The Collaborative Cross mouse model for dissecting genetic susceptibility to infectious diseases

Hanifa Abu Toamih Atamni¹ · Aysar Nashef^{2,3} · Fuad A. Iraqi¹

Received: 19 February 2018 / Accepted: 2 August 2018 / Published online: 24 August 2018
© Springer Science+Business Media, LLC, part of Springer Nature 2018

Abstract

Infectious diseases, also known as communicable diseases, refer to a full range of maladies caused by pathogen invasion to the host body. Host response towards an infectious pathogen varies between individuals, and can be defined by responses from asymptomatic to lethal. Host response to infectious pathogens is considered as a complex trait controlled by gene–gene (host–pathogen) and gene–environment interactions, leading to the extensive phenotypic variations between individuals. With the advancement of the human genome mapping approaches and tools, various genome-wide association studies (GWAS) were performed, aimed at mapping the genetic basis underlying host susceptibility towards infectious pathogens. In parallel, immense efforts were invested in enhancing the genetic mapping resolution and gene-cloning efficacy, using advanced mouse models including advanced intercross lines; outbred populations; consomic, congenic; and recombinant inbred lines. Notwithstanding the evident advances achieved using these mouse models, the genetic diversity was low and quantitative trait loci (QTL) mapping resolution was inadequate. Consequently, the Collaborative Cross (CC) mouse model was established by full-reciprocal mating of eight divergent founder strains of mice (A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HiLtJ, CAST/Ei, PWK/PhJ, and WSB/EiJ) generating a next-generation mouse genetic reference population (CC lines). Presently, the CC mouse model population comprises a set of about 200 recombinant inbred CC lines exhibiting a unique high genetic diversity and which are accessible for multidisciplinary studies. The CC mouse model efficacy was validated by various studies in our lab and others, accomplishing high-resolution (< 1 MB) QTL genomic mapping for a variety of complex traits, using about 50 CC lines (3–4 mice per line). Herein, we present a number of studies demonstrating the power of the CC mouse model, which has been utilized in our lab for mapping the genetic basis of host susceptibility to various infectious pathogens. These include *Aspergillus fumigatus*, *Klebsiella pneumoniae*, *Porphyromonas gingivalis* and *Fusobacterium nucleatum* (causing oral mixed infection), *Pseudomonas aeruginosa*, and the bacterial toxins Lipopolysaccharide and Lipoteichoic acid.

Background

The term infectious diseases (or communicable diseases) defines a wide range of health disorders resulting from the invasion of an infectious agent within the host body. The infectious agents comprise all the microorganisms or macro-organisms which are competent to invade the host body using multiple modes of transmission (directly or indirectly) to generate an infectious disease (Barreto et al. 2006; Webster et al. 2017). Infectious diseases, caused by diverse infectious pathogens (viruses, bacteria, fungi, and parasites) are considered to be within the top leading serious health conditions worldwide. In particular, pathogens involved in lower respiratory infections have been shown to be among the leading causes of death worldwide in the year 2015 (WHO 2017; GBD 2015 LRI Collaborators

✉ Fuad A. Iraqi
fuadi@post.tau.ac.il

Hanifa Abu Toamih Atamni
hanifaa@post.tau.ac.il

Aysar Nashef
aysar_nashef@hotmail.com

¹ Department of Clinical Microbiology and Immunology, Sackler Faculty of Medicine, Tel Aviv University, Ramat Aviv, Tel Aviv 69978, Israel

² Department of Prosthodontics, Dental school, The Hebrew University, Hadassah Jerusalem, Israel

³ Present Address: Department of Cranio-maxillofacial Surgery, Poria Medical Centre, The Azrieli School of Medicine, Bar Ilan University, Safed, Israel

2017). Effective pathogen invasion to the host body is dependent on multiple factors, which are mostly related to the host features and environmental conditions and partly related to the pathogen genotypic features (Casadevall and Pirofski 2017). Host susceptibility towards a specific infectious pathogen is a complex trait that is highly influenced by age, sex, immune system functionality, host microbiota, environmental health (food hygiene/personal hygiene), climate conditions, and the host genetic makeup (Yi Rang et al. 2018; Nikolich-Zugich 2018; Casadevall and Pirofski 2017; Belkaid and Harrison 2017). Although host susceptibility enables the occurrence of infection, significant variations are observed between individuals relating to the specific pathogen-generating asymptomatic infection in one individual versus lethal response in another, as well as in their response to antimicrobial treatment. Such variations are believed to be attributed to the host genetic components (Verhein et al. 2018; Hollox and Hoh 2014; Calcagno et al. 2017). With the emergence of the genetic study era, new tools including advanced sequencing techniques, DNA arrays, GWAS, and whole exome sequencing have enabled more studies to be performed in human populations to target the identification of the genetic architecture underlying host susceptibility or resistance, as well as to study the severity of the host response (Kenney et al. 2017; Tian et al. 2017; Abel et al. 2014; Manry and Quintana-Murci 2013). On the pathogen side, advanced molecular studies were performed targeting the pathogen genetic features determining strain, virulence, infectivity, pathways of pathogen invasion (receptor binding/inhibition), infection pathogenesis, pathogen susceptibility to antimicrobial treatments, and pathogen–host gene interactions (Lanza et al. 2018; Kucharski et al. 2016; Yang et al. 2008). Nonetheless, human genetic studies of complex diseases and traits encountered many constraints, mainly related to obtaining coherent and standardized environmental conditions with proper controls, due to conflicts and contradictions surrounding the ethical code for the protection of patients in human-subject studies (Barrow and Gossman 2017). To surmount these constraints, standard methodology in complex disease research adopts the utilization of recombinant inbred mouse strains and comparative and translational genomics approaches for mapping the genomic locations of the QTLs in linkage with the observed phenotypic variations between individuals (Crow 2007; Williams et al. 2001; Haendel et al. 2015). As expected, cross-species phenotyping, QTL mapping, and translational research introduced considerable advances in identification of the genetic architecture of complex traits but still required enhancement of the mapping resolution. Subsequently, a scientific panel on complex trait analysis proposed the development of the

Collaborative Cross (CC), a unique mouse model that imitates the level of genetic diversity observed in humans, to enable high-resolution mapping of the genetic basis of human complex diseases (Threadgill et al. 2002; Churchill et al. 2004). Full-reciprocal inter-crosses of eight founder mouse strains, A/J, C57BL/6J, 129S1/SvImJ, NOD/LtJ, NZO/HiLtJ, CAST/Ei, PWK/PhJ, and WSB/EiJ, generated the CC mouse model recombinant inbred lines, which now comprise a set of ~200 available CC lines (Iraqi et al. 2012; Roberts et al. 2007). At first, the genotypes of the CC lines were obtained using the high-density mouse diversity array (MDA), containing ~620,000 single nucleotide polymorphism (SNP) marker sets that captures the genetic diversity of the CC lines' founder strains, both classical and wild-derived (Yang et al. 2009). Subsequently, heterozygous SNPs, SNPs with missing genotypes within the eight CC founder strains, and SNPs with genotyping errors were eliminated, resulting in 170,935 SNPs, which were mapped onto build 37 of the mouse genome (Durrant et al. 2011). Advanced generations of the CC lines were re-genotyped using the mouse universal genotype array (MUGA) of ~7500 SNPs, and once again re-genotyped five generations later using the Mega-MUGA method of ~77,000 SNPs (Iraqi et al. 2012, 2008; Welsh et al. 2012). The particularly high diversity observed in the CC lines is attributed mainly to the inclusion of the three wild-derived strains (CAST/EiJ, PWK/PhJ, and WSB/EiJ) within the CC parental founders, representing three distant mouse progenitors: *M.m. castaneus*, *M.m. musculus*, and *M.m. domesticus*, respectively. Evidently, the wild-derived strains are more distant than are the classical strains from the C57BL/6J reference genome, where PWK and CAST vary at 17 million SNPs compared to four million SNP variations for the classical strains (Keane et al. 2011). The contribution of the additional sequence variants, not segregating within the classical strains, was believed to enhance the discovery of novel and high-resolution associations of disease phenotypes with polymorphisms (Valdar et al. 2006). To date, extensive genetic studies in many research areas have been successfully accomplished using the CC mouse model, achieving novel QTL mapping with high-resolution (~1 Mb), identification of candidate genes underlying the QTL, next-generation RNA-sequencing for gene expression variations, and estimation of founder effect size, merely by phenotyping a minimal number of CC lines (~50 CC lines), induced by certain environmental conditions, with no need for assessing the parental strains (Valdar et al. 2006; Kovacs et al. 2011; Durrant et al. 2011; Vered et al. 2014; Levy et al. 2015; Aylor et al. 2011; Philip et al. 2011; Kelada et al. 2011; Xiong et al. 2014; Ram et al. 2014; Gralinski et al. 2015; Rogala et al. 2014; Phillippi et al. 2014; Ferris et al. 2013; Thaisz et al.

