds-Mathes W, et al. Genetic analysis of complex traits in the emerging Collaborative Cross. *Genome Res*. 2011;21:1213-1222.
16. Dorman A, Baer D, Tomlinson I, Mott R, Iraqi FA. Genetic analysis of intestinal polyp development in Collaborative Cross mice carrying the *Apc* $^{Min/+}$ mutation. *BMC Genet*. 2016;17:46.
17. Durrant C, Tayem H, Yalcin B, et al. Collaborative Cross mice and their power to map host susceptibility to *Aspergillus fumigatus* infection. *Genome Res*. 2011;1:1239-1248.
18. Iraqi F, Churchill G, Mott R. The Collaborative Cross, developing a resource for mammalian systems genetics: a status report of the Wellcome Trust cohort. *Mamm Genome*. 2008;19:379-381.
19. Collaborative Cross Consortium. The genome architecture of the Collaborative Cross mouse genetic reference population. *Genetics*. 2012;190:389-401.

20. Levy R, Mott RF, Iraqi FA, Gabet Y. Collaborative cross mice in a genetic association study reveal new candidate genes for bone microarchitecture. *BMC Genom*. 2015;16:1013.
21. Nashef A, Qabaja R, Salaymeh Y, et al. Integration of murine and human studies for mapping periodontitis susceptibility. *J Dent Res*. 2018;97:537-546.
22. Philip VM, Sokoloff G, Ackert-Bicknell CL, et al. Genetic analysis in the Collaborative Cross breeding population. *Genome Res*. 2011;21:1223-1238.
23. Vered K, Durrant C, Mott R, Iraqi FA. Susceptibility to *Klebsiella pneumoniae* infection in collaborative cross mice is a complex trait controlled by at least three loci acting at different time points. *BMC Genom*. 2014;15:865.
24. Welsh CE, Miller DR, Manly KF, et al. Status and access to the Collaborative Cross population. *Mamm Genome*. 2012;23:706-712.
25. Yang H, Ding Y, Hutchins LN, et al. A customized and versatile high-density genotyping array for the mouse. *Nat Methods*. 2009;6:663-666.
26. Mott R, Talbot CJ, Turri MG, Collins AC, Flint J. A method for fine mapping quantitative trait loci in outbred animal stocks. *Proc Natl Acad Sci USA*. 2000;23:12649-12654.
27. Moser AR, Mattes EM, Dove WF, Lindstrom MJ, Haag JD, Gould MN. ApcMin, a mutation in the murine Apc gene, predisposes to mammary carcinomas and focal alveolar hyperplasias. *Proc Natl Acad Sci USA*. 1993;90:8977-8981.
28. Rubinfeld B, Souza B, Albert I, et al. Association of the *APC* gene product with beta-catenin. *Science*. 1993;262:1731-1734.
29. Sheng JQ, Cui WJ, Fu L, et al. *APC* gene mutation in Chinese familial adenomatous polyposis patients. *World J Gastroenterol*. 2010;16:1522-1526.
30. Silverman KA, Koratkar RA, Siracusa LD, Buchberg AM. Exclusion of Madh2, Madh4, and Madh7 as candidates for the modifier of Min 2 (Mom2) locus. *Mamm Genome Res*. 2003;14:119-129.
31. Suraweera N, Haines J, McCart A, et al. Genetic determinants modulate susceptibility to pregnancy-associated tumourigenesis in a recombinant line of Min mice. *Human Mol Genet*. 2006;15:3429-3435.
32. Dietrich WF, Lander ES, Smith JS, et al. Genetic identification of Mom-1, a major modifier locus affecting Min-induced intestinal neoplasia in the mouse. *Cell*. 1993;75:631-639.
33. Harada N, Tamai Y, Ishikawa T, et al. Intestinal polyposis in mice with a dominant stable mutation of the beta-catenin gene. *EMBO J*. 1999;18:5931-5942.
34. Hirohashi S, Kanai Y. Cell adhesion system and human cancer morphogenesis. *Cancer Sci*. 2003;94:575-581.
35. Joslyn G, Carlson M, Thliveris A, et al. Identification of deletion mutations and three new genes at the familial polyposis. *Cell*. 1991;66:601-613.
36. Kinzler K, Nilbert M, Su L, et al. Identification of FAP locus genes from chromosome 5q21. *Science*. 1999;253:661-665.
37. Rudling R, Hassan AB, Kitau J, Mandir N, Goodlad RA. A simple device to rapidly prepare whole mounts of murine intestine. *Cell Prolif*. 2006;39:415-420.
