Gut microbiota composition is associated with SARS-CoV-2 vaccine immunogenicity and adverse events

Siew C Ng,1,2,3,4 Ye Peng,5,6 Lin Zhang,1,2,4,7 Chris KP Mok,3,8 Shilin Zhao,5,6 Amy Li,1 Jessica YL Ching,1,9 Yingzhi Liu,4,7 Shuai Yan,4,7 Dream L S Chan,4 Jie Zhu,5,6 Chunken Chen,3,8 Adrian CH Fung,9 Kenneth KY Wong,9 David SC Hui,1,10 Francis KL Chan1,1,2,3,4 Hein M Tun, HKU-Pasteur Research Pole, School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China; heimtun@hku.hk and Professor Francis KL Chan, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong SAR, China; fkchan@cuhk.edu.hk

SCN, YP and LZ contributed equally.

Correspondence to Dr Hein M Tun, HKU-Pasteur Research Pole, School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China; heimtun@hku.hk and Professor Francis KL Chan, Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong SAR, China; fkchan@cuhk.edu.hk

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ABSTRACT

Objective The gut microbiota plays a key role in modulating host immune response. We conducted a prospective, observational study to examine gut microbiota composition in association with immune responses and adverse events in adults who have received the inactivated vaccine (CoronaVac; Sinovac) or the mRNA vaccine (BNT162b2; BioNTech; Comirnaty).

Design We performed shotgun metagenomic sequencing in stool samples of 138 COVID-19 vaccinees (37 CoronaVac and 101 BNT162b2 vaccinees) collected at baseline and 1 month after second dose of vaccination. Immune markers were measured by SARS-CoV-2 surrogate virus neutralisation test and spike receptor-binding domain IgG ELISA.

Results We found a significantly lower immune response in recipients of CoronaVac than BNT162b2 vaccines (p<0.05). Bifidobacterium adolescentis was persistently higher in subjects with high neutralising antibodies to CoronaVac vaccine (p=0.023) and their baseline gut microbiome was enriched in pathways related to carbohydrate metabolism (linear discriminant analysis (LDA) scores >2 and p<0.05). Neutralising antibodies in BNT162b2 vaccinees showed a positive correlation with the total abundance of bacteria with flagella and fimbriae including Roseburia faecis (p=0.028). The abundance of Prevotella copri and two Megamonas species were enriched in individuals with fewer adverse events following either of the vaccines indicating that these bacteria may play an anti-inflammatory role in host immune response (LDA scores >3 and p<0.05).

Conclusion Our study has identified specific gut microbiota markers in association with improved immune response and reduced adverse events following COVID-19 vaccines. Microbiota-targeted interventions have the potential to complement effectiveness of COVID-19 vaccines.

INTRODUCTION

Vaccination elicits protective immune responses against SARS-CoV-2 and provides hope for containing the COVID-19 pandemic. As of 17 January 2022, more than 9.3 billion doses of vaccine have been administrated worldwide with substantial efficacy.1–4 Recent observational studies reported a steady decline of antibody levels among vaccinated individuals which implied a growing risk of breakthrough infection over time.5–8 but factors influencing immunogenicity and durability of vaccine remains poorly understood. Evidence from clinical or animal studies suggested that the composition and functions of the gut microbiota are crucial in modulating immune responses of vaccination.7,8 Mucosal or systemic microbiota exposure shapes T and B cell repertoires that have an important implication for regulating responses to vaccination.9,10 Whether host microbiota composition influences
responses of COVID-19 vaccines in humans has not been determined. We conducted a prospective observational study of adults who have received either the inactivated vaccine (CoronaVac; Sinovac) or the mRNA vaccine (BNT162b2; BioNTech; Comirnaty) to examine gut microbiota determinants of vaccine immune responses and vaccine-related adverse events.

MATERIALS AND METHODS

Study cohorts
Participants were volunteers receiving the mRNA COVID-19 vaccine (BNT162b2; N=101) or the inactivated COVID-19 vaccine (CoronaVac; N=37) recruited for serial blood and stool donations at the Prince of Wales Hospital of the Chinese University of Hong Kong (CUHK), the Queen Mary Hospital of the University of Hong Kong (HKU) or the community between 1 April 2021 and 31 August 2021. Eligible participants were aged 18 or above with no history of SARS-CoV-2 infection receiving either BNT162b2 or CoronaVac vaccine. Exclusion criteria included the presence of clinical signs and symptoms suggestive of acute infection with a positive reverse transcription PCR results for SARS-CoV-2 in saliva, or a positive COVID-19 serology. All participants provided written informed consent and completed both doses of vaccines.

Collection of stool and blood samples
One stool sample in DNA preservative and ~10 mL of blood in anticoagulant were collected from the participants at baseline (within 3 days of the first dose) and 1 month after second dose of vaccination.12 Stool samples were self-collected in DNA preservative tube at home and transferred at room temperature to −80°C until DNA extraction. Blood samples were collected at hospital clinics and transported to laboratories for separation of plasma for serological tests.

