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ORIGINAL ARTICLE

Assessing the host genetic background effects on type 2 diabetes and obesity development in response to mixed–oral bacteria and high-fat diet using the collaborative cross mouse model

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Abstract

Background: Host genetic background and sex, play central roles in defining the pathogenesis of type 2 diabetes (T2D), obesity and infectious diseases. Our previous studies demonstrated the utilization of genetically highly diverse inbred mouse lines, namely collaborative cross (CC), for dissecting host susceptibility for the development of T2D and obesity, showing significant variations following high-fat (42% fat) diet (HFD). Here, we aimed to assessing the host genetic background and sex effects on T2D and obesity development in response to oral-mixed bacterial infection and HFD using the CC lines.

Materials and Methods: Study cohort consists of 97 mice from 2 CC lines (both sexes), maintained on either HFD or Standard diet (CHD) for 12 weeks. At week 5 a group of mice from each diet were infected with *Porphyromonas gingivalis* (*Pg*) and *Fusobacterium nucleatum* (*Fn*) bacteria (control groups without infection). Body weight (BW) and glucose tolerance ability were assessed at the end time point of the experiment.

Results: The CC lines varied ($P < .05$) at their BW gain and glucose tolerance ability (with sex effect) in response to diets and/or infection, showing opposite responses despite sharing the same environmental conditions. The combination of diet and infection enhances BW accumulation for IL1912, while restraints it for IL72. As for glucose tolerance ability, only females (both lines) were deteriorated in response to infection.

Conclusions: This study emphasizes the power of the CC mouse population for the characterization of host genetic makeup for defining the susceptibility of the individual to development of obesity and/or impaired glucose tolerance.

Luna Karkar and Hanifa Abu-Toamih Atamni contributed equally to this work.

KEYWORDS

collaborative cross (CC) mouse model, high-fat diet (HFD), mixed oral bacteria (*PG* and *Fn*), obesity, sex-differences, type 2 diabetes (T2D)

1 | INTRODUCTION

Type 2 diabetes (T2D) is one of the most ancient human diseases, which has been proven to be a multifactorial and polygenic disease, usually accompanied by related health complications. Based on epidemiological studies, 2.2 million deaths in the year 2012 were attributed to hyperglycemia, while in the year 2015; an estimated number of 1.6 million deaths were directly caused by diabetes.[1,2] The World Health Organization (WHO) projections for the year 2030 indicate that diabetes will be the seventh leading cause of death worldwide.[1,2] One of the major risk factors for T2D development is obesity, which is defined as a medical condition in which excess body fat has accumulated to the extent that it may have negative health effects. These reports show that overall obesity by body weight (BW), body length (for BMI calculation) and central obesity can be a strong predictor for T2D development.[3-5]

Nowadays, obesity is considered a serious public health condition that is increasing worldwide[6] and requires treatment and prevention strategies to avoid severe health complications.[7,8] Hence, obesity increases the risk of chronic disease development and progress such as diabetes mellitus, cardiovascular disease, stroke, non-alcoholic fatty liver disease and certain cancers. And yet, obesity's impact on health varies between individuals, and not all obese people develop health complications at the same level of severity.[9]

Previous studies showed that the etiology of T2D and obesity is influenced by genetic and environmental factors and their interactions.[10]

Hence, the host genetic background plays a central role in defining the phenotypic response of the host to certain environmental conditions, such as consumption of fat/carbohydrates-rich diets, sedentary lifestyle, environmental stressors (infectious pathogens/air pollution, etc.). Therefore, it is suggested that due to genetic background differences among individuals, there are significant response variations to a given phenotype, despite sharing the same environmental conditions.[11]

In some cases, the progress of T2D and obesity can be balanced/delayed by environmental interventions, mainly involving a change of lifestyle (diet, physical activity, etc.). Yet, the success of the environmental interventions is also influenced by the host genetic background, where individuals with a certain genetic makeup may respond positively (improved glucose tolerance and reduce obesity) to a certain diet, while others (from a different genetic makeup) shows no effects or even worsened features of the diseases.[12]

Another factor that may influence the development and progress of obesity and T2D is infectious pathogens, such as pathogenic oral bacteria. A previous study by Demmer and group, 2015, have reported that oral exposure of certain bacteria, including *Porphyromonas gingivalis* (*Pg*) was positively associated with pre-diabetes conditions in human populations.[13]