2012; Bottomly et al. 2012; Mathes et al. 2011; Gelinas et al. 2011; Zombeck et al. 2011; Abu-Toamih Atamni et al. 2016a, b; Abu-Toamih Atamni et al. 2017; Nashef et al. 2017). Additionally, broad-sense heritability (H^2) was assessed as the extent of phenotypic variety related to the differences between CC lines using the formula: $H^2 = Vg/(Vg/Ve)$ and the analysis of variance (ANOVA) test of each trait, as detailed in our previous publication (Iraqi et al. 2014). Hence, the CC mouse genetic resource population adheres to the paradigm “genotype once, phenotype many times,” where the genotypic data are available for use in multiple studies of complex trait diseases (Iraqi et al. 2012). Herein, we present the implementation of the powerful CC mouse model in our lab for dissecting the genetic basis of host susceptibility towards various infectious pathogens, including *Aspergillus fumigatus* (Durrant et al. 2011), *Klebsiella pneumoniae* (Vered et al. 2014), co-infection with *Porphyromonas gingivalis* and *Fusobacterium nucleatum* (Shusterman et al. 2013a, b; Nashef et al. 2018), *Pseudomonas aeruginosa* (Lorè et al. 2015), and host response towards microbial toxins (Lipopolysaccharide (LPS) and Lipoteichoic acid (LTA)) (Nashef et al. 2017) for studying Sepsis.

Aspergillus fumigatus pathogen

Aspergillus fumigatus (*A. fumigatus*) is a ubiquitous human pathogenic and opportunistic fungus, leading to acute Aspergillosis in immunocompromised patients, which initially appears as primary pulmonary infection and evolves into severe systemic damage and high mortality rates

(Latgé 1999; Soubani and Chandrasekar 2002; Ghazaei 2017). Patients who are immunocompromised as a result of HIV infection, neutropenia, oncological, or organ transplants conditions, are highly prone towards virulent *A. fumigatus* pulmonary Aspergillosis due to their inadequate innate immunity (Maschmeyer et al. 2007). However, host response towards specific pathogens, in this case variants of *A. fumigatus*, is a complex trait which varies between patients largely due to host genetic components contributing to susceptibility or resistance response and to severity of disease (van de Veerdonk et al. 2017; Nivoix et al. 2008; Li et al. 2016). These phenotypic variations between the hosts were demonstrated in a successful study in our lab by phenotyping 371 immune-competent mice from 66 CC lines post *A. fumigatus* infection challenge. Results of this study showed the mapping of eight QTLs, of which five were contributed mainly by the wild-derived strains. Host response was evaluated by survival time post-infection and varied significantly ($p < 0.05$) between the CC lines (Fig. 1), presenting a wide profile of responses ranging from 4 to 28 days of survival (Durrant et al. 2011). These results confirm the essential role of genetic variability among the CC lines and the extensive advantages of the CC genetic resource in providing high-resolution mapping of QTLs affecting a wide variety of traits, including susceptibility to a spectrum of infectious diseases, in naïve non-immunocompromised mice (Iraqi et al. 2014; Yang et al. 2009; Durrant et al. 2011; Vered et al. 2014; Nashef et al. 2018). To our knowledge, this is the first report of a murine study to assess host response to *A. fumigatus* and enabling successful mapping of three QTLs in naïve non-immunocompromised

Fig. 1 Mean survival time (days) of different CC lines in response to *Aspergillus fumigatus* infection (Durrant et al. 2011). The X-axis represents the different CC lines while the Y-axis represents the mean

survival time in days ($\pm$ SEM). Full details of the analysis are presented in Durrant et al. (2011)

mice. In fact, since founder effects were contributed mainly by the wild-derived strains, without these founders it may not be possible to dissect such complex disease. These findings and conclusions were also confirmed in recent published studies using the CC mouse model for different complex traits, including host response to multiple infectious diseases such as West Nile virus (Green et al. 2017), Influenza A viruses (Elbahesh and Schughart 2016), Influenza H3N2 (Leist et al. 2016), *Klebsiella pneumoniae* (Vered et al. 2014), Ebola hemorrhagic fever (Rusmussen et al., 2014), and *Aspergillus fumigatus* (Durrant et al. 2011).

***Klebsiella pneumoniae* pathogen**

Klebsiella pneumoniae (*K. pneumoniae*) is gram-negative bacteria, a common multi-drug-resistant opportunistic pathogen that is associated with nosocomial (hospital-acquired) infections including pneumonia, urinary tract infections, gastroenteritis, soft tissue infections, and sepsis (Jarvis et al. 1985; Gerding et al. 1979; Podschun and Ullmann 1998). Moreover, classical *K. pneumoniae* is a major cause of ventilator-associated pneumonia with high morbidity and mortality rates, particularly in intensive care units (ICUs) (Patil and Patil 2017). Notwithstanding the immense association of *K. pneumoniae* with nosocomial infections, emerging hypervirulent strains of *K. pneumoniae* (hvKPs) such as K1/K2 serotypes, have the capability to cause severe and life-threatening community-acquired infections in healthy young patients (Shon et al. 2013; Lee et al. 2017). Several factors determine *K. pneumoniae* pathogenicity and virulence levels, of which LPS and capsular polysaccharide (CPS) play highly significant roles in bacterial resistance to host immune response (phagocytosis) and drug resistance (Lee et al. 2017; Podschun and Ullmann 1998). Furthermore, host genetic background plays a significant factor in determining infection effectiveness and pathogenicity, whereas both sides contribute host–pathogen interactions. Accordingly, we have assessed the host genetic basis for susceptibility or resistance to infectious *K. pneumoniae* (K2 serotype) using 328 CC mice generated from 73 CC lines challenged with an intraperitoneal (IP) infection dose of 104 CFU/ml (colony forming units per ml) (Vered et al. 2014). The recorded phenotype for post-infection response was mouse survival time (days) and body weight changes (g), monitored daily for 15 days post-infection. Study findings revealed significant variations in survival time (Fig. 2a) between CC lines ($p < 0.05$), but not for body weight changes (data not shown). The observed survival profiles between susceptible CC lines (≤ 2 days survival) and resistant CC lines that survived 15 days post-infection demonstrated the high genetic diversity of the CC lines, which contained a variety of responses. Interestingly,

day 7 post-infection appears to be a critical time-window in infection pathogenesis; a host that survived day 7 eventually survived until day 15 and completed the challenge (resistant). Moreover, broad-sense heritability of the survival time trait was high, reaching the value of 0.45, which emphasizes the major role of the host hereditary factors in determining host response towards the K2 serotype of *K. pneumoniae*. Using the phenotypic and genotypic data of 48 CC lines, we have mapped three significant time-specific QTLs in linkage with survival time (days) of the host following *K. pneumoniae* infection (Fig. 2b). The three QTLs were named *Klebsiella pneumoniae*-resistant locus 1, 2, and 3 (*Kprl1*, *Kprl2*, and *Kprl3*), and are located on Chromosomes 4, 8, and 18, respectively. These QTLs were each mapped for specific time of survival; *Kprl1* QTL mapped to survival time until day 2 post-infection (susceptible), while *Kprl2* and *Kprl3* QTL mapped to day 8, suggesting a time-dynamic genetic host response that activates different genes during the different stages of disease progression. Founder effects estimation determined that the observed variations between the CC lines post-infection were contributed mainly by the wild-derived founder strains. Furthermore, examination of the mapped genomic intervals using the mouse genome database (<http://www.informatics.jax.org>) revealed several candidate genes that were highly associated with host susceptibility or resistance towards *K. pneumoniae*. The suggested candidate genes also included well-known protein-coding genes that play significant roles in pathways of pathogen invasion to the host body. These include the *catenin alpha-like 1* (*Cnnal1*) gene of the *Kprl1* QTL, which plays central roles in cell adhesion, as well as the *actin-like 7a* and *7b* (*Actl7a* and *Actl7b*) candidate genes of the *Kprl1* QTL which are involved in phagocytosis and in eliminating bacterial infection. The mapped QTLs in linkage with the host response phenotype towards *K. pneumoniae* infection are independent of the *A. fumigatus* mapped QTLs, proposing distinct genetic networks in the host in association with specific pathogens, which should be further investigated to identify advanced levels of gene expression variation between controls (naïve) and post-infection individuals. To our knowledge, this is the first report of a murine study to assess host response towards the *K. pneumoniae* pathogen, enabling successful mapping of three QTLs in naïve, non-immunocompromised mice. This achievement is believed to be due mainly to the presence of the three wild-derived strains (*CAST/Ei* (*M.m.castaneus*), *PWK/PhJ* (*M.m.musculus*), and *WSB/EiJ* (*M.m.domesticus*)) within the eight CC founder strains, enriching the known structure of genetic variations among the resulting mouse strains, due to their genetic distinction from classical laboratory strains.