Collection of demographic and epidemiological data
Standardised questionnaires were used to capture basic demographics and adverse events after both doses of vaccine. Demographics included age, gender, weight, height, comorbidities (hypertension, diabetes mellitus, allergy, diarrhoea, any other comorbidities), medication (antibiotics, hormone, immunomodulator), probiotics, vaccination in the past year, diet, alcohol intake (within 2 weeks prior to the first vaccination) and regular exercise (strenuous/moderate). Overweight or obese (OWOB) cut-off point of body mass index (BMI) ≥23 kg/m². Adverse events questionnaires are summarised in the online supplemental table S1.

Serological tests
SARS-CoV-2 surrogate virus neutralisation test (sVNT) and spike receptor-binding domain (RBD) IgG ELISA were used to assess antibody levels in plasma collected at baseline and 1 month after second dose of vaccination. sVNT kits were obtained from GenScript, NJ, USA (Catalogue No. L00847-A) and tests were carried out according to manufacturer’s instructions. SARS-CoV-2 spike RBD IgG ELISA was carried out as previously described13 14 (online supplemental methods).

Stool metagenomic sequencing
Faecal DNA was extracted from the pellet using Maxwell RSC PureFood GMO and Authentication Kit (Promega, Madison, Wisconsin, USA). Faecal DNA was subjected to library construction using Nextera DNA Flex Library Preparation kit (Illumina, San Diego, California, USA)15 16 following manufacturer’s instructions (online supplemental methods). Libraries were sequenced on an in-house sequencer Illumina NovaSeq 6000 (250 base pairs paired-end) at the Microbiota I-Centre, Hong Kong, China. Sequence data processing and analysis were fully stated in online supplemental methods.

Statistical analysis
The primary analysis was to compare the relationship between microbiome profile and immune response to COVID-19 vaccines. Detailed statistical analysis can be found in online supplemental methods.

RESULTS

SARS-CoV-2 vaccine cohort
Between 1 April 2021 and 31 August 2021, we recruited 138 adults who have received two doses of either the inactivated vaccines (CoronaVac; n=37) or the mRNA vaccine (BNT162b2; n=101) from CUHK and HKU (figure 1A). The participants ranged in age from 18 to 67 years (median=47 years, IQR 31.2–55.0) and 32.6% were male. 38.4% was classified as OWOB (ie, BMI ≥23) (table 1). Compared with BNT162b2 vaccinees, CoronaVac vaccinees were older in age (55.0 (CoronaVac) vs 42.0 (BNT162b2); p=0.003) and a higher proportion had hypertension (18.9% (CoronaVac) vs 6.9% (BNT162b2), p=0.035). Plasma SARS-CoV-2 sVNT and spike RBD IgG ELISA before vaccination were negative in all participants. At 1 month after completion of two doses of vaccines, CoronaVac vaccinees had a significantly lower immune response against SARS-CoV-2 compared with BNT162b2 vaccinees (sVNT: 57.6% vs 95.2%, p<0.001; anti-RBD: 1725.0 vs 8696.0, p<0.001) (table 1 and online supplemental figure 1A,B) based on adjusted linear regression and propensity score matching analysis matched for age and comorbidities (p<0.001, (online supplemental tables S2, S3). Moreover, sVNT were negatively correlated with BMI in the CoronaVac group (BMI; Spearman’s r=−0.385, p=0.018, (online supplemental table S4), and it was significant in both males and females (r=−0.817, p=0.007 and r=−0.403, p=0.033, respectively).

Gut microbiota composition in CoronaVac and BNT162b2 vaccinees
We performed shotgun metagenomic analysis on stool samples to determine whether baseline gut microbiome composition was associated with immune response to COVID-19 vaccines. In total, 272 stool samples were sequenced to generate an average of 7.7 Gb (33.7 M reads) per sample. We observed a significant change in the gut microbiome composition including shifts in beta diversity (figure 1B) and a decrease in alpha diversity (figure 1C) at 1 month after the second dose of vaccination compared with baseline samples in both vaccine groups. These changes were not significantly different between the two vaccine groups. Baseline gut microbiome was significantly associated with several comorbidities, antibiotic use within 3 months prior to vaccination, regular exercise and recent symptoms of diarrhoea (online supplemental table S5). At the species level, only the abundance of Bacteroides caccae was found to be increased in CoronaVac vaccinees whereas BNT162b2 vaccinees had increased abundances of both B. caccae and Alistipes shahii, 1 month after two doses of vaccination. On the other hand, a relative decline in abundances of common bacterial species including Adlercreutzia equolificans, Asaccharobacter celatus, Blautia obeum, Blautia wexlerae, Dorea formicigenerans, Dorea
longicatena, Coprococcus comes, Streptococcus vestibularis, Collinsella aerofaciens, and Ruminococcus obeum CAG 39 (figure 1D) were observed in both vaccine groups. A significant decline in Actinobacteria and Firmicutes abundances could be explained by altered physiological functions and drastic inflammation during vaccine regimen. Importantly, none of the participants reported significant dietary changes during the study period. Among 72 randomly selected participants, no significant changes in detailed dietary intake were recorded at baseline and 1 month after second dose of vaccination (p>0.05; online supplemental table S6).

