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Hannah R Wardill, Joanne M Bowen, Ysabella ZA Van Sebille, Rachel J GibsonBoyle

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ROUTINE ASSESSMENT OF THE GUT MICROBIOME TO PROMOTE PRECLINICAL RESEARCH REPRODUCIBILITY AND TRANSPARENCY

Hannah R Wardill[1,2], Joanne M Bowen[1], Ysabella ZA Van Sebille[1] and Rachel J Gibson[3].

1. School of Medicine, University of Adelaide
2. Centre for Nutrition and Gastrointestinal Diseases, South Australian Health and Medical Research Institute (SAHMRI)
3. Division of Health Sciences, University of South Australia

Corresponding Author

Dr Hannah Wardill

South Australian Health and Medical Research Institute (SAHMRI); Department of Nutrition and Metabolism

North Terrace

Adelaide, 5000

South Australia

AUSTRALIA

Email: hannah.wardill@adelaide.edu.au

Phone: 08 8128 4842

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Abbreviations: TLR4 = toll like receptor 4; GI = gastrointestinal

The irreproducibility of preclinical, biomedical research is becoming increasingly problematic, as recently highlighted by Omary et al., (2016) in the June issue of *Gut*. As discussed in this paper, variations in study design, mouse strain, sex and age are important factors that should be adequately described to promote study reproducibility [1]. However recent speculation suggests the gut microbiome may also confound preclinical research outcomes [2]. Adequate recognition of this potential confounder is particularly important with the large volume of studies now reporting associations between the gut microbiome and various pathologies, of both gastrointestinal (GI) and non-GI origin.

The gut microbiome is highly plastic, with its composition dependent on genotype and environment [2]. *In vivo* embryo transfer, with genetically distinct embryos, results in similar microbial profiles regardless of genetic background [3]. Similarly, relocation of infant mice dramatically changes the native microbial community [3]. In adult mice, viable counts of the total bacterial load have revealed large differences in the gut microbiome among animals from different facilities [4]. Furthermore, differences in the relative abundance of particular microbes have been identified in animals originating from different breeding rooms, within the same facility [2]. This critically highlights the need to routinely characterise the composition of the gut microbiome to promote study reproducibility.

We have previously shown shown genetic knockout of the innate immune receptor, Toll-like receptor 4 (TLR4; BALB/c-$Tlr4^{-/-billy}$), alters the composition of the caecal microbiota. Following introduction of a new breeding facility for this genetically modified strain, we assessed the composition of the gut microbiome in this new population. In keeping with previous recommendations, efforts were made to reduce environmental confounders. Female mice (n=12, BALB/c background, 18-20 g, 8-10 weeks) were group housed in ventilated cages, in the same rack/room, with six animals per cage. All animals were allowed to acclimitise for 1 week (at the University of Adelaide) during which they were exposed to the same dark/light conditions (12 h) and given access to water and food *ad libitum*. The first population of BALB/c- $Tlr4^{-/-billy}$ mice were obtained from the University of Adelaide Laboratory Animal Service (TLR4KO1). The second population of BALB/c-$Tlr4^{-/-billy}$ mice were sourced from

University of Newcastle (TLR4KO2). Both populations were originally sourced from Osaka, Japan. The caecal contents were aseptically collected and sent for genetic sequencing at the Australian Genomics Research Facility (Brisbane, Australia).

Consistent with previous *in vivo* research, our data indicate that breeding facility alters the composition of the gut microbiome. In this instance, TLR4KO1 has significantly lower levels of Bacteroidetes compared to TLR4KO2 (** $P = 0.009$, Figure 1). TLR4KO1 mice displayed higher levels of gram-positive Actinobacteria (TLR4KO1 2.23 ± 0.63 %; TLR4KO2 60.38 ± 0.07 % Figure 1, * $P = 0.03$) and the pathogenic microbe, Proteobacteria (TLR4KO1 3.54 ± 0.90 %; TLR4KO2 1.03 ± 0.25 % Figure 1, * $P = 0.03$). Fewer total species were also reported in TLR4KO1 (total = 69) compared TLR4KO2 (total = 104), although no changes were seen in alpha diversity (Figure 2). Given efforts to reduce additional confounders, this supports previous research showing altered microbial phenotype in mice from different breeding facilities and even different rooms within the same facility [2] and suggests the gut microbiome reflects the cumulative effects of various environmental factors. The mechanism(s) underlying these changes are unclear, however our results highlight the importance of consistent rodent husbandry when designing and conducting preclinical studies, and the need to routinely characterise the composition of the gut microbiome. This is especially important with current calls to address the irreproducibility of preclinical research, and standardise reporting of animal research data presentation [1].

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Declarations

The authors have no conflicts of interest to declare.

Figure Legends

Figure 1 Breeding conditions affect gut microbiome composition in the BALB/c mouse. **(A)** Relative abundance (%) of bacteria phyla in n = 6 TLR4KO1 and n = 6 TLR4KO2 mice. **(B)** Mean percentage of each bacteria phyla in TLR4KO1 and TLR4KO2 populations. Differences were identified in Actinobacteria (TLR4KO1 2.23 ± 0.63 %; TLR4KO2 60.38 ± 0.07 %, * P = 0.03), Bacteroidetes (TLR4KO1 13.34 ± 3.47 %; TLR4KO2 27.75 ± 2.75 %, ** P = 0.009) and Proteobacteria (TLR4KO1 3.54 ± 0.90 %; TLR4KO2 1.03 ± 0.25 %, * P = 0.03). Data presented as individual data points with mean ± SEM.

Figure 2 Shannon's Diversity Index for TLR4KO1 and TLR4KO2 caecal microbiome species. An unpaired t-test with Welch's correction showed no significant difference between populations of BALB/c-$Tlr4^{-/-billy}$ mice. Data presented as individual data points with mean ± SEM; n = 6 per group, P > 0.05.

References

1 Omary MB, Cohen DE, El-Omar E, Jalan R, Low M, Nathanson M*, et al.* Not All Mice Are the Same: Standardization of Animal Research Data Presentation. Gut 2016;**65**:894-5.
2 Hufeldt MR, Nielsen DS, Vogensen FK, Midtvedt T, Hansen AK. Variation in the gut microbiota of laboratory mice is related to both genetic and environmental factors. Comp Med 2010;**60**:336-47.
3 Friswell MK, Gika H, Stratford IJ, Theodoridis G, Telfer B, Wilson ID*, et al.* Site and strain-specific variation in gut microbiota profiles and metabolism in experimental mice. PLoS One 2010;**5**:e8584.
4 Ericsson AC, Davis JW, Spollen W, Bivens N, Givan S, Hagan CE*, et al.* Effects of vendor and genetic background on the composition of the fecal microbiota of inbred mice. PLoS One 2015;**10**:e0116704.