Fig. 2 Genetic dissection of host response to *Klebsiella pneumoniae* infection. **a** Shows phenotypic profile of mean survival time (days) of the different CC lines and four inbred strains after infection with *Klebsiella pneumoniae*. The X-axis represents the CC lines, while the Y-axis represents the mean survival time in days ($\pm$ SEM). **b** and **c** present the 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; one QTL on day 2 on chromosome 4 named *Kprl1* (**b**) and two QTLs on day 8 named *Kprl2* and *Kprl3* on chromosomes 8 and 18, respectively (**c**). The X-axis shows the 19 autosomes plus the X chromosomes, while the Y-axis shows the log *p* value of the linkage analysis. (Vered et al. 2014)

***Pseudomonas aeruginosa* pathogen**

Pseudomonas aeruginosa (*P. aeruginosa*) is a gram-negative, multi-drug-resistant (MDR), opportunistic and nosocomial pathogen widespread in hospital ICUs and healthcare systems worldwide (10–15% of cases). It is ranked second in the WHO list of critical-priority drug-resistant bacteria on which to focus for the development of new therapeutic strategies (Tacconelli et al. 2017; Majumdar and Padiglione 2012; Vincent 2003). *P. aeruginosa* infection leads to high morbidity and mortality rates particularly among immunocompromised patients, post-invasive medical procedure patients, and cystic fibrosis (CF) patients (Wieland et al. 2018; Stefani et al. 2017; Winstanley et al. 2016). *P. aeruginosa* incidence in ICUs constitutes a global major threat due to higher risk for severe ventilator-associated pneumonia and sepsis associated with multi-systemic damage and high mortality (Gellatly and Hancock 2013). However, significant phenotypic variations were reported between individuals' morbidity and mortality rates in response to *P. aeruginosa* infection, indicating the important role of host genetic variants in determining the outcome of the host–pathogen interplay. Consequently, various pathogen-oriented genetic studies were performed, targeting the identification of *P. aeruginosa* genetic features associated with host response phenotypic variations in disease progress and pathogenesis (Ramanathan et al. 2017; Bianconi et al. 2011; Cigana et al. 2009; Bragonzi et al. 2009; Nguyen and Singh 2006). On the other hand, host-oriented genetic studies focused on dissection of the host genetic architecture contributing to the observed phenotypic variations in host response to certain sub-types of *P. aeruginosa*, assessing both human populations and animal models (Wang et al. 2017; Di Paola

et al. 2017; Alhazmi et al. 2018; Weiler and Drumm 2013). Additionally, various studies using different mouse strains infected with certain sub-types of *P. aeruginosa* demonstrated highly significant host phenotypic variations post-infection between the different strains, evident support for the role of host genetics in infection pathogenesis (Bragonzi 2010; De Simone et al. 2014). A recent study focusing on mapping host genetic features in linkage with susceptibility or resistance to *P. aeruginosa* was performed using the Collaborative Cross (CC) mouse population. In this study, 17 CC lines (92 mice: 50 males, 42 Females) and a susceptible control group of A/J mice were challenged with intratracheal injection of *P. aeruginosa*, and were subsequently monitored for 7 days post-infection (Lorè et al. 2015). Host phenotypic responses were measured in terms of modifications in survival time (days) and body weight (gram) throughout the 7-day post-infection observation period. Survival time varied significantly between CC lines, showing a wide range of survival profiles (Fig. 3a), from highly susceptible CC lines with a lethal response (1.5 days survival) to highly resistant (7 days survival). Moreover, similar phenotypic variations between the CC lines were recorded for body weight modification post-infection, showing CC lines with severe body weight loss versus CC lines with body weight recovery 5 days post-infection (Fig. 3b). Heritability (H^2) calculations for the measured traits were 0.54 for survival time and 0.28 for body weight modifications (Table 1). A similar study for mapping the genetic components underlying the host response to *P. aeruginosa* was previously performed by De Simone et al. (2016), using an F2 intercross population by mating A/J (as susceptible) with C3H/HeOuJ (as resistant). This study revealed mapping of a significant locus associated with susceptibility

Fig. 3 Response to *P. aeruginosa* airway infection of CC lines (Lorè et al. 2015). **a** Shows the mean survival time in days ($\pm$ SEM). **b** Shows percentage changes in body weight following the infection.

The X-axis represents the CC lines, while the Y-axis represent the mean survival time in days ($\pm$ SEM) (**a**) and % change in body weight (**b**)

Table 1 Estimated broad-sense heritability (H^2) of a variety of phenotypic host responses to different infectious pathogens assessed using the CC mouse model

Infectious Pathogen	Trait	H^2
<i>Aspergillus fumigatus</i> (Durrant et al. 2011)	Survival time (days)	0.78
<i>Klebsiella pneumoniae</i> (Vered et al. 2014)	Survival time (days)	0.45
<i>P. gingivalis</i> and <i>F. nucleatum</i> (Nashef et al. 2018)	Control bone volume	0.42
	Residual bone volume	0.45
	Percentage alveolar bone loss	0.33
<i>Pseudomonas aeruginosa</i> (Lorè et al. 2015)	Survival time (days)	0.54
	Body weight loss (g)	0.28
<i>Lipopolysaccharide</i> (Nashef et al. 2017)	Survival time (h)	0.19

towards *P. aeruginosa* on chromosome 6 and designated as *Pair1* for *P. aeruginosa* infection-resistance locus 1, positioned at genomic location 90.8 Mbp with a genomic interval of 20.7 Mbp (81.5–102.2 Mbp). The genomic interval of *Pair1* is relatively large, compared to extremely low intervals (> 3 Mbp) achieved in different studies when using the CC mouse model, although not using this specific pathogen. QTL analysis of the current study is ongoing and is expected to be published soon. These findings add evidence for the strong role of host genetic features in determining infection pathogenesis.

Bacterial Sepsis

As stated by the Sepsis-3 task force, the updated definition and criteria for sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection (Singer et al. 2016). Sepsis is a complex nosocomial disease caused by the host's immune response triggered by an infectious pathogen, most likely gram-negative bacteria (bacterial sepsis) leading to systemic health complications and high mortality, particularly in elderly patients, preterm infants, or immunocompromised patients (Levy et al. 2003; Munford 2006; Collins et al. 2018). Notwithstanding the ongoing advances in therapeutic strategies and medical technologies, worldwide sepsis-related morbidity and mortality are constantly increasing, and are estimated to be one of the major leading causes of death in ICUs worldwide, albeit to a great extent in low-middle income countries (Friedman et al. 1998; Fleischmann et al. 2016; Reinhart et al. 2017). Pathogenesis of sepsis is determined by complex host–pathogen interplay, which is known to be controlled by genetic features of both the pathogen and the host, together generating the multifaceted characteristic features of sepsis in different patients (Goh et al. 2017; Christaki and Giamarellos-Bourboulis 2014; Davenport et al. 2016). Bacterial sepsis is principally caused by a host systemic immune system severe response towards the infectious pathogen virulence factors, such as the microbial toxins LPS featured in gram-negative bacteria and LTA in gram-positive bacteria (Chandler and Ernst