Baseline gut microbiome composition predicts immune response at one month after COVID-19 vaccine

Consistent with previous findings,18 19 our study showed a high correlation between neutralising antibody by sVNT and anti-spike RBD IgG measured by ELISA (Spearman’s r=0.85, p<0.001 in CoronaVac; r=0.48, p<0.001 in BNT162b2, (online supplemental figure S1C,D), thus, we focused our analysis using results of sVNT. Khoury et al reported that 50% protection from neutralisation was related to antibody levels that were 20% of convalescent antibody titers.20 People with a sVNT lower than 50% may prone to re-infection. Since there was waning of antibody from peak titres observed at 1 month after second dose of vaccination, we set our target titre achieved at 1 month after second dose of vaccination to be twice the 50% protection titre which corresponded to sVNT inhibition of 60%.19 Among CoronaVac vaccinees, 21 of 37 (56.8%) who showed sVNT lower than 60% (low-responders) had a distinct baseline gut microbiome from those with sVNT higher than 60% (high responders). We observed that certain baseline gut microbiota species were associated with antibody response to COVID-19 vaccines. In particular, a total of 15 bacterial species in the baseline gut microbiome were identified, of which Bifidobacterium adolescentis was enriched in high-responders while Bacteroides vulgatus, Bacteroides thetaiotaomicron and Ruminococcus gnavus were more abundant in low-responders (figure 2A). B. adolescentis which was present in 64.9% of subjects showed a significant correlation with sVNT% in the CoronaVac group (table 2). At 1 month after second dose of vaccination, seven species including B. adolescentis, A. equolibaciens and A. celatus were more abundant whereas B. vulgatus

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Figure 1  Study design and changes in beta diversity, alpha diversity and bacterial species from baseline to 1 month after second dose of vaccination. (A) Study design. (B) Beta diversity was significantly different between baseline and 1 month after completion of vaccination (CoronaVac baseline, n=37; BNT162b2 baseline, n=101; CoronaVac 1 month, n=36; BNT162b2 1 month, n=98). P values were given by PERMANOVA and Wilcoxon rank-sum test (two sided), and adjusted for FDR, respectively. (C) Alpha diversity decreased significantly from baseline to 1 month after completion of vaccination for CoronaVac (n=36) and BNT162b2 (n=98). P values were given by paired Wilcoxon rank-sum test (two sided). (D) Differentially abundant species between baseline and 1 month after completion of vaccination for CoronaVac (n=36) and BNT162b2 (n=98). Differentially abundant species were detected using paired Wilcoxon rank-sum test (FDR corrected p<0.05). Elements on boxplots: centre line, median; box limits, upper and lower quartiles; whiskers, 1.5×IQR; points, outliers. FDR, false discovery rate; NMDS, non-metric multi-dimensional scaling; PERMANOVA, permutation multivariate analysis of variance.
Table 1  Baseline characteristics of study population

