Gut microbiome and dietary fibre intake strongly associate with IgG function and maturation following SARS-CoV-2 mRNA vaccination

The first study to investigate potential associations between gut microbiota composition and SARS-CoV-2 vaccine immunogenicity was recently published in *Gut*.[1] This study demonstrated a statistically significant reduction in alpha diversity and a shift in gut microbiota composition following BNT162b2 vaccination, characterised by reductions in Actinobacteriota, *Blautia, Dorea, Adlercreutzia, Asacchaobacter, Coprococcus, Streptococcus, Collinsella* and *Ruminococcus* spp and an increase in *Bacteroides cacaae* and *Alistipes shahii*. Our prospective observational study (n=52; figure 1A, online supplemental table S1) similarly showed a shift in gut microbiota after the first BNT162b2 vaccine dose (p=0.016; online supplemental figure S1A), including a reduction in Actinobacteria, *Blautia* spp (p<0.01; figure 1B), and alpha diversity (p=0.078; online supplemental figure S1B). Our data support the findings by Ng *et al*,[1] reinforcing the link between SARS-CoV-2 mRNA vaccine immunogenicity and the gut microbiota.

Ng *et al*[1] also identified strong associations between baseline gut microbiota composition and serological IgG responses to BNT162b2 vaccination. After stratifying participants as low or high vaccine responders, they showed higher abundances of *Eubacterium rectale, Roseburia faces, Bacteroides thetaiotaomicron* and *Bacteroides spp OM05-12* were associated with stronger BNT162b2 vaccine responses. Correspondingly, in our cohort, several baseline bacterial taxa significantly differed between participants with low versus high BNT162b2 vaccine responses. Specifically, we observed higher baseline counts of *Prevotella*, *Haemophilus, Veillonella* and *Ruminococcus gnavus* taxa in participants with higher RBD and Spike competitive binding antibody and IgG levels (p<0.01; figure 1C). Additional studies are needed to ascertain the clinical significance of these findings. However, together with Ng *et al*,[1] these findings further support that differences in microbiome composition and/or function modulate antibody responses to SARS-CoV-2 vaccination. Interestingly, a study recently discovered that SARS-CoV-2 specific T cells can also cross-react with microbial peptides from commensals (including *Prevotella* spp) and undefined faecal lysates.[2] Considering that individuals with milder COVID-19 showed higher frequencies of cross-reactive SARS-CoV-2 T cells[3] it is plausible that microbe-based stimulation of SARS-CoV-2-reactive T or B cells could modulate SARS-CoV-2 vaccine responses. Consistent with Ng *et al*[1] we demonstrated an association between a gut microbiome signature at baseline and SARS-CoV-2 vaccine immunogenicity; however, the specific bacterial