Additional studies published by our research group showed in separate experiments the significant contribution of the host genetic background on determining the host susceptibility or resistance in response to high-fat diets (HFDs), and in response to infection with mixed pathogenic oral bacteria, using the unique CC mouse model genetic reference population (GRP) as an appropriate animal murine model for exploring the genetics of complex trait diseases.[14-17] Using the collaborative cross (CC) mouse GRP in these studies, confirmed that sex and diet effects, significantly varied between the different CC lines, where some CC lines, that is certain genetic backgrounds showed excessive BW gain and impaired glucose tolerance in response to HFD, while other genetic backgrounds were resistant to these environmental challenges. These studies showed and confirmed that sex differences, the gap between males to females within a CC line, in terms of BW and glucose tolerance ability, varied significantly between the different CC lines.[17] In a separate study, significant variation in response to mixed-oral infection was observed between the different CC lines, at the level of alveolar bone resorption.[18] In previous reports, studying diet and infection separately, raise the question of diet and infection combination impact on development of obesity and T2D at 1 level (within CC line in different environments), and the influence of host genetic backgrounds on these effects at second level (comparison between CC lines in response to the same environmental condition). Herein, we present a novel study model using the multi-challenges approach on 2 different genetic backgrounds of the CC mouse model, with the presentation of both sexes. The multi-challenge in our study consists of HFD (42% Fat) vs CHD (11% Fat) with or without (placebo) infection with oral mixed bacteria (*P. gingivalis* and *Fusobacterium nucleatum*), while host responses were measured for bodyweight accumulation and glucose tolerance ability following 12 weeks of the experimental period. Thereby, our research presents a murine model for examining the hypothesis that T2D and obesity as chronic inflammatory diseases can be induced to different levels by HFD (inflammatory diet) and infection with mixed oral bacteria, and may be controlled by common or distinct genetic components of the host. The outcome of this study provides a unique platform for future research, aiming for mapping and identification of new susceptibility/resistance host genes that control obesity and T2D in response to multi-challenge environments, while using comparative mapping approach, which subsequently will propose candidate human genetic components, predisposing T2D and obesity and eventually leading to a better understanding of the etiology of comorbidity and personalized sex-specific treatment and prevention strategies.

2 | MATERIALS AND METHODS

2.1 | Ethical statement

All experimental mice and procedures were approved by the Institutional Animal Care and Use Committee (no. 01-19-013) of Tel-Aviv University (TAU), which adhered to the Israeli guidelines that follow the National Institutes of Health of USA animal care and use protocols.

2.2 | CC lines

Study cohort consisted of 97 mice (50 females and 47 males, detailed N of mice in Table 1) generated from the 2 different CC lines, named IL72 and IL1912, provided by the small animal facility at TAU (full details of the breeding colony available at Iraqi et al. 2008 and 2012). At the age of 3-week-old, mice were weaned to separate cages by sex and line, and housed on hardwood chip bedding in open-top cages, maintained at a 12:12-h light: dark cycle at a temperature of 21-23°C with free access to standard rodents chow diet (CHD) of Altromin 1324 IRR, and water ad libitum.

TABLE 1 Summary table of the total number (N) of mice from each CC line (IL72 and IL1912), males and females separately, assessed in each study group

	CHD (11% fat)				HFD (42% fat)				
	Inf (−)		Inf (+)		Inf (−)		Inf (+)		
CC line	♀	♂	♀	♂	♀	♂	♀	♂	Total
IL72	3	4	4	5	5	5	3	3	32
IL1912	10	12	8	5	9	4	8	9	65
Total	13	16	12	10	14	9	11	12	97

2.3 | Dietary challenge

Dietary challenges of the experiment consisted of CHD (as a control group) provided from Altromin 1324 IRR. (Altromin Spezialfutter GmbH & Co Germany), which consists of 11% Kcal from fat, 24% from Protein, and 65% from carbohydrates, and HFD considered as Western diet, TD. 88137 (Teklad Global, Harlan Inc.), which consists of 42.0% kcal from fat, 15.3% from protein, and 42.7% from Carbohydrates (primarily sucrose).