38. Latgé JP *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol Rev*. 1999;12:310-350.
39. Soubani AO, Chandrasekar PH. The clinical spectrum of pulmonary aspergillosis. *Chest*. 2002;121:1988-1999.
40. Williams RC. Periodontal disease. *N Engl J Med*. 1990;322:373-382.
41. Wilson M. Biological activities of lipopolysaccharides from oral bacteria and their relevance to the pathogenesis of chronic periodontitis. *Sci Prog*. 1995;78:19-34.
42. Baker PJ, Dixon M, Roopenian DC. Genetic control of susceptibility to *Porphyromonas gingivalis*-induced alveolar bone loss in mice. *Infect Immun*. 2000;68:5864-5868.
43. Baker PJ, Roopenian DC. Genetic susceptibility to chronic periodontal disease. *Microbes Infect*. 2002;4:1157-1167.
44. Polak D, Wilensky A, Shapira L, et al. Mouse model of experimental periodontitis induced by *Porphyromonas gingivalis/Fusobacterium nucleatum* infection: bone loss and host response. *J Clin Periodontol*. 2009;36:406-410.
45. Wilensky A, Gabet Y, Yumoto H, Houri-Haddad Y, Shapira L. Three-dimensional quantification of alveolar bone loss in *Porphyromonas gingivalis*-infected mice using micro-computed tomography. *J Periodontol*. 2005;76:1282-1286.
46. Gellatly SL, Hancock RE *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. *Pathog Dis*. 2013;67:159-173.
47. Bianconi I, Milani A, Cigana C, et al. Positive signature-tagged mutagenesis in *Pseudomonas aeruginosa*: tracking patho-adaptive mutations promoting airways chronic infection. *PLoS Pathog*. 2011;7:e1001270.
48. Bragonzi A. Murine models of acute and chronic lung infection with cystic fibrosis pathogens. *Int J Med Microbiol*. 2010;300:584-593.
49. Bragonzi A, Paroni M, Nonis A, et al. *Pseudomonas aeruginosa* microevolution during cystic fibrosis lung infection establishes clones with adapted virulence. *Am J Respir Crit Care Med*. 2009;180:138-145.
50. Cigana C, Curcurù L, Leone MR, et al. *Pseudomonas aeruginosa* exploits lipid A and muropeptides modification as a strategy to lower innate immunity during cystic fibrosis lung infection. *PLoS ONE*. 2009;4:e8439.
51. Nguyen D, Singh PK. Evolving stealth: Genetic adaptation of *Pseudomonas aeruginosa* during cystic fibrosis infections. *Proc Natl Acad Sci USA*. 2006;103:8305-8306.
52. Weiler CA, Drumm ML. Genetic influences on cystic fibrosis lung disease severity. *Front Pharmacol*. 2013;4:40.
53. De Simone M, Spagnuolo L, Lorè NI, et al. Host genetic background influences the response to the opportunistic *Pseudomonas aeruginosa* infection altering cell-mediated immunity and bacterial replication. *PLoS ONE*. 2014;9:e106873.
54. Lorè NI, Iraqi FA, Bragonzi A. Host genetic diversity influences the severity of *Pseudomonas aeruginosa* pneumonia in the Collaborative Cross mice. *BMC Genet*. 2015;16:106.
55. Levy MM, Fink MP, Marshall JC, et al. 2001 CCM/ESICM/ACCP/ATS/SIS International Sepsis Definitions Conference. *Crit Care Med*. 2003;31:1250-1256.
56. Friedman G, Silva E, Vincent JL. Has the mortality of septic shock changed with time? *Crit Care Med*. 1998;26:2078-2086.
57. Mattsson E, Verhage L, Rollof J, Fleer A, Verhoef J, van Dijk H. Peptidoglycan and teichoic acid from *Staphylococcus epidermidis* stimulate human monocytes to release tumour necrosis factor-alpha, interleukin-1 beta and interleukin-6. *FEMS Immunol Med Microbiol*. 1993;7:281-287.
58. Van der Poll T, Opal SM. Host–pathogen interactions in sepsis. *Lancet Infect Dis*. 2008;8:32-43.
59. Iraqi FA, Athamni H, Dorman A, et al. Heritability and coefficient of genetic variation analyses of phenotypic traits provide strong basis for high-resolution QTL mapping in the Collaborative Cross mouse genetic reference population. *Mamm Genome*. 2014;25:109-119.
60. Shusterman A, Salyma Y, Nashef A, et al. Genotype is an important determinant factor of host susceptibility to periodontitis in the Collaborative Cross and inbred mouse populations. *BMC Genet*. 2013;14:68.

How to cite this article: Abu-Toamih Atamni HJ, Iraqi FA. Efficient protocols and methods for high-throughput utilization of the Collaborative Cross mouse model for dissecting the genetic basis of complex traits. *Animal Model Exp Med*. 2019;2:137–149. https://doi.org/10.1002/ame2.12074