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall (N=138)</th>
<th>BNT162b2 (N=101)</th>
<th>CoronaVac (N=37)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristics</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, years, (median (IQR))</td>
<td>47 (31.2–55.0)</td>
<td>42 (29.0–53.0)</td>
<td>55 (44.0–57.0)</td>
<td>0.003</td>
</tr>
<tr>
<td>Female*</td>
<td>93 (67.9)</td>
<td>65 (65.0)</td>
<td>28 (75.7)</td>
<td>0.304</td>
</tr>
<tr>
<td>BMI, kg/m², (median (IQR))</td>
<td>21.8 (20.2–24.5)</td>
<td>21.8 (20.1–24.6)</td>
<td>22.2 (20.4–23.7)</td>
<td>0.946</td>
</tr>
<tr>
<td>Overweight or obese†</td>
<td>53 (38.7)</td>
<td>38 (38.0)</td>
<td>15 (40.5)</td>
<td>0.844</td>
</tr>
<tr>
<td>Obese‡</td>
<td>27 (19.7)</td>
<td>22 (22.0)</td>
<td>5 (13.5)</td>
<td>0.338</td>
</tr>
<tr>
<td>Presence of comorbidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypertension</td>
<td>14 (10.1)</td>
<td>7 (6.9)</td>
<td>7 (18.9)</td>
<td>0.055</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>4 (2.9)</td>
<td>3 (3.0)</td>
<td>1 (2.7)</td>
<td>1.000</td>
</tr>
<tr>
<td>Allergy ever</td>
<td>49 (35.5)</td>
<td>40 (39.6)</td>
<td>9 (24.3)</td>
<td>0.111</td>
</tr>
<tr>
<td>Diarrhoea (past 3 months to current)</td>
<td>55 (40.4)</td>
<td>42 (42.0)</td>
<td>13 (36.1)</td>
<td>0.560</td>
</tr>
<tr>
<td>Other comorbidities‡</td>
<td>15 (10.9)</td>
<td>13 (12.9)</td>
<td>2 (5.4)</td>
<td>0.354</td>
</tr>
<tr>
<td>Current medication</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antibiotic intake (past 3 months and/or currently)</td>
<td>6 (4.3)</td>
<td>6 (5.9)</td>
<td>0 (0.0)</td>
<td>0.192</td>
</tr>
<tr>
<td>Hormone therapy</td>
<td>4 (2.9)</td>
<td>4 (4.0)</td>
<td>0 (0.0)</td>
<td>0.574</td>
</tr>
<tr>
<td>Immunomodulator</td>
<td>3 (2.2)</td>
<td>3 (3.0)</td>
<td>0 (0.0)</td>
<td>0.564</td>
</tr>
<tr>
<td>Probiotics</td>
<td>18 (13.1)</td>
<td>12 (12.0)</td>
<td>6 (16.2)</td>
<td>0.572</td>
</tr>
<tr>
<td>Vaccination in the past year</td>
<td>53 (38.7)</td>
<td>38 (38.0)</td>
<td>15 (40.5)</td>
<td>0.844</td>
</tr>
<tr>
<td>Dietary habit</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vegetarian</td>
<td>1 (1.0)</td>
<td>1 (1.0)</td>
<td>0 (0.0)</td>
<td>1.000</td>
</tr>
<tr>
<td>Diet change during vaccination</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>0 (0.0)</td>
<td>–</td>
</tr>
<tr>
<td>Alcohol intake (within 2 weeks prior to first vaccine dose)</td>
<td>31 (22.5)</td>
<td>25 (24.8)</td>
<td>6 (16.2)</td>
<td>0.361</td>
</tr>
<tr>
<td>Exercise</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Regular exercise (strenuous/moderate)</td>
<td>86 (62.3)</td>
<td>62 (61.4)</td>
<td>24 (64.9)</td>
<td>0.843</td>
</tr>
<tr>
<td>SARS-CoV-2 antibody response</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AUC of spike RBD IgG level (median (IQR))‡</td>
<td>7889.5 (3110.8–9588.5)</td>
<td>8696.0 (7628.0–11048.0)</td>
<td>1725.0 (1418.0–2459.0)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sVNT (&gt;60%)</td>
<td>116 (84.1)</td>
<td>100 (99.0)</td>
<td>16 (43.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>sVNT (inhibition %) (median (IQR))§</td>
<td>93.9 (79.7–95.9)</td>
<td>95.2 (92.1–96.4)</td>
<td>57.6 (42.1–69.3)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Any adverse events¶</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>After the first dose</td>
<td>116 (84.7)</td>
<td>93 (93.0)</td>
<td>23 (62.2)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>After the second dose</td>
<td>120 (87.6)</td>
<td>95 (95.0)</td>
<td>25 (67.6)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Categorical data are presented as number (percentage) and continuous data as median (IQR). Within-group valid percentages are shown.

* One participant requested concealment of gender.

† BMI between 23.0 and 25.0 kg/m² is classified as overweight and BMI above 25.0 kg/m² is classified as obese.

‡ Any other comorbidities: asthma, depression, eczema, high cholesterol, systemic lupus erythematosus, attention deficit hyperactivity disorder.

§ Plasma IgG antibody binding to SARS-CoV-2 RBD was reported as area under the curve.

¶ Any adverse events: injection site pain/burn, fatigue, fever, injection site swelling/pruritus/erythema/induration, myalgia, drowsiness, headache, chills, dizziness, arthralgia, loss of appetite, abdominal pain, rhinorhoea, sore throat, diarrhoea, pruritus, coughing, constipation, abdominal distension, nausea, flushing, hypersensitivity, muscle spasms, nasal congestion, oedema, vomiting, tremor, eyelid oedema, nosebleeds, hypoaesthesia, ocular congestion, low back pain, increase of appetite, muscle pain, rib pain, eye pain, palpitations.

AUC, area under the curve; BMI, body mass index; DM, diabetes mellitus; RBD, receptor-binding domain; sVNT, surrogate virus neutralisation test.

remained less abundant in high responders (online supplemental figure S2A). Using mixed effect modeling,21 we showed that B. adolescentis was persistently higher while B. vulgatus was persistently lower from baseline to 1 month after second dose in high-responders (online supplemental table S7). We further interrogated functional pathways (online supplemental table S8) in the baseline gut microbiome and found that CoronaVac vaccinees with sVNT > 60% had higher abundances of pathways related to carbohydrate metabolism and most of these pathways were positively correlated with abundance of B. adolescentis (figure 2A). In contrast, low responders had a relatively higher abundance of L-ornithine biosynthesis II pathway which was positively correlated with abundances of B. vulgatus and B. thetaiotaomicron at baseline (figure 2A).