2.4 | Study design

The total period of the experiment was 12 weeks, including the 2 environmental challenges of HFD and oral infection with mixed-oral bacteria (infographic scheme in Figure 1). At the zero-time point of the experiment (8-week old mice), BW was recorded using an electronic scale (0.1 g accuracy), and consequently mice divided into 2 dietary groups, in which HFD (42% fat) provided for the experimental group and CHD (11% fat) for the control group. At week 5 of the experiment (13 weeks old), mice from both dietary conditions were divided into 2 groups for the infection challenge, where experimental groups were orally infected with mixed-oral bacteria by gavage, and control groups were placebo-infected without bacteria as a control group. At week 12 of the experiment, glucose tolerance ability was assessed by intraperitoneal (IP) glucose tolerance test (IPGTT), and after overnight recovery, mice were weight and sacrificed. Summary table of the four study groups, named

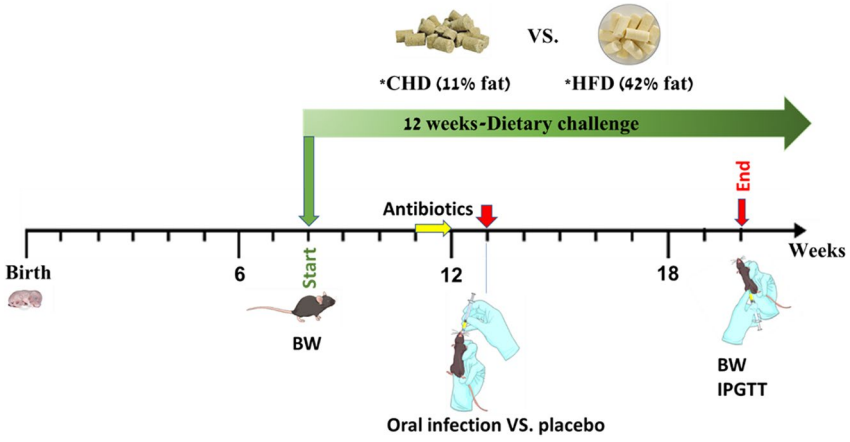

FIGURE 1 Study design scheme showing timescale and study groups of the experiment procedures, starting from the age of 8 weeks old (start point) until the age of 20 wk old, that is, 12 wk period. At the start time point, BW was recorded and consequently, mice divided into 2 dietary groups of HFD (*42% fat) or CHD (*11% fat) as the control group. At week 5 of the experiment (13-wk-old), perorally infection was performed with mixed oral bacteria and placebo-infection without bacteria for control groups. At week 12 of the experiment, glucose tolerance ability was assessed by IPGTT, and mice sacrifice. CHD—Chow diet; BW—BW; HFD—high-fat diet; IPGTT—Intraperitoneal glucose tolerance test. *% kcal/kg from fat (metabolized energy)

(a) CHD/non-infected, (b) CHD/Infected, (c) HFD/non-infected, (d) HFD/Infected, presented in Table 2.

2.5 | IP glucose tolerance test

The glucose tolerance test measures the clearance ability of an intraperitoneally injected glucose load from the bloodstream, during 180 min following a glucose load, to detect disturbances in glucose metabolism that can be linked to diabetes or pre-diabetic conditions. Following 6 hours (06:00-12:00 AM), fasting with free access to water, fasting blood glucose levels were measured (time zero) and consequently a solution of glucose (2.5 mg glucose per g mouse body mass) was administered by IP injection. Thereafter, blood glucose levels were measured at different time points (15, 30, 60, 120, and 180 minutes after glucose injection), using the Accu-Check Performa glucometer (AC PERFORMA KIT 53597 by Roche Ltd.) and glucose strips (AC Performa 50 F2 24049 by Roche Ltd.).

2.6 | Bacterial cultivation

Porphyromonas gingivalis (*Pg*) strain ATCC 33277 and *F. nucleatum* (*Fn*) strain PK 1594 were grown in peptone yeast extract containing hemin and vitamin K (Wilkins Chalgren broth, Oxoid Ltd), in an anaerobic chamber with 85% N2, 5% H2, and 10% CO2, followed by 3 washes in 1% phosphate-buffered saline (PBS). Bacterial concentration was measured using the spectrophotometer standardized to OD_{650nm} = 0.1 for Pg, corresponding to 10^{10} bacteria/mL; and OD_{660nm} = 0.26 for *Fn*, to 10^{9} bacteria/mL. Quality control was tested by a confocal microscope (to eliminate contaminations), pictures of *Pg* and *Fuso* showed in Figure 2. Before the infection, both strains of bacteria were mixed with the addition of carboxymethyl cellulose (CMC) (two-thirds of total volume) to the ratio of 1:1 (*Pg*: *Fn*).