2017; van der Poll and Opal 2008; Mattsson et al. 1993). Moreover, host septic responses towards bacterial virulence factors, including LTA and LPS, vary between individuals in prevalence, severity, progress, and outcome, indicating the major role of host genetic features associated with bacterial sepsis pathogenesis. Furthermore, studies of sex differences in sepsis are contradictory due to the complexity of the disease and the involvement of multiplex genetic factors of the host and pathogen; several studies suggest no difference, while others suggest higher risk in females, or higher risk in males, which will require further investigation (Failla and Connelly 2017). Mapping of the host genetic susceptibility components will lead to advances in the direction of personalized medicine for prevention and medical cure of sepsis to halt the increase in sepsis morbidity and mortality. Two ongoing studies in our lab are aimed at mapping the host genetic response towards LPS and LTA, separately, using the CC lines challenged by LPS or LTA injections. The LPS study consists of 296 mice generated from 16 CC lines, injected (15 mg/1 kg mouse) with IP LPS (L2630 Sigma) from *Escherichia coli* (*E. coli* 0111:B4) dissolved in PBS and monitored for 72 h post-infection to assess the host phenotypic response measuring survival time (h), body temperature (°C), and weight (g) modifications (unpublished data). Body weight was measured using digital balance and rectal body temperature using an EcoScan temp4 thermometer with a special probe designed for small animals, at different time points post injection (0, 2, 4, 6, 24, 30, 48, 54, 72 h). The CC lines varied significantly in their phenotypic responses at all levels, including survival time (Fig. 4a), body weight (Fig. 4d), and temperature modifications (Fig. 4c). Interestingly, sex differences within the CC lines in their host response were significant ($p < 0.05$) and varied between the CC lines, showing for several CC lines that females are much more resistant towards LPS than males of the same CC line (Fig. 4b). Altogether, these data support the accumulated evidence for the complexity of sepsis, despite the controlled environmental conditions and the use of a microbial specific toxin. At the time of writing this report, further CC lines are under assessment

Fig. 4 Septic response towards infection of CC lines with the microbial toxic component Lipopolysaccharide (LPS). **a** Shows CC lines mean ($\pm$ SEM) survival time (Hr) profile during the 72 h after the infection, **b** shows the data split by sex in each CC line due to the presence of a significant sex effect. The white columns represent females and the blue columns represent males. The X-axis represents the CC lines, while the Y-axis represents the mean survival time in

hours ($\pm$ SEM). **c** and **d** show 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 a percentage of the initial value. The X-axis represents time after LPS injection (Hr), the Y-axis represents percentage of the initial body temperature (**c**) / weight (**d**) (Nashef et al. 2017)

for LPS and LTA in order to increase the study population size which should enable QTL mapping of sepsis-related genomic features.

Insights into human periodontal disease using the CC population

Periodontitis (dental infection) is a polygenic inflammatory disease that compromises the integrity of the tooth-supporting tissues with an adverse impact on systemic health and a complex etiology at multiple levels (Hajishengallis 2014). The transition from periodontal health state to disease (periodontitis) is associated with a dramatic shift from a symbiotic to dysbiotic microbial community in the oral cavity. However, although independent microbial dysbiosis may not necessarily precipitate periodontitis, it could initiate the disease in conjunction with the co-occurrence of other risk factors such as host genotype, diet, and environmental factors such as smoking (Hajishengallis 2014;

Hajishengallis et al. 2012a, b). Within the mentioned risk factors, host genetic background is a major determinant for host susceptibility to periodontitis, inasmuch as alterations in gene expression levels or regulation contribute to the disease development, progress, and severity.

Various GWAS studies in human were previously carried out to identify biomarkers for periodontal disease susceptibility at the host genotypic level (Divaris et al. 2012; Shimizu et al. 2015; Hashim et al. 2015; Schaefer et al. 2010; Teumer et al. 2013; Ernst et al. 2010). However, lack of proper control and standardization of human studies along with the high heterogeneity of periodontitis itself constitutes a major constraint in the mapping of the genetic basis for periodontitis (Vaithilingam et al. 2014). Hence, we have assessed recently the power of the CC population in conjunction with a genetic analysis of the human orthologous chromosomal regions to dissect the genetic basis of periodontitis. Our study was performed by integrating QTLs associated with experimental periodontitis

Fig. 5 **a** The means of the alveolar bone volumes (+SEM) of 25 different CC lines. The X-axis represents the different CC lines while the Y-axis represents the mean of the alveolar bone volume in mm³ evaluated by micro CT. The black bars represent the mean of the control alveolar bone volume (CBV) while the gray bars represent the mean of residual bone volume after mixed infection (RBV). Asterisks

represent the significant differences between the two groups (p value < 0.05). **b** Percent alveolar bone volume loss (PBL) due to the mixed infection (+SEM) of 25 different CC lines. The X-axis represents the different CC lines while the Y-axis represents the mean percent of alveolar bone volume loss among the different lines. (Nashef et al. 2018)

in the CC population, with imputed human genotype data from two large case–control clinical sub-types of periodontitis: aggressive periodontitis (AgP) and chronic periodontitis (CP) (Nashef et al. 2018). The initial stage of the study assessed 25 CC lines (286 mice) for host response towards a mixed culture of periodontal pathogens (*Porphyromonas gingivalis* and *Fusobacterium nucleatum*) based on the well-established mixed infection model (Baker et al. 1994; Polak et al. 2009). Phenotypic host response was estimated by measuring the alveolar bone loss phenotype, as a major hallmark of periodontitis development and progress, using the Compact fan-Beam-type Computerized Tomography system (μ CT) (Wilensky et al. 2005). The CC lines showed significant wide variations in their phenotypic response to the mixed infection challenge, ranging between moderate and high levels of bone loss for the different CC lines, as well as post-infection bone formation for some CC lines, similar to condensing osteitis (Fig. 5). Subsequently, linkage analysis was conducted for QTLs associated with percentage Alveolar Bone Loss using the phenotypic and genotypic data of the different CC lines. The QTL analysis revealed two significant QTLs (Table 2) in linkage with percentage alveolar bone loss, located on chromosome 1: 180–181.5 Mbp (1.5 Mb) and chromosome 14: 93.5–96.5 Mbp (3 Mb) and designated as *Perio3* (Periodontitis) and *Perio4* in continuation of the previously reported QTLs (Shusterman et al. 2013b). In addition to

that, eight suggestive QTLs were mapped and designated as *Perio3* to *Perio10* (Table 3). Interestingly, the *Perio3* QTL overlaps with the previously mapped *Perio3* QTL in a similar study by our group using the progenies of the A/Jx BALB/cJ intercross F2 population (Shusterman et al. 2013b). Using 408 F2 mice, Shusterman et al. (2013b) mapped two significant QTLs in linkage with periodontitis located on chromosomes 5 (*Perio1* QTL) and 3 (*Perio2* QTL), and a suggestive QTL located on chromosome 1 (*Perio3* QTL) at 50% genome-wide significance ($\log p = 2.3$) with $\log p$ 2.47. As expected, performing the study using the CC mouse model enhanced the significance level of *Perio3* to make it highly significant (at 95% genome-wide significance) with higher resolution (Fig. 6a). Moreover, due to the contribution of the wild-derived strains among the CC founder strains, a new QTL was mapped when using this model (*Perio4*) (Fig. 6a). A mouse genome database (<http://www.informatics.jax.org>) search for candidate genes located within the genomic intervals of *Perio3* and *Perio4* QTL revealed in total 80 candidate genes. Next, we used the Merge analysis approach (Durrant et al. 2011) to identify variants that give rise to the significant QTLs *Perio3* and *Perio4*. The merge analysis revealed six causal-effect variants and two linked-effect variants at both *Perio3* and *Perio4*. Estimation of founder haplotype effect showed that the most significant loci on chr1 and chr14 were shown to be less affected by WSB/EiJ (wild-derived

Table 2 The locations of the mouse QTLs associated with host susceptibility to periodontitis, their genomic intervals, and their corresponding human orthologues are listed in the table, according to significance threshold of QTL. (Nashef et al. 2018)

	QTL	Chr.	Position (interval)	No. Genes	Human Chr.	No. human genes	p value of QTL	Total human genes
Significant QTL	Perio3	1	180–181.5 Mbp (1.5 Mb)	70	1	28	$-\log p = 5.3$	31
					2	1		
Suggestive QTL	Perio4	14	93.5–96.5 Mbp (3 Mb)	10	13	2	$-\log p = 3.52$	685
	Perio3`	1	172.6–182.2 (9.6 Mb)	226	1	83		
					2			
	Perio4`	14	71.6–73.03 (1.43 Mb)	43	13	13		
			81.83–86.2 (4.37 Mb)					
			91.29–99.88 (8.59 Mb)					
	Perio5	15	63.08–65.68 (2.6 Mb)	9	8	7		
	Perio6	15	93.5–99.2 (5.7 Mb)	105	12	68		
	Perio7	14	36.4–45.1 (8.7 Mb)	91	14	26		
					10			
	Perio8	2	66.01–70.19 (4.18 Mb)	648	1	373		
			78.61–102.58 (23.97 Mb)		2			
					11			
	Perio9	7	3.1–12.8 (9.7 Mb)	184	19	112		
	Perio10	17	9.8–11.9 (2.1 Mb)	3	6	3		