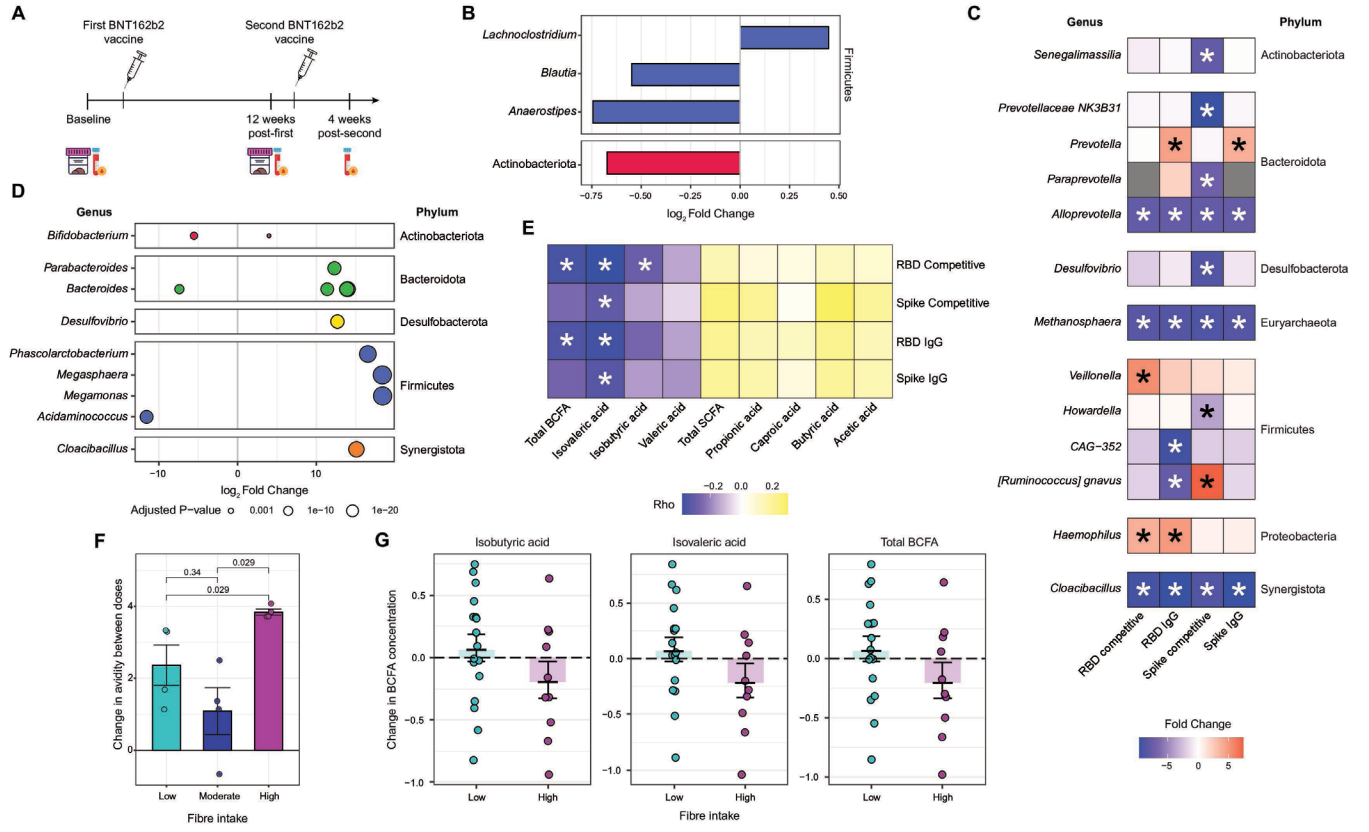

Figure 1 Impact of the mRNA SARS-CoV-2 BNT162b2 vaccine on gut microbiota composition as well as the gut microbiome and dietary factors that affect BNT162b2 vaccine response. (A) Schematic of the study design and blood and stool sample collection timepoints. (B) A significant reduction in Actinobacteriota (p<0.0001), *Anaerostipes* (p=0.00161) and *Blautia* (p=0.00103) and an increase in *Lachnoclostridium* (p=0.00179) were observed after the first BNT162b2 vaccine. (C) Heat-map depicting several significantly higher (red) or lower (blue) (*p<0.05; Wald test) baseline microbial counts in high (quartile 4) vs low (quartile 1) BTN162b2 vaccine responders across several immune parameters. (D) Several gut microbiota species were significantly positively or negatively associated (p<0.01; Wald test) with higher total relative fractional avidity after the second dose of the BNT162b2 vaccine in a subset of participants (n=15). Refer to online supplemental table S2 for the specific species names and p values. (E) Baseline total branched-chain fatty acid (BCFA) concentrations were negatively correlated with RBD IgG (p=0.02, r=−0.35; Spearman rank) and Spike IgG levels (p=0.017, r=−0.36; Spearman rank), isovaleric acid concentrations were negatively associated with RBD IgG (p=0.015, r=−0.37; Spearman rank) and competitive binding antibody (p=0.026, r=−0.34; Spearman rank), and Spike IgG (p=0.013, r=−0.38; Spearman rank) and competitive binding antibody levels (p=0.034, r=−0.32; Spearman rank), and baseline isobutyric acid concentrations were negatively associated with Spike IgG levels (p=0.047, r=−0.29; Spearman rank). (F) High dietary fibre consumers had a significantly greater change in total relative fractional avidity from the first to second BNT162b2 dose compared with low and moderate dietary fibre consumers (p=0.029; Mann-Whitney). (G) High dietary fibre consumers experienced a reduction in total BCFAs (p=0.164, adjusted t-test), isovaleric (p=0.189, adjusted t-test) and isobutyric acid (p=0.213 adjusted t-test) concentrations (−0.186 to −0.198 and −0.177 mean fold change, respectively) after BNT162b2 vaccination, whereas low dietary fibre consumers experienced an increase in total BCFAs, isovaleric and isobutyric acid concentrations (0.08, 0.08 and 0.08 mean fold change, respectively).

taxa associated with vaccine responses differed between cohorts. This is possibly due to differences in geography (Hong Kong vs Canada), dietary habits and/or microbiome sampling/analysis methods.