TABLE 2 Summary table of the 4 study groups by diet and infection challenges

Study group[a]	Diet	Infection
1	CHD (11% Fat)	No
2	CHD (11% Fat)	Yes
3	HFD (42% Fat)	No
4	HFD (42% Fat)	Yes

[a]Males and females were assessed separately for each study group, that is in total 8 study groups presented here.

2.7 | Oral infection challenge

Before the infection mice were treated with antibiotics to standardize the oral microbiota status of the different mice, using sulfamethoxazole (10 mL/500 mL) water administration for 10 days, followed by 3 days recovery (antibiotic-free). Then infection challenge started, by oral infection with 400 µL per-mouse of the mixed-oral bacteria (*Pg* and *Fn*). The infection procedure was repeated every other day 3 times during 5 days of week 5. In parallel, control groups of the placebo infection were treated with 400 µL of 2% CMC in distilled water and 1% PBS (ratio of 2:1 for CMC: PBS).

2.8 | Data Analysis

Data analysis were performed using the IBM SPSS (statistical package for the social sciences) software platform Version 24 using the One-way analysis of variance tests (ANOVA) to assess the significance level of the observed variations between the CC lines and sex cross diet effects, respectively. Independent *T*-test was performed to assess the significance level of the observed variations between study groups, $P < .05$ considered significant

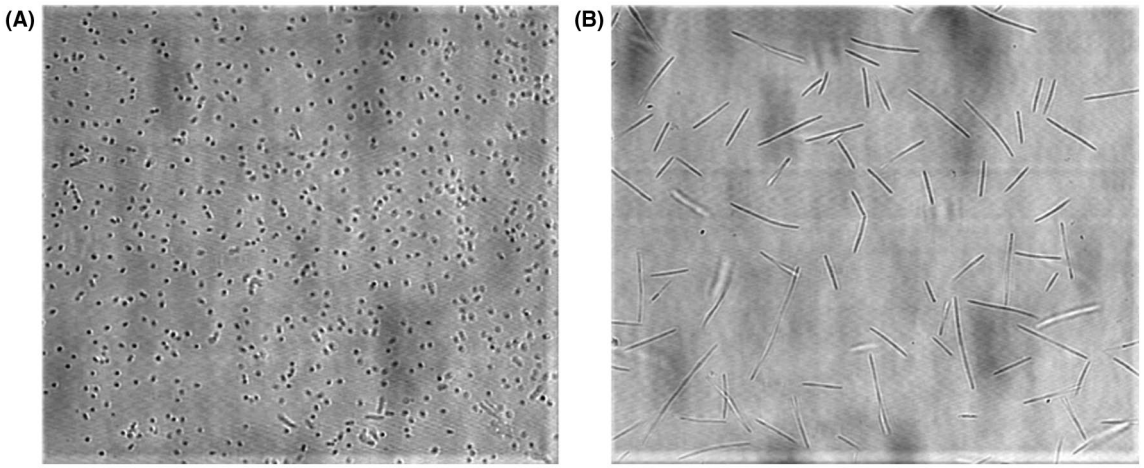

FIGURE 2 Confocal microscopy images for oral bacteria samples. A, *Porphyromonas gingivalis* (PG) and B, *Fusobacterium nucleatum* (Fn)

2.9 | Area under the curve calculation

To assess the glucose tolerance ability status, area under the curve (AUC) of the IPGTT results was calculated according to the trapezoid role between time 0 and 180 minutes, as a quantitative measure of glucose clearance activity, using the formula below:

$$\mathrm{AUC}_{\text{time a–time b}} = (b_{\min} - a_{\min}) \times (\text{glucose levels at time } a + b)/2$$

$$\text{Total AUC} = \mathrm{AUC}_{0-180} = \mathrm{AUC}_{0-15} + \mathrm{AUC}_{15-30} + \mathrm{AUC}_{30-60} + \mathrm{AUC}_{60-120} + \mathrm{AUC}_{120-180}.$$

3 | RESULTS

In total, our study consisted of 8 experimental groups, including a challenge with HFD or Infection, separately or jointly groups and their control groups with CHD and No-Infection, with both sexes. Data analysis were performed with different directions of analysis, reporting independent effects at first levels, such as sex effects within a line, diet effects only (HFD vs. CHD) or infection effects only (Infection vs. No-infection on the same diet), and interaction between the challenges at second level, that is combination of HFD with infection.