Chr. chromosome

Table 3 Candidate genes that were revealed through integration of mouse QTL associated with periodontitis and human GWAS

QTL analysis in mice			Human GWAS			
QTL	Candidate Gene	<i>p</i> value of QTL	AgP, best-associated SNP (<i>p</i> value, OR/Beta)	CP mod, best-associated SNP (<i>p</i> value, OR/Beta)	CP sev, best-associated SNP (<i>p</i> value, OR/Beta)	Reported trait association-Ref (SNP, <i>p</i> value, OR/Beta)
Perio3	<i>coiled-coil domain containing 121</i> (<i>CCDC121</i>)	$-\log p = 5.3$	rs35605899 (0.027, 1.16/0.144)	rs3749147 (0.207, 0.93/-0.0699)	rs7382 (0.313, 1.070/0.068)	CP-Teumer et al. 2013 (rs1115713647.98 $\times 10^{-6}$, 3.46)
Perio7	<i>neuregulin 3</i> (<i>NRG3</i>)	$-\log p = 3.52$	rs2250828 (7.5×10^{-5} , 1.41/0.344)	rs9971278 (7.2×10^{-5} , 0.74/-0.303)	rs10509436 (8.3×10^{-5} , 1.39/0.33)	–
Perio9	<i>zinc finger ZNF579</i>		rs310432 (0.134, 1.09/0.088)	rs2902925 (0.34, 1.11/0.106)	rs10421563 (0.11, 0.899/-0.107)	CP-Teumer et al. 2013 (rs1495467605.12 $\times 10^{-6}$, 0.34)
	<i>zinc finger (FIZ1)</i>		rs3803890 (0.078, 1.14/0.13)	rs520125 (0.168, 0.87/-0.140)	rs310469 (0.155, 0.885/-0.122)	CP-Teumer et al. 2013 (rs1409000464.44 $\times 10^{-6}$, 0.34)
	<i>zinc finger (ZNF524)</i>					
Perio10	<i>Parkin RBR E3 ubiquitin protein ligase (PARK2)</i> <i>PARK2 co-regulated (PACRG)</i>		rs4709556 (0.0013, 1.27/0.242)	rs9458343 (0.0003, 0.80/-0.222)	rs2803101 (0.0076, 1.199/0.182)	–

Gene symbols, their mouse QTL significance value, and the significance of the best-associated SNP marker in the human CP/AgP data are listed in the table (Nashef et al. 2018)

CP chronic periodontitis, AgP aggressive periodontitis, OR Odds Ratio

strain) than by the rest of the parental strains, which seem to have quite similar effects on the trait (Fig. 6b). Subsequently, candidate genes identified by merge analysis (Fig. 6c, d), significant QTLs, and suggestive QTLs were tested for their association to periodontal diseases in the GWAS-Catalog (Welter et al. 2014), as well in the available data of the human case–control samples. Briefly, orthologous human chromosomal regions were analyzed using available imputed genotype data (OmniExpress BeadChip arrays) derived from case–control samples of aggressive periodontitis (AgP; 896 cases, 7104 controls) and chronic periodontitis (CP; 2746 cases, 1864 controls) of northwest European and European American descent, respectively.

Three out of seven candidate genes that were selected based on merge analysis and 14 out of 31 human orthologous genes of the significant QTLs, previously showed gene-centric associations of periodontal sub-phenotypes (Rhodin et al. 2014). In addition, we found that one of the suggested orthologous genes, *CCDC121*, within the *Perio3* QTL, is located 5 kb upstream of a previously reported risk variant of chronic periodontitis (*p* value = 8.0×10^{-6} , OR = 3.46) (Teumer et al. 2013). However, we could not replicate these associations in our available AgP and CP samples. Further analysis was performed using the corresponding human genes with an additional 1229 mouse genes located within the genomic intervals of the suggestive QTLs. Data analysis revealed six candidate genes (*NRG3*, *ZNF579*, *FIZ1*, *ZNF524*, *PARK2*, and *PACRG*) showing nominal significant

associations with either AgP or CP. Regional association plots of three genes (*NRG3*, *PARK2*, and *PACRG*) that showed associations with both AgP and CP in our human data are shown in Fig. 7. Eventually, our study revealed seven candidate genes based on the integration of mouse QTLs and human GWAS (Table 3).

Overall, our findings confirm that utilization of the CC mouse model populations is a powerful method for mapping the susceptibility to alveolar bone loss using a minimal number of 25 CC lines. The high genetic diversity of the CC mouse model enabled successful mapping of two significant QTLs to a particularly narrow region of ~1.5 to ~3 Mb compared to previous findings of ~35 Mb revealed by using an F2 approach (Shusterman et al. 2013b). Furthermore, our observation of several candidate genes (suggestive QTLs) which were replicated in AgP, moderate CP, and severe periodontitis emphasizes the potential of the CC mouse model for dissection of the genetic basis of human complex diseases. Phenotype–genotype and gene expression data from larger human and CC mouse cohorts will be required for enhanced identification of true positive associations related to the complex etiology of periodontitis.

Conclusions

Herein, we have described a notable assemblage of genetic studies in our lab targeting identification of the genetic basis of host susceptibility or resistance towards various infectious

Fig. 6 **a** Genome scans of susceptibility to alveolar bone volume loss percentage in 25 different CC lines. The X-axis represents genome location; the Y-axis represents the minus log p value of the test of association between locus and percentage of bone loss. Two QTLs associated with percentage of bone loss after infection mapped on chromosome 1 with genome coordinate (181.5–182.5 Mbp) and chromosome 14 with genome coordinate (93.5–96.5 Mbp). **b** Founder effect estimated haplotype effects at Perio3 and Perio4 QTLs for alveolar bone loss after mixed infection with *P. gingivalis* and *F. nucleatum*. Effects are shown as deviations

relative to WSB/EiJ, which is arbitrarily assigned the trait effect of 0. The X-axis of each plot shows the founder strains; the Y-axis shows the estimated haplotype effects of the CC founders. **c** Merge analysis of sequence variants at Perio3 and **d** Perio4. The X-axis is genome location; the Y-axis is the minus log p value of the test of association between locus and alveolar bone loss phenotype. The continuous black lines are sections of the genome scans **a** while gray dots are the results of ANOVA tests of sequence. Reproduced with permission from (Nashef et al. 2018)

pathogens using the Collaborative Cross (CC) mouse population. The CC is a next-generation mouse genetic reference population designed for the genetic study of human complex trait diseases and livestock agriculture, identification of candidate genes associated with the host phenotypic variations, and characterization of the gene-networks involved in disease pathogenesis. Our studies emphasize the leading role of host genetic background in determining infection potency, severity, and pathogenesis using the powerful CC

mouse genetic reference population. Given the ability to map QTLs associated with any given trait with high resolution in the CC population, it will now be possible to identify genes within the mapped loci responsible for phenotypes of interest by using a specific gene knockout approach. Furthermore, it will now be possible to translate CC mouse results to human by using human GWAS analysis to identify human genes orthologous to those mapped in the CC population, a successful approach described by Nashef et al. (2018).

Fig. 7 Association plots of imputed genotypes at the NRG3, PARK2, and PACRG loci. Regional association plots of imputed genotypes for aggressive periodontitis (**a**) severe chronic periodontitis (**b**) and moderate chronic periodontitis (**c**) for the chromosomal region spanning NRG3. Regional association plots of imputed genotypes for aggressive periodontitis, (**d**) severe chronic periodontitis, (**e**) and moder-

ate chronic periodontitis (**f**) for the chromosomal region spanning PARK2, and PACRG. The minus log p values of the analyzed SNPs were plotted as a function of the genomic SNP position. SNP annotation provided by Locus Zoom databases. Reproduced with permission from (Nashef et al. 2018)

Acknowledgements The funding was supported by Israel Science Foundation (Grant Nos. 429/09, 961/15), United States-Israel Binational Fund (Grant No. 2015/077), Italian CF Research Foundation (Grant No. 11/2015), German Research Foundation DFG (Grant No. 1582/3-1), Wellcome Trust (Grant Nos. 085906/Z/08/Z, 075491/Z/04), Wellcome Trust core funding by Tel-Aviv University (Grant No. 090532/Z/09/Z) and Dental school at Hadassah.