Moving beyond antibody levels, we explored the associations between BNT162b2 vaccine-induced antibody avidity maturation (ie, the ratio of low to high IgG antibody avidity to the Spike protein[4 5]) and specific gut microbiome signatures, in a subset of participants (n=15). Multiple bacterial taxa were negatively (ie, *Bifidobacterium bifidum*, *Acidaminococcus intestini*) or positively (ie, *Bifidobacterium animalis*, *Bacteroides plebeius*, *Bacteroides ovatus*) associated with enhanced antibody avidity (p<0.01; figure 1D), with several species having known immunomodulatory properties. Most notably, *Bacteroides ovatus* induces increased production of IgM and IgG antibodies specific to human cancer cells.[6] Additionally, *Bifidobacterium animalis* can significantly increase vaccine-specific IgG production after seasonal influenza vaccination.[7] Thus, these observations provide further evidence that gut bacterial species may enhance functional binding of IgG elicited by BNT162b2 vaccination.

We also examined the potential link between the functional capacity of the gut microbiome and habitual diets, with BNT162b2 vaccine response. Our data suggest that microbial-derived branched-chain fatty acids (BCFA) isovaleric and isobutyric acids, produced via protein fermentation, may reduce vaccine responses (p<0.05; figure 1E). BCFA concentrations are known to be higher in patients with immune-mediated conditions such as inflammatory bowel disease[8]; however, little is known about the mechanisms by which BCFAs modulate inflammation or antibody-mediated vaccine responses. Interestingly, *Megasphaera* spp, which were negatively associated with vaccine responses, are prominent isovaleric and isobutyric acid producers.[9] Future research is needed to explore the

potential impact of BCFAs on SARS-CoV-2 vaccine immunogenicity.

Lastly, no research has elucidated the role distinct dietary intakes have on SARS-CoV-2 vaccine responses. Therefore, we determined whether differing dietary fibre (microbial substrate) intakes affected IgG binding strength. We observed that the change in avidity between the first and second BNT162b2 dose was significantly greater in high fibre consumers (p=0.029; figure 1F). This further suggests that dietary fibre intakes may modulate BNT162b2 vaccine response maturation. Interestingly, high fibre consumers also experienced a reduction in total BCFAs post vaccination (p=0.164; figure 1G), signifying a potential mechanistic link between fibre intake, BCFA production and SARS-CoV-2 vaccine immunogenicity.

In summary, these data, while exploratory, validate findings from Ng *et al*,[1] reinforcing a potential link between the gut microbiome and SARS-CoV-2 mRNA vaccine antibody responses. This study further expands on these findings and shows, for the first time, that BCFA levels may negatively impact, while dietary factors, such as fibre intake, may enhance BNT162b2 immunogenicity. Studies are currently underway to explore the therapeutic benefit of microbiome-modulating interventions to enhance SARS-COV-2 vaccine immunogenicity.[10] Considering that the effectiveness of most SARS-CoV-2 vaccines are high, but relatively short-lived, especially in vulnerable age and medical groups, the gut microbiome could represent a simple, yet powerful way to optimise long-term protection or improve recovery after infection.

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Correction notice This article has been corrected since it published Online First. The first author's name has been corrected and figure 1 replaced.

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Contributors GRH, PML and BAV conceived and designed the study. LG coordinated patient recruitment and assisted with blood sample processing with AM. LG undertook the vaccine response experimental design, execution, and analyses. GRH assembled stool collection kits, undertook the dietary fibre analysis, and prepared stool samples for sequencing and short-chain fatty acid analysis. AS undertook the statistical and bioinformatic analyses. GRH wrote the manuscript with significant input from all co-authors.

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