3.1 | Sex effects within the CC line vary between IL72 and IL1912

Sex effects for BW and glucose tolerance ability, at week 12, in response to dietary and infection challenges, varied between IL72 and IL1912. In all studied groups, for both CC lines, males showed higher values than females (to different distinct) for BW (g) (Figure 3), and AUC (min × mg/dL), except in the group of IL72 on HFD/Infection (Figure 4B), where females showed higher values of AUC than males. One-way ANOVA for sex effects revealed a highly significant ($P < .05$) variations between males to females for IL1912, for BW and glucose tolerance ability (AUC) in all the studied groups/conditions (diet and infection). For IL72 the sex effects within the line were significant for all the groups for BW, except the experiment group of HFD with infection (HFD/Inf). At the levels of glucose

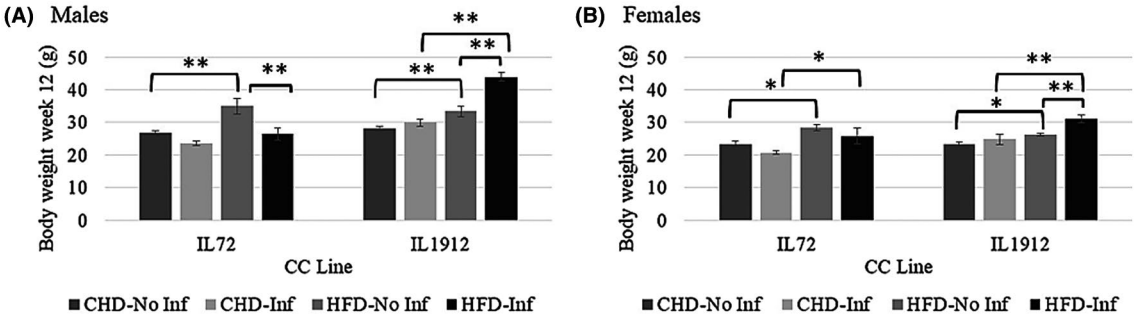

FIGURE 3 Body Weight (g) measures at the end time point (wk 12) of IL72 and IL1912 mice, separately for females and males following 12 wk on Chow diet (CHD, 11% fat) vs high-fat diet (HFD, 42% fat) and with or without infection. A, The BW (means ± SE) for female mice. B, BW (means ± SE) of males of the four studied groups. The *X*-axis represents the different CC lines; the *Y*-axis represents BW (g) at the end time point of the experiment (week 12)

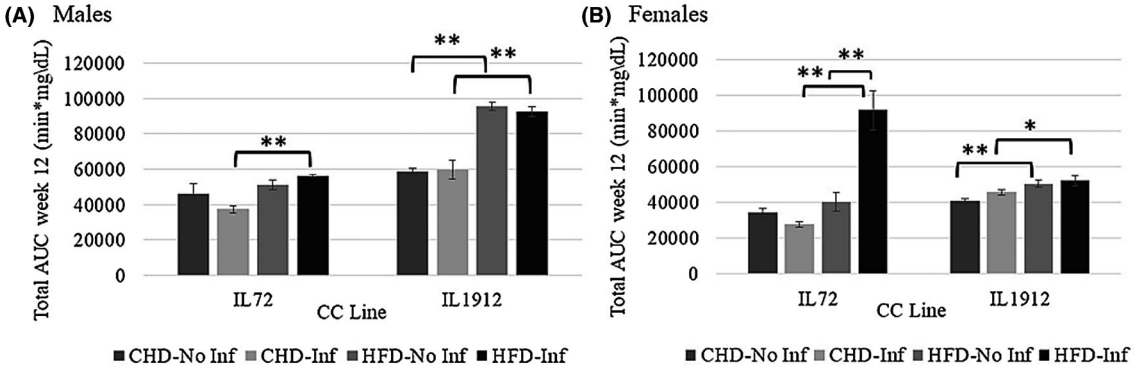

FIGURE 4 Blood glucose levels (mg/dL) during intraperitoneal glucose tolerance test (IPGTT) of IL72 and IL1912 mice, separately for females and males, after 12 weeks on Chow diet (CHD, 11% fat) and on high-fat diet (HFD, 42% fat) measured at time 0, 15, 30, 60, 120, and 180 min after glucose injection. A, (means ± SE) of the total area under the curve (AUC) for the female mice of the 4 studied groups, including maintaining mice on CHD and HFD and with and without the infection condition. B, (means ± SE) of total AUC for the male mice of the 4 studied groups. The *X*-axis represents the different CC lines; the *Y*-axis represents AUC (min × mg/dL) at week 12