References

- Abel L, Alcaïs A, Schurr E (2014) The dissection of complex susceptibility to infectious disease: bacterial, viral and parasitic infections. *Curr Opin Immunol* 30:72–78
- Abu-Toamih Atamni HJ, Botzman M, Mott R, Gat-Viks I, Iraqi FA (2016a) Mapping liver fat female-dependent quantitative trait loci in collaborative cross mice. *Mamm Genome* 27(11–12):565–573
- Abu-Toamih Atamni HJ, Mott R, Soller M, Iraqi FA (2016b) 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* 17(1):10
- Abu-Toamih Atamni HJ, Ziner Y, Mott R, Wolf L, Iraqi FA (2017) Glucose tolerance female-specific QTL mapped in collaborative cross mice. *Mamm Genome* 28(1–2):20–30
- Alhazmi A (2018) NOD-like receptor(s) and host immune responses with *Pseudomonas aeruginosa* infection. *Inflamm Res*. <https://doi.org/10.1007/s00011-018-1132-0>
- Aylor DL, Valdar W, Foulds-Mathes W, Buus RJ, Verdugo RA et al (2011) Genetic analysis of complex traits in the emerging Collaborative Cross. *Genome Res* 21:1213–1222
- Baker PJ, Evans RT, Roopenian DC (1994) Oral infection with *Porphyromonas gingivalis* and induced alveolar bone loss in immunocompetent and severe combined immunodeficient mice. *Arch Oral Biol* 39(12):1035–1040
- Barreto ML, Teixeira MG, Carmo EH (2006) Infectious diseases epidemiology. *J Epidemiol Community Health* 60(3):192–195
- Barrow JM, Gossman WG (2017) Ethics, Research. StatPearls [Internet]. StatPearls Publishing, Treasure Island (FL)
- Belkaid Y, Harrison OJ (2017) Homeostatic immunity and the microbiota. *Immunity* 46(4):562–576
- Bianconi I, Milani A, Cigana C, Paroni M, Levesque RC et al (2011) Positive signature-tagged mutagenesis in *Pseudomonas aeruginosa*: tracking patho-adaptive mutations promoting airways chronic infection. *PLoS Pathog* 7(2):e1001270
- Bottomly D, Ferris MT, Aicher LD, Rosenzweig E, Whitmore A et al (2012) Expression quantitative trait Loci for extreme host response to influenza A in pre-collaborative cross mice. *G3* 2(2):213–221
- Bragonzi A (2010) Murine models of acute and chronic lung infection with cystic fibrosis pathogens. *Int J Med Microbiol* 300(8):584–593
- Bragonzi A, Paroni M, Nonis A, Cramer N, Montanari S et al (2009) *Pseudomonas aeruginosa* microevolution during cystic fibrosis lung infection establishes clones with adapted virulence. *Am J Respir Crit Care Med* 180(2):138–145
- Broman KW (2005) The genomes of recombinant inbred lines. *Genetics* 169(2):1133–1146
- Calcagno A, Cusato J, D'Avolio A, Bonora S (2017) Genetic polymorphisms affecting the pharmacokinetics of antiretroviral drugs. *Clin Pharmacokinet* 56(4):355–369
- Casadevall A, Pirofski LA (2017) What is a host: the attributes of individual susceptibility. *Infect Immun* 86(2):e00636–e00617
- Chandler CE, Ernst RK (2017) Bacterial lipids: powerful modifiers of the innate immune response. *F1000Res*. <https://doi.org/10.12688/f1000research.11388.1>
- Christaki E, Giamarellos-Bourboulis EJ (2014) The beginning of personalized medicine in sepsis: small steps to a bright future. *Clin Genet* 86(1):56–61
- Churchill G, Airey D, Allayee H, Angel J, Attie A et al (2004) The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat Genet* 36:1133–1137
- Cigana C, Curcurù L, Leone MR, Ieranò T, Lorè NI et al (2009) *Pseudomonas aeruginosa* exploits lipid A and mucopeptides modification as a strategy to lower innate immunity during cystic fibrosis lung infection. *PLoS ONE* 4(12):e8439
- Collins A, Weitkamp JH, Wynn JL, Et al (2018) Why are preterm newborns at increased risk of infection? *Arch Dis Child Fetal Neonatal Ed* 103(4):F391–F394
- Crow JF (2007) Haldane, Bailey, Taylor and recombinant-inbred lines. *Genetics* 176(2):729–732
- Davenport EE, Burnham KL, Radhakrishnan J, Humburg P, Hutton P et al (2016) Genomic landscape of the individual host response and outcomes in sepsis: a prospective cohort study. *Lancet Respir Med* 4(4):259–271
- De Simone M, Spagnuolo L, Lorè NI, Rossi G, Cigana C et al (2014) Host genetic background influences the response to the opportunistic *Pseudomonas aeruginosa* infection altering cell-mediated immunity and bacterial replication. *PLoS ONE* 9(9):e106873
- De Simone M, Spagnuolo L, Lorè NI, Cigana C, De Fino I et al (2016) Mapping genetic determinants of host susceptibility to *Pseudomonas aeruginosa* lung infection in mice. *BMC Genomics* 17:351
- Di Paola M, Park AJ, Ahmadi S, Roach EJ, Wu YS et al (2017) SLC6A14 is a genetic modifier of cystic fibrosis that regulates *Pseudomonas aeruginosa* attachment to human bronchial epithelial cells. *mBio*. 8(6):e02073-17. <https://doi.org/10.1128/mBio.02073-17>
- Divaris K, Monda KL, North KE, Olshan A, Lange EM, Moss K et al (2012) Genome-wide association study of periodontal pathogen colonization. *J Dent Res* 91(7 Suppl):S21–S28
- Durrant C, Tayem H, Yalcin B, Cleak J, Goodstadt L et al (2011) Collaborative Cross mice and their power to map host susceptibility to *Aspergillus fumigatus* infection. *Genome Res* 21:1239–1248
- Elbahesh H, Schughart K (2016) Genetically diverse CC-founder mouse strains replicate the human influenza gene expression signature. *Sci Rep* 6:26437
- Ernst FD, Linden GJ, Homuth G, Kocher T (2010) Replication of the association of chromosomal region 9p21.3 with generalized aggressive periodontitis (gAgP) using an independent case-control cohort. *BMC Med Genet* 11:119
- Failla KR, Connelly CD (2017) Systematic review of gender differences in sepsis management and outcomes. *J Nurs Scholarsh* 49(3):312–324
- Ferris MT, Aylor DL, Bottomly D, Whitmore AC, Aicher LD et al (2013) Modeling host genetic regulation of influenza pathogenesis in the collaborative cross. *PLoS Pathog* 9(2):e1003196
- Fleischmann C, Scherag A, Adhikari NK, Hartog CS, Tsaganos T et al (2016) Assessment of global incidence and mortality of hospital-treated sepsis. Current estimates and limitations. *Am J Respir Crit Care Med* 193(3):259–272
- Friedman G, Silva E, Vincent JL (1998) Has the mortality of septic shock changed with time. *Crit Care Med* 26(12):2078–2086
- GBD 2015 LRI Collaborators (2017) Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory tract infections in 195 countries: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet Infect Dis* 17(11):1133–1161