The sVNT kit has a ceiling of detection limit using the standard dilution.22 Studies showed that most people who received BNT162b2 vaccine reached this detection limit 1 month after two doses of vaccination.24 Only one participant who received BNT162b2 vaccine had very low sVNT inhibition (29.3%) (online supplemental figure S1A). The participant was overweight, had a history of kidney transplant and was on corticosteroids and antihypertensive therapy. Similar to CoronaVac low responders, the gut microbiota of BNT162b2 low responders had a persistently low level of Actinobacteria particularly B. adolescentis (online supplemental figure S3). To further differentiate response among the participants, we performed sVNT using plasma samples after 200-fold of dilution to differentiate neutralising antibody level from samples of BNT162b2 (online supplemental figure S1B). We then defined the quartiles from the sVNT results of BNT162b2 cohort. Four specific bacteria in the baseline gut microbiome including Eubacterium rectale, Roseburia faecis and two Bacteroides species, B. thetaiotaomicron and Bacteroides sp OM05-12 were significantly increased in the highest-tier responders with top 25% of sVNT level (figure 2B). Abundance of these species except Bacteroides sp OM05-12 also significantly correlated with the sVNT%
Interestingly, a higher relative abundance of bacteria with flagella in the baseline gut microbiome was associated with a higher antibody response to BNT162b2 vaccine. *R. faecis* is one of the major contributors to gut bacterial motility, according to both bacterial phenotype databases and Gene Ontology annotation (GO:0009289). Among these bacterial biomarkers, two *Bacteroides* species remained persistently enriched at 1 month after BNT162b2 vaccination in highest-tier responders. AUROC values of models based on individual biomarkers and a combined model based on all biomarkers for high responders (n=16) vs low responders (n=21) among CoronaVac vaccinees. AUROC, area under the receiver operating characteristic curve; FDR, false discovery rate; LEfSe, linear discriminant analysis effect size; sVNT, surrogate virus neutralisation test.

Figure 2  Baseline gut bacterial species and functions associated with high and low responders to vaccines at 1 month after second dose of vaccination. (A) Baseline bacterial species and pathways associated with high responders among CoronaVac vaccinees (n=37) (sVNT of 10-fold diluted plasma >60%). Differential baseline gut bacterial species and pathways were detected by LEfSe. Pairwise correlations between selected bacterial species and pathways markers with FDR corrected p<0.05 were shown. (B) Baseline bacterial species and pathways for highest-tier responders among BNT162b2 vaccinees (n=101) (the first quartile (Q1) of sVNT of 200-fold diluted plasma). sVNT-10: sVNT level of 10-fold diluted plasma; sVNT-200: sVNT level of 200-fold diluted plasma. Differential baseline gut bacterial species and pathways were detected by LEfSe. Pairwise correlations between selected bacterial species and pathways markers with FDR corrected p<0.05 were shown. Full names of differentially abundant pathways between high/low responders in (A, B) are described in online supplemental table S7C, AUROC (95% CI) values of models based on individual biomarkers and a combined model based on all biomarkers for high responders (n=16) vs low responders (n=21) among CoronaVac vaccinees. (D) AUROC (95% CI) values of models based on individual biomarkers and a combined model based on all biomarkers for the highest-tier responders (n=25) vs others (n=76) among BNT162b2 vaccines. Each AUROC was presented as an orange dot with a bar showing the 95% CI.

(table 2). Interestingly, a higher relative abundance of bacteria with flagella in the baseline gut microbiome was associated with a higher antibody response to BNT162b2 vaccine. *R. faecis* is one of the major contributors to gut bacterial motility, according to both bacterial phenotype databases and Gene Ontology annotation (GO:0009289). Among these bacterial biomarkers, two *Bacteroides* species remained persistently enriched at 1 month after BNT162b2 vaccination in highest-tier responders.
supplemental figure S2B). Enriched pathways for biosynthesis of several menaquinols were found in highest-tier responders’ samples collected before but not after vaccination. There was decreased abundance of pathways for adenosine27 ribonucleotide biosynthesis and peptidoglycan biosynthesis (figure 2B) in the baseline gut microbiome.

We further tested predictive power of the abovementioned baseline gut bacterial species markers based on area under the receiver operating characteristic curve (AUROC) to each type of vaccine. Predictive power of B. adolescentis alone (AUROC (95% CI): 0.780 (0.624 to 0.935) was higher than other bacterial species including, A. celatus, B. adolescentis, and A. equolifaciens and A. celatus (figure 4). However, compared with normal weight people with high abundances of B. adolescentis and A. celatus, the risk of being low responders was not significant for OWOB people if they had a high abundance of the same bacterial species (model 2: adjusted OR 0.27, 95% CI 0.02 to 2.51 and OR 0.43, 95% CI 0.04 to 4.23, respectively). These results suggest that the beneficial effect of these bacteria on the immune responses to CoronaVac vaccine was attenuated in OWOB people. Therefore, we further identified specific bacterial species in the high BMI population. LEfSe analysis showed enrichment of three bacterial species including, Butyrivibrio virosa, Alistipes putredinis, and Adlecreutzia equilifaciens in CoronaVac high responders who were OWOB (online supplemental figure S7).