tolerance ability for IL72, the sex effects were significant ($P < .05$) for both diets (CHD and HFD), only with the combination of infection. As shown in Figure 3, the BW differences between males and females within a line were greater on HFD, for the groups without infection, thus for IL72 the difference between males to females was 3 g when maintained on CHD (Figure 3A,B), while reached 7 g difference when maintained on HFD (Figure 3A,B), and similarly for IL1912, that is 5 vs 7 g. As, for IL1912, the gap between males to females on HFD with or without infection was highly significant ($P < .01$) and increased from 7 to 13 g when maintained on HFD with the infection (Figure 3A,B). Moreover, as shown in Figure 3A,B for IL72, infection with HFD had significantly reduced the BW gap between males to females, changing from significant ($P < .05$) difference of 7 g, on HFD without infection to non-significant difference of only 1 g on HFD with infection. As for glucose tolerance ability, presented in Figure 4A,B, as the AUC at week 12, sex effects were significant ($P < .05$) for IL1912 in all study groups, while for IL72 the sex effects were significant ($P < .05$), only for studied groups with infection (HFD/Infected and CHD/Infected). Focusing on the sex effects within a line in CHD vs HFD, without infection, presented in Figure 4A,B, revealed non-significant and consistent differences (~9.6% change) between males to females for IL72 on both diets (11 898 vs 10 751 minutes × mg/dL for CHD vs HFD, respectively). While, for IL1912 the sex differences reach greater levels (extra 151% units) when maintained on HFD (45 096 minutes × mg/dL) vs CHD (17 937 minutes × mg/dL), without infection. Interestingly, at the level of infection effects on the observed sex differences, comparing Figure 4A vs 4B, showed that the differences between males to females in IL72 and IL1912 is reduced to lower levels in response to the infection, that is 11 898 vs 9648 minutes × mg/dL for IL72; and 17 937 vs 13 774 minutes × mg/dL for IL1912 in CHD/non-infected vs CHD/Infected, respectively. However, on the HFD groups, the infection influenced the sex effects and IL72, showed higher values of AUC for females vs males, 10 751 minutes × mg/dL vs −35 588 minutes × mg/dL gap between males to females when maintained on HFD with infection (Figure 4A,B). While IL1912 showed the same pattern of sex effects (males higher than females) in all the studied groups with very slight changes in the same direction. Apparently, males and females within each CC line differ significantly in most of the cases in their levels of BW (g) and AUC (min × mg/dL) and, therefore, will be assessed and discussed separately.

3.2 | Opposite effects on BW and glucose tolerance in response to infection between IL72 and IL1912

Interestingly, the combination of infection with both diets lead to different responses between the 2 CC lines. Yet, HFD vs CHD consumption (both with infection) leads to higher BW accumulation within and between a line (Figure 3A,B) for both CC lines. However, the BW gain was significant ($P < .01$) only for IL1912, for both sexes, showing 43.8 ± 1.28 vs 29.9 ± 1.07 g for males (Figure 3A) and 31.1 ± 1.27 vs 24.8 ± 1.53 g for females (Figure 3B) when maintained on HFD vs CHD, respectively. IL72 shows an increase for both sexes but was significant ($P < .05$) only for females (Figure 3B) showing 25.8 ± 2.38 vs 20.75 ± 0.61 g. As for variations between the CC lines in response to CHD vs HFD with infection, IL1912 (both sexes) showed higher BW gain than IL72, when maintained on HFD. Statistical analysis showed significance level ($P < .01$) only for male groups and showed 29.9 ± 1.07 vs 23.5 ± 0.69 g for IL1912 vs IL72, when maintained on CHD with infection, and 43.8 ± 1.28 vs 26.4 ± 1.88 g, respectively, when maintained on HFD. Moreover, IL72 and IL1912 showed opposite responses to infection at the level of BW gain for both diets, as presented in Figure 3A,B, CHD/without and with infection, respectively, and HFD/without and with infection, respectively, with both sexes. The infection challenge decelerated BW gain for IL72, while oppositely accelerates BW gain for IL1912, on both diets to a different extent. Furthermore, when IL1912 maintained on CHD or HFD with infection, the calculated BW gain (delta BW = BW_{wk12}-BW_{wk0}) increases from 5.1 to 6.88 g gain for males and 4.41 to 5.81 g gain for females, without vs with infection on CHD, respectively, and to greater extent when maintained on HFD, increasing from 10.36 to 20.96 g for males and 7.3 to 12.10 g gain for females, without vs with infection (Figure 3A for males and Figure 3B for females). Oppositely, IL72 males and females showed a lower BW gain when exposed to infection, on both diets, hence BW gain for males decreased from 3.66 to 0.41 and for females from 3.53 to 0.93 g decrease when maintained on CHD without vs with infection, respectively, and from 11.77 to 3.26 g decrease for males and 8.59-5.98 for females when maintained on HFD without vs with infection.