- Gelinas R, Chesler EJ, Vasconcelos D, Miller DR, Yuan Y et al (2011) A genetic approach to the prediction of drug side effects: bleomycin induces concordant phenotypes in mice of the collaborative cross. *Pharmacogenomics Pers Med* 4:35–45
- Gellatly SL, Hancock RE (2013) *Pseudomonas aeruginosa*: new insights into pathogenesis and host defenses. *Pathog Dis* 67(3):159–173
- Gerding DN, Buxton AE, Hughes RA, Cleary PP, Arbaczawski J, Stamm WE (1979) Nosocomial multiply resistant *Klebsiella pneumoniae*: epidemiology of an outbreak of apparent index case origin. *Antimicrob Agents Chemother* 15(4):608–615
- Ghazaei C (2017) Molecular insights into pathogenesis and infection with *Aspergillus Fumigatus*. *Malays J Med Sci* 24(1):10–20
- Goh C, Knight JC et al (2017) Enhanced understanding of the host-pathogen interaction in sepsis: new opportunities for omic approaches. *Lancet Respir Med* 5(3):212–223
- Gralinski LE, Ferris MT, Aylor DL, Whitmore AC, Green R et al (2015) Genome wide identification of SARS-CoV susceptibility loci using the Collaborative Cross. *PLoS Genet* 11(10):e1005504
- Green R, Wilkins C, Thomas S, Sekine A, Hendrick DM, Voss K et al (2017) Oas1b-dependent immune transcriptional profiles of West Nile virus infection in the Collaborative Cross. *G3 (Bethesda)* 7(6):1665–1682
- Haendel MA, Vasilevsky N, Brush M, Hochheiser HS, Jacobsen J, Oellrich A et al (2015) Disease insights through cross-species phenotype comparisons. *Mamm Genome* 26(9–10):548–555
- Hajishengallis G (2014) Periodontitis: from microbial immune subversion to systemic inflammation. *Nat Rev Immunol* 15(1):30–44
- Hajishengallis G, Lamont RJ (2012b) Beyond the red complex and into more complexity: the polymicrobial synergy and dysbiosis (PSD) model of periodontal disease etiology. *Mol Oral Microbiol* 27(6):409–419
- Hajishengallis G, Darveau RP, Curtis MA (2012a) The keystone-pathogen hypothesis. *Nat Rev Microbiol* 10(10):717–725
- Hashim NT, Linden GJ, Ibrahim ME, Gismalla BG, Lundy FT, Hughes FJ et al (2015) Replication of the association of GLT6D1 with aggressive periodontitis in a Sudanese population. *J Clin Periodontol* 42(4):319–324
- Hollox EJ, Hoh BP (2014) Human gene copy number variation and infectious disease. *Hum Genet* 133(10):1217–1233
- Iraqi FA, Churchill G, Mott R (2008) The Collaborative Cross, developing a resource for mammalian systems genetics: a status report of the Wellcome Trust cohort. *Mamm Genome* 19:379–381
- Iraqi FA, Mahajne M, Salaymah A, Sandovsky H, Tayem H et al (2012) The genome architecture of the Collaborative Cross mouse genetic reference population. *Genetics* 190(2):389–402
- Iraqi FA, Athamni H, Dorman A, Salymah Y, Tomlinson I et al (2014) 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* 25(3–4):109–119
- Jarvis WR, Munn VP, Highsmith AK, Culver DH, Hughes JM (1985) The epidemiology of nosocomial infections caused by *Klebsiella pneumoniae*. *Infect Control* 6(2):68–74
- Keane TM, Goodstadt L, Danecek P, White MA, Wong K et al (2011) Mouse genomic variation and its effect on phenotypes and gene regulation. *Nature* 477(7397):289–294
- Kelada SN, Aylor DL, Peck BC, Ryan JF, Tavarez U et al (2012) Genetic analysis of hematological parameters in incipient lines of the collaborative cross. *G3* 2(2):157–165
- Kenney AD, Dowdle JA, Bozzacco L, McMichael TM, St Gelais C, Panfil AR et al (2017) Human Genetic Determinants of Viral Diseases. *Annu Rev Genet* 51:241–263
- Kovacs A, Ben-Jacob N, Tayem H, Halperin E, Iraqi FA. Et al (2011) Genotype is a stronger determinant than sex of the mouse gut microbiota. *Microb Ecol* 61(2):423–428
- Kucharski AJ, Andreasen V, Gog JR (2016) Capturing the dynamics of pathogens with many strains. *J Math Biol* 72(1–2):1–24
- Lanza VF, Baquero F, Martínez JL, Ramos-Ruiz R, González-Zorn B, Andrement A et al (2018) In-depth resistome analysis by targeted metagenomics. *Microbiome* 6(1):11
- Latgé JP (1999) *Aspergillus fumigatus* and aspergillosis. *Clin Microbiol Rev* 12(2):310–350
- Lee CR, Lee JH, Park KS, Jeon JH, Kim YB, Cha CJ et al (2017) Antimicrobial resistance of hypervirulent *klebsiella pneumoniae*: epidemiology, hypervirulence-associated determinants, and resistance mechanisms. *Front Cell Infect Microbiol* 7:483
- Leist SR, Pilzner C, van den Brand JM, Dengler L, Geffers R, Kuiken T et al (2016) Influenza H3N2 infection of the collaborative cross founder strains reveals highly divergent host responses and identifies a unique phenotype in CAST/EiJ mice. *BMC Genomics* 17:143
- Levy MM, Fink MP, Marshall JC, Abraham E, Angus D et al (2003) CCM/ESICM/ACCP/ATS/SIS international sepsis definitions conference. *Critical Care Med* 29(4):1250–1256
- Levy R, Mott RF, Iraqi FA, Gabet Y (2015) Collaborative cross mice in a genetic association study reveal new candidate genes for bone microarchitecture. *BMC Genom* 16(1):1013
- Li B, Dewey CN (2011) RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 12:323
- Li Y, Oosting M, Deelen P, Ricaño-Ponce I, Smeekens S (2016) Inter-individual variability and genetic influences on cytokine responses to bacteria and fungi. *Nat Med* 22(8):952–960
- Lorè NI, Iraqi FA, Bragonzi A (2015) Host genetic diversity influences the severity of *Pseudomonas aeruginosa* pneumonia in the Collaborative Cross mice. *BMC Genet* 16:106
- Majumdar S, Padiglione A (2012) Nosocomial infections in the intensive care unit. *Anaesth Intensive Care Med* 13(5):204–208
- Manry J, Quintana-Murci L (2013) A genome-wide perspective of human diversity and its implications in infectious disease. *Cold Spring Harb Perspect Med* 3(1):a012450
- Maschmeyer G, Haas A, Cornely OA (2007) Invasive aspergillosis: epidemiology, diagnosis and management in immunocompromised patients. *Drugs* 67(11):1567–1601
- Mathes WF, Aylor DL, Miller DR, Churchill GA, Chesler EJ et al (2011) Architecture of energy balance traits in emerging lines of the Collaborative Cross. *Am J Physiol Endocrinol Metab* 300(6):E1124–E1134
- Mattsson E, Verhage L, Roloff J, Fleer A, Verhoef J et al (1993) Peptidoglycan and teichoic acid from *Staphylococcus epidermidis* stimulate human monocytes to release tumour necrosis factor- α , interleukin-1 β and interleukin-6. *FEMS Immunol Med Microbiol* 7(3):281–287
- Mott R, Talbot CJ, Turri MG, Collins AC, Flint J J (2000) A method for fine mapping quantitative trait loci in outbred animal stocks. *Proc Natl Acad Sci USA* 23(7):12649–12654
- Munford RS (2006) Severe sepsis and septic shock: the role of gram-negative bacteremia. *Annu Rev Pathol* 1:467–496
- Na YR, Je S, Seok SH (2018) Metabolic features of macrophages in inflammatory diseases and cancer. *Cancer Lett* 413:46–58
- Nashef A, Agbaria M, Shusterman A, Lorè NI, Bragonzi A, Wiess E et al (2017) Dissection of host susceptibility to bacterial infections and its toxins. *Methods Mol Biol* 1488:551–578
- Nashef A, Qabaja R, Salaymeh Y, Botzman M, Munz M, Dommisch H et al (2018) Integration of murine and human studies for mapping periodontitis susceptibility. *J Dent Res* 97(5):537–546