**Effect of beneficial bacteria on immune response to inactivated vaccine is modified by BMI**

Gut microbiome is known to be influenced by host physiological status and lifestyle factors. Reciprocally, gut microbiome orchestrates host immune system and modulates responses to vaccines.7 We found that sVNT levels were correlated with BMI (online supplemental table S4 and figure 4) and abundance of certain bacteria in the CoronaVac group. This observation prompted us to further investigate the potential role of weight as an effect modifier of bacteria-immune response relationship. Based on comparison between strata of weight status and abundance of bacterial species markers of the baseline gut microbiome, associations of the four bacterial species with immune response were significantly influenced by body weight. Positive associations between the four bacterial biomarkers with immune response were compromised in OWOB people. These species included two short-chain fatty acid (SCFA) producers, B. adolescentis and Butyrivibrio virosa, and A. equilifaciens and A. celatus (figure 4). None of the participants had serious adverse events that led to hospitalisation. Consistent with the previous report,28 a greater proportion of BNT162b2 vaccinees reported adverse events than CoronaVac vaccinees. Compared with CoronaVac vaccinees, more BNT162b2 vaccinees developed injection site pain, fatigue, fever, myalgia, drowsiness, headache and chills (table 1 and

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Prevalence (%)</th>
<th>Spearman correlation</th>
<th>Crude correlation</th>
<th>Adjusted for age correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>r</td>
<td>P value</td>
<td>r</td>
</tr>
<tr>
<td><strong>CoronaVac</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacterium adolescentis</td>
<td>64.9</td>
<td>0.354</td>
<td>0.032</td>
<td>0.329</td>
</tr>
<tr>
<td>Alistipes putredinis</td>
<td>78.4</td>
<td>0.380</td>
<td>0.020</td>
<td>0.294</td>
</tr>
<tr>
<td>Adlercreutzia equilifaciens</td>
<td>81.1</td>
<td>0.202</td>
<td>0.230</td>
<td>0.154</td>
</tr>
<tr>
<td>Oscillibacter sp S7 20</td>
<td>73</td>
<td>0.261</td>
<td>0.118</td>
<td>0.207</td>
</tr>
<tr>
<td>Asaccharobacter celatus</td>
<td>78.4</td>
<td>0.204</td>
<td>0.227</td>
<td>0.175</td>
</tr>
<tr>
<td>Ruminococcus sp CAG 330</td>
<td>8.1</td>
<td>0.300</td>
<td>0.071</td>
<td>0.257</td>
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<tr>
<td>Intestinibacter bartletti</td>
<td>37.8</td>
<td>0.276</td>
<td>0.099</td>
<td>0.228</td>
</tr>
<tr>
<td>Lactococcus petauri</td>
<td>8.1</td>
<td>0.211</td>
<td>0.211</td>
<td>0.212</td>
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<tr>
<td>Mitsuokella multaciida</td>
<td>8.1</td>
<td>0.253</td>
<td>0.131</td>
<td>0.147</td>
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<tr>
<td>Butyrivibrio virosa</td>
<td>59.5</td>
<td>0.136</td>
<td>0.423</td>
<td>0.046</td>
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<tr>
<td>Blautia hydrogenotrophica</td>
<td>27</td>
<td>−0.399</td>
<td>0.014</td>
<td>−0.388</td>
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<tr>
<td>Paraprevotella xylaniphila</td>
<td>32.4</td>
<td>−0.310</td>
<td>0.062</td>
<td>−0.273</td>
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<tr>
<td>Ruminococcus gravis</td>
<td>59.5</td>
<td>−0.281</td>
<td>0.092</td>
<td>−0.198</td>
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<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>100</td>
<td>−0.074</td>
<td>0.662</td>
<td>−0.015</td>
</tr>
<tr>
<td>Bacteroides vulgatus</td>
<td>100</td>
<td>−0.147</td>
<td>0.385</td>
<td>−0.127</td>
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<tr>
<td><strong>BNT162b2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Eubacterium rectale</td>
<td>71.3</td>
<td>0.227</td>
<td>0.023</td>
<td>0.223</td>
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<tr>
<td>Roseburia faecis</td>
<td>76.2</td>
<td>0.214</td>
<td>0.031</td>
<td>0.215</td>
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<tr>
<td>Bacteroides thetaiotaomicron</td>
<td>95</td>
<td>0.191</td>
<td>0.056</td>
<td>0.204</td>
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<tr>
<td>Bacteroides sp OM05 12</td>
<td>13.9</td>
<td>0.101</td>
<td>0.317</td>
<td>0.088</td>
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<tr>
<td>Fusobacterium mortiferum</td>
<td>13.9</td>
<td>−0.167</td>
<td>0.096</td>
<td>−0.161</td>
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<tr>
<td>Clostridium saccharolyticum</td>
<td>25.7</td>
<td>−0.097</td>
<td>0.335</td>
<td>−0.085</td>
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<tr>
<td>Parabacteroides merdae</td>
<td>70.3</td>
<td>−0.276</td>
<td>0.005</td>
<td>−0.273</td>
</tr>
</tbody>
</table>

Partial Spearman correlation was used to adjust for age.

sVNT, surrogate virus neutralisation test.