As for the effect of the infection on glucose tolerance ability (AUC levels), data analysis revealed an increase in AUC levels within line and sex (ie impairment of glucose tolerance ability) in response to HFD consumption in both conditions of infection and placebo Figure 4A for males and 4B for females , CHD without vs with infection, and HFD without vs with infection, respectively), all increases were significant ($P < .01$) except for the IL1912 females' group on CHD vs HFD with infection. Nonetheless, a comparison between the CC lines reveals opposite effects of the infection on AUC levels between IL72 and IL1912 for both sexes when maintained on CHD (Figure 4, thus IL72 males and females showing amelioration of the glucose tolerance ability (ie decrease in AUC values) in response to infection, with delta AUC (delta $AUC_{\text{Infection effect}}$ = $AUC_{\text{CHD/No-Infection}}$–$AUC_{\text{CHD/Infection}}$) 8811 and 6562 minutes × mg/dL for males and females, respectively. Oppositely, on the same diet (CHD), IL1912 males and females showed deterioration (ie an increase of AUC levels) of the glucose tolerance ability in response to infection, in which delta AUC of the infection effect for males was −895 and to greater extent −5058 in females. Interestingly, these responses to infection were reversed when mice were maintained on HFD (except for IL1912 females, which still show deterioration, but moderate), unlike the described amelioration on CHD condition, males and females of IL72 show here (HFD) a significant ($P < .01$) deterioration of glucose tolerance ability in response to infection, delta AUC of −4949 and −51 287 minutes × mg/dL for males and females, respectively, while males of IL1912 show a significant ($P < .01$)

amelioration (delta AUC of 3321 minutes × mg/dL) of glucose tolerance ability in response to infection when maintained on HFD. In all groups and conditions, AUC levels of IL1912 were significantly ($P < .01$) higher than IL72 levels, for males and females. Furthermore, focusing on CHD with infection group (Figure 4A,B), the AUC levels of IL1912 females' were significantly higher than the IL72 females' group, (45 780.94 ± 1628.3) vs (27 748.13 ± 1459.64) minutes × mg/dL, respectively, and similarly for males groups with AUC levels of (59 554.5 ± 5376.6) vs (37 396.5 ± 1932.40) minutes × mg/dL for IL1912 vs IL72, respectively; likewise, for the male's groups on HFD with infection as presented in Figure 4A, where AUC levels were (92 350.83 ± 2863.96) vs (56 180 ± 507.01) minutes × mg/dL for IL1912 vs IL72, respectively. Interestingly, however, this relationship between IL1912 to IL72 of AUC levels drastically change for the female's groups when maintained on HFD with infection (Figure 4B), where IL72 females' group show a significantly ($P < .01$) high deterioration of glucose tolerance ability in response to infection, to exceed the AUC levels of IL1912 females' on CHD with infection (Figure 4B), reaching (91 767.5 ± 10 867.79) vs (52 312.5 ± 2949.80) minutes × mg/dL, respectively, and as well exceeding the AUC levels of IL72 males (on both CHD and HFD with infection) and IL72 AUC levels of CHD with infection females'.