- Nguyen D, Singh PK (2006) Evolving stealth: genetic adaptation of *Pseudomonas aeruginosa* during cystic fibrosis infections. *Proc Natl Acad Sci USA* 103(22):8305–8306
- Nikolich-Zugich J (2018) The twilight of immunity: emerging concepts in aging of the immune system. *Nat Immunol* 19(1):10–19
- Nivoix Y, Veltens M, Letscher-Bru V, Moghaddam A, Natarajan-Amé S et al (2008) Factors associated with overall and attributable mortality in invasive aspergillosis. *Clin Infect Dis* 47(9):1176–1184
- Patil HV, Patil VC (2017) Incidence, bacteriology, and clinical outcome of ventilator-associated pneumonia at tertiary care hospital. *J Nat Sci Biol Med* 8(1):46–55
- Philip VM, Sokoloff G, Ackert-Bicknell CL, Striz M, Branstetter L et al (2011) Genetic analysis in the Collaborative Cross breeding population. *Genome Res* 21:1223–1238
- Phillippi J, Xie Y, Miller DR, Bell TA, Zhang Z et al (2014) Using the emerging Collaborative Cross to probe the immune system. *Genes Immun* 15(1):38–46
- Podschn R, Ullmann U (1998) *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* 11(4):589–603
- Polak D, Wilensky A, Shapira L, Halabi A, Goldstein D et al (2009) Mouse model of experimental periodontitis induced by *Porphyromonas gingivalis*/Fusobacterium nucleatum infection: bone loss and host response. *J Clin Periodontol* 36(5):406–410
- Ram R, Mehta M, Balmer L, Gatti DM, Morahan G (2014) Rapid identification of major-effect genes using the collaborative cross. *Genetics* 198(1):75–86
- Ramanathan B, Jindal HM, Le CF, Gudimella R, Anwar A et al (2017) Next generation sequencing reveals the antibiotic resistant variants in the genome of *Pseudomonas aeruginosa*. *PLoS ONE* 12(8):e0182524
- Rasmussen AL, Okumura A, Ferris MT, Green R, Feldmann F, Kelly SM, Scott DP, Safronetz D, Haddock E, LaCasse R, Thomas MJ, Sova P, Carter VS, Weiss JM, Miller DR, Shaw GD, Korth MJ, Heise MT, Baric RS, de Villena FP, Feldmann H, Katze MG (2014) Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance. *Science* 346(6212):987–991
- Reinhart K, Daniels R, Kissoon N, Machado FR, Schachter RD (2017) Recognizing sepsis as a global health priority—a WHO resolution. *N Engl J Med* 377(5):414–417
- Rhodin K, Divaris K, North KE, Barros SP, Moss K, Beck JD et al (2014) Chronic periodontitis genome-wide association studies: gene-centric and gene set enrichment analyses. *J Dent Res* 93(9):882–890
- Roberts A, Villena FP, Wang W, McMillan L, Threadgill DW (2007) The polymorphism architecture of mouse genetic resources elucidated using genome-wide resequencing data. *Mamm Genome* 18(6):473–481
- Rogala AR, Morgan AP, Christensen AM, Gooch TJ, Bell TA et al (2014) The Collaborative Cross as a resource for modeling human disease: CC011/Unc, a new mouse model for spontaneous colitis. *Mamm Genome* 25(3–4):95–108
- Schaefer AS, Richter GM, Nothnagel M, Manke T, Dommisch H, Jacobs G et al (2010) A genome-wide association study identifies GLT6D1 as a susceptibility locus for periodontitis. *Hum Mol Genet* 19(3):553–562
- Shimizu S, Momozawa Y, Takahashi A, Nagasawa T, Ashikawa K, Terada Y et al (2015) A genome-wide association study of periodontitis in a Japanese population. *J Dent Res* 94(4):555–561
- Shon AS, Bajwa RP, Russo TA (2013) Hypervirulent (hyper-mucoviscous) *Klebsiella pneumoniae*: a new and dangerous breed. *Virulence* 4(2):107–118
- Shusterman A, Salymah Y, Nashef A, Soller M, Wilensky A et al (2013a) Genotype is an important determinant factor of host susceptibility to periodontitis in the Collaborative Cross and inbred mouse populations. *BMC Genet* 14:68–79
- Shusterman DC, Mott R, Polak D, Schaefer A, Weiss EI et al (2013b) Host susceptibility to periodontitis: mapping murine genomic regions. *J Dent Res* 92(5):438–443
- Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D et al (2016) The third international consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 315(8):801–810
- Soubani AO, Chandrasekar PH (2002) The clinical spectrum of pulmonary aspergillosis. *Chest* 121(6):1988–1999
- Stefani S, Campana S, Cariani L, Carnovale V, Colombo C et al (2017) Relevance of multidrug-resistant *Pseudomonas aeruginosa* infections in cystic fibrosis. *Int J Med Microbiol* 307(6):353–362
- Tacconelli E, Carrara E, Savoldi A, Harbarth S, Mendelson M, Monnet DL et al (2017) Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis* 18(3):318–327
- Teumer A, Holtfreter B, Völker U, Petersmann A, Nauck M, Biffar R et al (2013) Genome-wide association study of chronic periodontitis in a general German population. *J Clin Periodontol* 40(11):977–985
- Thaisz J, Tsaih SW, Feng M, Philip VM, Zhang Y et al (2012) Genetic analysis of albuminuria in collaborative cross and multiple mouse intercross populations. *Am J Physiol Renal Physiol* 303(7):F972–F981
- Threadgill DW, Hunt KW, Williams RW (2002) Genetic dissection of complex and quantitative traits: from fantasy to reality via a community effort. *Mamm Genome* 16:344–355
- Tian C, Hromatka BS, Kiefer AK, Eriksson N, Noble SM, Tung JY et al (2017) Genome-wide association and HLA region fine-mapping studies identify susceptibility loci for multiple common infections. *Nat Commun* 8(1):599
- Vaithilingam RD, Safii SH, Baharuddin N, Ng CC, Cheong SC, Bartold PM et al (2014) Moving into a new era of periodontal genetic studies: relevance of large case-control samples using severe phenotypes for genome-wide association studies. *J Periodontol Res* 49(6):683–695
- Valdar W, Flint J, Mott R (2006) Simulating the collaborative cross: power of quantitative trait loci detection and mapping resolution in large sets of recombinant inbred strains of mice. *Genetics* 172(3):1783–1797
- van de Veerdonk FL, Gresnigt MS, Romani L, Netea MG, Latgé JP (2017) *Aspergillus fumigatus* morphology and dynamic host interactions. *Nat Rev Microbiol* 15(11):661–674
- Van der Poll T, Opal SM (2008) Host-pathogen interactions in sepsis. *Lancet Infect Dis* 8(1):32–43
- Vered K, Durrant C, Mott R, Iraqi FA (2014) 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 Genomics* 15:865
- Verheine KC, Vellers HL, Kleeberger SR (2018) Inter-individual variation in health and disease associated with pulmonary infectious agents. *Mamm Genome*. <https://doi.org/10.1007/s00335-018-9733-z>
- Vincent JL (2003) Nosocomial infections in adult intensive-care units. *Lancet* 361(9374):2068–2077
- Wang JB, Lu HL, St Leger RJ (2017) The genetic basis for variation in resistance to infection in the *Drosophila melanogaster* genetic reference panel. *PLoS Pathog* 13(3):e1006260
- Webster JP, Borlase A, Rudge JW (2017) Who acquires infection from whom and how? Disentangling multi-host and multi-mode transmission dynamics in the ‘elimination’ era. *Philos Trans R Soc Lond B*. 372(1719):20160091
- Weiler CA, Drumm ML (2013) Genetic influences on cystic fibrosis lung disease severity. *Front Pharmacol* 4:40
- Welsh CE, Miller RD, Manly KF, Wang J, McMillan L, Morahan G, Mott R, Iraqi FA, Threadgill DW (2012) Status and access to the Collaborative Cross population. *Pardo-Manuel de Villena F Mammalian Genome* 23:706–712

- Welter D, MacArthur J, Morales J, Burdett T, Hall P, Junkins H et al (2014) The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. *Nucleic Acids Res* 42(Database issue):D1001–D1006
- Wieland K, Chhatwal P, Vonberg RP (2018) Nosocomial outbreaks caused by *Acinetobacter baumannii* and *Pseudomonas aeruginosa*: Results of a systematic review. *Am J Infect Control* 46(6):643–648
- Wilensky A, Gabet Y, Yumoto H, Houry-Haddad Y, Shapira L (2005) Three-dimensional quantification of alveolar bone loss in *Porphyromonas gingivalis*-infected mice using micro-computed tomography. *J Periodontol* 76(8):1282–1286
- Wilensky A, Shapira L, Halabi A, Goldstein D, Weiss EI et al (2009) Mouse model of experimental periodontitis induced by *Porphyromonas gingivalis*/*Fusobacterium nucleatum* infection: bone loss and host response. *J Clin Periodontol* 36(5):406–410
- Williams RW, Gu J, Qi S, Lu L (2001) The genetic structure of recombinant inbred mice: high-resolution consensus maps for complex trait analysis. *Genome Biol* 2(11):RESEARCH0046
- Winstanley C, O'Brien S, Brockhurst MA (2016) *Pseudomonas aeruginosa* evolutionary adaptation and diversification in cystic fibrosis chronic lung infections. *Trends Microbiol* 24(5):327–337
- World Health Organization (2017) The top 10 causes of death, Fact sheet (Updated January 2017). <http://www.who.int/mediacentre/factsheets/fs310/en/>. Accessed 9 Jan 2018
- Xiong H, Morrison J, Ferris MT, Gralinski LE, Whitmore AC et al (2014) Genomic profiling of collaborative cross founder mice infected with respiratory viruses reveals novel transcripts and infection-related strain-specific gene and isoform expression. *G3* 4(8):1429–1444
- Yang X, Yang H, Zhou G, Zhao GP (2008) Infectious disease in the genomic era. *Annu Rev Genomics Hum Genet* 9:21–48
- Yang H, Ding Y, Hutchins LN, Szatkiewicz J, Bell TA (2009) A customized and versatile high-density genotyping array for the mouse. *Nat Methods* 6(9):663–666
- Zombeck JA, Deyoung EK, Brzezinska WJ, Rhodes JS (2011) Selective breeding for increased home cage physical activity in collaborative cross and Hsd:ICR mice. *Behav Genet* 41(4):571–582