**Gut microbiota composition is associated with vaccine-related adverse events**

None of the participants had serious adverse events that led to hospitalisation. Consistent with the previous report, a greater proportion of BNT162b2 vaccinees reported adverse events than CoronaVac vaccinees. Compared with CoronaVac vaccinees, more BNT162b2 vaccinees developed injection site pain, fatigue, fever, myalgia, drowsiness, headache and chills (table 1 and
We hypothesised that gut microbiome composition may associate with adverse events caused by vaccination. Among BNT162b2 vaccinees, participants who reported any adverse effect after the first dose of vaccination had a significant decrease in observed bacterial species richness \((p=0.011)\) (online supplemental figure S8). To assess whether specific baseline bacterial species were associated with vaccine-related adverse events, we applied partitioning around medoids clustering, which optimally clustered the gut microbiome composition of CoronaVac vaccinees into two distinct groups (online supplemental figure S9). Consistent with previous studies including Asian populations, two distinct gut microbiota clusters can be distinguished primarily by levels of \(Bacteroides\) and \(Prevotella\). The cluster associated with fewer adverse events after CoronaVac vaccination had a higher abundance of \(Prevotella\ copri\) and two \(Megamonas\) species (\(M.\ funiformis\) and \(M.\ hypermegale\)) in their baseline gut microbiome (online supplemental figure S9D). Similarly, baseline gut microbiota cluster enriched by \(F.\ copri\) and two \(Megamonas\) species was associated with fewer adverse events in BNT162b2 vaccinees (online supplemental figure S9F), indicating that these species may play an anti-inflammatory role in both vaccine groups. Interestingly, symptoms of fatigue after the first dose of vaccination were associated with a higher sVNT inhibition in BNT162b2 vaccinees but lower inhibition in CoronaVac vaccinees (online supplemental tables S10, S11).

**DISCUSSION**

To our knowledge, this is the first human study to show that baseline gut microbiota composition reflects immunogenicity and adverse events of COVID-19 vaccines. We found that different baseline bacterial species were associated with higher vaccine response. Specifically, the presence of an immunomodulatory bacteria, \(B.\ adolescentis\), was associated with higher neutralising

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**Figure 3** Association of baseline gut bacterial motility and fimbrial gene abundance with neutralising antibody response to CoronaVac and BNT162b2 vaccines at 1 month after second dose of vaccination. (A) Association of baseline gut bacterial motility (based on bacterial relative abundance and bacterial motility phenotype, the Methods section) with neutralising antibody response at 1 month after second dose of vaccination. (B) Association of flagellum-dependent cell motility \((GO:0071973)\) of baseline gut microbiome with neutralising antibody response at 1 month after second dose of vaccination. CoronaVac \((n=37)\): high responders, \(n=16\); low responders, \(n=21\). BNT162b2 \((n=101)\): highest tier, \(n=25\); others, \(n=76\). sVNT-10: sVNT level of 10-fold diluted plasma; sVNT-200: sVNT level of 200-fold diluted plasma. Correlation between motility/fimbrial gene abundance and sVNT data was examined using Spearman’s correlation test. Regression lines with 95% CI (grey area) were shown on scatter plots. Comparison between high versus low responder groups/highest tier versus others was made using Wilcoxon’s rank-sum test (two-sided). Elements on boxplots: centre line, median; box limits, upper and lower quartiles; whiskers, 1.5×IQR; points, outliers. sVNT, surrogate virus neutralisation test.
Figure 4  Weight status modifies the associations between baseline gut bacterial species and immune response in CoronaVac vaccinees at 1 month after second dose of vaccination. Immune response and ORs to be high responders separated by baseline bacterial abundance within weight strata (A) by *Bifidobacterium adolescentis* abundance. (B) By *Butyricimonas virosa* abundance (C) by *Adlercreutzia equolifaciens* abundance. (D) by *Asaccharobacter celatus* abundance. sVNT-10: sVNT of 10-fold diluted plasma. Sample size per group was indicated on the figure. Comparisons between subgroups were done using Dunn’s test (one sided) with FDR correction. Model 1: crude model. Model 2: adjusted for age. Reference group: NW with high bacterial abundance. Elements on boxplots: centre line, median; box limits, upper and lower quartiles; whiskers, 1.5×IQR; points, outliers. Each OR was presented as an orange dot with a bar showing the 95% CI. NW, normal weight; FDR, false discovery rate; OWOB, overweight or obese; sVNT, surrogate virus neutralisation test.
antibodies to CoronaVac suggesting that this bacteria may serve as an adjuvant to potentially overcome waning immunity of inactivated vaccine. Interestingly, abundance of \textit{P. copri} and two \textit{Megamonas} species were found to be more enriched in the baseline gut microbiome of participants with fewer adverse events after inactivated and mRNA vaccines.