4 | DISCUSSION

The current study represents a novel and unique murine model for addressing multiple complex diseases of various human aspects of T2D and obesity, in combinations of different host genetic backgrounds and environmental challenges, which emphasizes the power of genetically diverse mouse reference population, the CC lines. The study findings promise to elucidate the nature of the genes involved in resistance or susceptibility to the development of diet-induced diabetes and obesity at first level and infection-induced at the second level, and the possible interactions of diet cross-infection affected by host genetic background. The results of this study model justify the expansion of assessing further CC lines, to identify genetic risk factors to be used for the prediction of an individual to develop these diseases under certain environmental conditions, which may lead to the development of a genetically based strategy for their prevention and treatment. Moreover, our study design includes the representation of both sexes, and the results emphasize the importance of studying males and females, separately for these diseases, in which sex effects are proven to be significant within the same CC line in response to common environmental conditions.

Pg and *Fn* are part of the oral flora, which is considered as the gateway and part of the gastrointestinal tract. These bacteria are also known as pathogenic for periodontal disease (PD), initiating a chronic inflammation that triggers inflammatory host immune responses at local and most likely systemic levels. Oral pathogens are traditionally regarded as the principal cause of periodontal inflammation, which is related to obesity and T2D in certain populations. Rather than the specific appearance of individual pathogens, a shift in the global balance of the microbial flora is attributed to the transition from health to disease.[19] Oral administration of *Pg* was shown to lead changes in oral and gut microbiota which are sensitive as well to the nutrient environment, and it was proposed that particular microbial configurations can promote or prevent inflammatory immune responses that drive metabolic dysfunction and inflammation. Likewise, HFD may act directly or indirectly leading to metabolic inflammation in susceptible obese individuals, which subsequently leads to an inflammatory state in the oral mucosa, eventually causing changes of the oral and gut microbiome. These changes would trigger a long-lasting immune reaction, eventually resulting in the development of obesity and T2D, independently or as comorbidity. A HFD plays as a risk factor for both T2D and PD through biological mechanisms including inflammation. Indeed, our study assumes and explains that the inflammation is the major drive and underlining the development and expression of these studied phenotypes. Nonetheless, we believe that the inflammation mechanisms, which are developed by HFD vs bacterial infection, are different, therefore the host response might be in different routes and levels with HFD and Bacterial infection, separately, vs the combined together, which may introduce synergism in the response.

The immune response to excess saturated fatty acids varies between individuals living in the same nutritional environment. This variation is explained to a significant extent by genetic variability. The understanding of how this genetic variation translates into different clinical manifestations will highlight pathways through which dietary composition may initiate or accelerate inflammatory disease processes and indicate mechanism through which disease can potentially prevent.

System genetics is an approach to understand the flow of biological information that underlies complex traits. The advantage of system genetics is that it allows an analysis of molecular interactions in a context that is the most relevant to the clinical trait, namely, multiple genetic perturbations (as in a natural population) rather than an individual genetic perturbation (as in a transgenic mouse).[20] Comparative mapping showed that mouse models can recapitulate human conditions and that the majority of genes in mice have orthologous in the human genome.[21] It was demonstrated that genetically highly diverse sets of recombinant inbred mouse lines (RIL) can be used as a tool for the identification of risk genes in complex human diseases.[22] Especially, highly inbred RILs such as were provided by the CC,[23-25] the next generation of mouse GRP, allow time and cost-efficient mapping of target regions as quantitative trait loci that are responsible for the genetic variance of a specific complex trait.

Finally, the CC mouse GRP will provide a unique and excellent platform and resource for studying the comorbidity and influence of T2D development due to HFD and Periodontitis, and identifying the genetic factors underlying this comorbidity.

To the extent of our knowledge, the proposed study represents a first-ever, novel and unique design of addressing multiple complex diseases of various human aspects of metabolic syndrome and its complications including, obesity, T2D, and periodontitis, on the same host genetic background, simultaneously using a power genetically

diverse mouse reference population. The expected data promise to elucidate the nature of the genes involved in resistance and rate of development of periodontitis induced by high-fat T2D induced by HFD and obesity. Once obtained, such data can be used to predict individual risk to develop these diseases and allow the development of the genetically based strategy for their prevention and treatment.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTIONS

Luna Karker involved in executing the project, data recording and analysis, and MS preparation. Hanifa J. Abu-Toamih Atamni and was involved in data analysis and MS preparation. Asal Milhem was involved in executing the project and data analysis. Yael Houri-Haddad was involved in the project design. Fuad Iraqi was involved in the project design, data analysis and MS preparation and approving its final version.

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