Data from clinical studies and animal models suggest that gut microbiota composition plays a crucial role in modulating immune responses to vaccines but mechanisms by which the gut microbiota modulate immune responses to different vaccines in different populations are poorly understood. One potential mechanism is via the provision of natural adjuvants that enhances responses to vaccination. Commonly used vaccine adjuvants can directly or indirectly activate antigen-presenting cells such as dendritic cells via pattern recognition receptors (PRRS) like TLRs or NOD-like receptors. Flagellin and peptidoglycan produced by the gut microbiota can act as natural adjuvants to vaccines and can be sensed by PRRs. For example, TLR5-mediated sensing of flagellin has been shown to be required for optimal antibody response to influenza vaccine. Moreover, adhesion portion of bacterial fimbriae can induce innate immune system via TLR4, which is one of the immune activator proteins that has been proposed as an effective adjuvant for mRNA vaccines. Consistently, a higher relative abundance of bacteria with flagella and fimbriae \textit{(E. rectale} and \textit{R. fæcis}) was associated with a higher antibody response to mRNA vaccine. Microbiota-derived SCFAs enhance B cell metabolism and gene expression to support optimal homeostatic and pathogen-specific antibody responses. \textit{E. rectale} and \textit{R. fæcis} which produce butyrate may in part account for the elevated immunogenicity in highest-tier BNT162b2 responders. These bacterial species may play a beneficial role in vaccine immunogenicity serving as adjuvants through immunomodulatory TLR agonists. With waning antibody levels, whether microbiota-derived flagella/fimbriae or SCFAs can contribute to sustaining long-term COVID-19 immunisation efficacy deserves further investigation.

Consistent with previous reports supporting the immunomodulatory properties of \textit{B. adolescentis}, \textit{E. retale}, and \textit{R. fæcis}, we observed enriched \textit{B. adolescentis} in CoronaVac high-responders and increased abundances of \textit{E. retale}, \textit{R. fæcis}, \textit{B. thetaiotaomicron} and \textit{Bacteroides}, sp OM05-12 in BNT162b2 highest-tier responders. Moreover, reduced abundance of \textit{B. adolescentis} was identified in a single BNT162b2 vaccinee with low level of sVNT. Studies in infants have shown that the abundance of Bifidobacteria was associated with CD4 T cell responses and increased antibody responses to several vaccines. A recent study also reported that vaccine-induced T cell responses showed broad cross-reactivity against SARS-CoV-2 variants. Thus, gut microbiota-associated T cell responses would benefit not only vaccine immunogenicity but also cross-protection against multiple variants. Apart from higher abundance of \textit{B. adolescentis}, we also observed enriched carbohydrate metabolic pathways in CoronaVac high-responders. Carbohydrates play a crucial role in appropriate stimulation of the immune response, hence association of \textit{B. adolescentis} with higher antibody response could be explained by carbohydrate-driven immunopotentiation effects. These data indicate that vaccinees with a higher abundance of these beneficial bacteria may have an optimal immune response and potentially stronger protection.

Obesity is often associated with an adverse impact on the immune system. A recent study reported an inverse correlation between titre of antibody against SARS-CoV-2 spike protein and BMI in men who received BNT162b2 vaccine. Herein, we observed that immune response based on percent inhibition in sVNT correlated with BMI and the abundance of certain bacteria (\textit{B. adolescentis}, \textit{B. virosa}, \textit{A. equalifaciens} and \textit{A. celatus}) in CoronaVac vaccinees. These results suggest that beneficial effects of these bacteria on immune response to CoronaVac vaccine was modified by body weight. We identified baseline gut microbiota species (\textit{R. tongues, E. ventriosum} and \textit{S. salivarius}) that were associated with high-responders.

Gut microbiota cluster with a higher abundance of \textit{P. copri} and \textit{Megamonas} species was associated with less adverse events to both types of vaccines likely mediated through their anti-inflammatory functions. A higher prevalence of \textit{P. copri} has been reported in non-westernised populations. \textit{P. copri} also enhanced farnesoid X receptor signalling via modulating bile acid metabolism. Among the \textit{Megamonas} species, \textit{M. furiniformis} could ferment glucose into acetate and propionate which are beneficial for immune homeostasis whereas \textit{M. hypermegale} can regulate the balance between regulatory T cell and type 17 helper T cells (Th17).

Although BNT162b2 vaccine induced over 90% neutralising antibody response, waning of pike-antibody levels has been reported in infection-naïve individuals over a period of 3–10 weeks after second vaccine dose. Both Spike-antibody and neutralising antibody levels at 1 month after the second dose of mRNA vaccine also positively correlated with vaccine efficacy. Longitudinal assessment of the gut microbiota profile and antibody response beyond 1 month after the second dose of vaccines will further delineate how gut microbiota influences immunogenicity and long term durability of vaccine response.

In a prospective study, we found that baseline gut microbiota was significantly associated with immunogenicity and adverse events of COVID-19 vaccines. These novel findings have potential in facilitating microbiota-targeted interventions to optimise vaccine immune response and enhance durability of protection.

**Author affiliations**

1Department of Medicine and Therapeutics, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
2State Key Laboratory of Digestive Disease, Institute of Digestive Disease, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
3Li Ka Shing Institute of Health Sciences, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
4Microbiota I-Center (MagIC), The Chinese University of Hong Kong, Hong Kong SAR, China
5HKU-Pasteur Research Pole, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China
6School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China
7School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China
8Department of Anaesthesia and Intensive Care, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
9Jockey Club School of Public Health and Primary Care, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
10Department of Surgery, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong SAR, China
11Stanley Ho Centre for Emerging Infectious Diseases, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong SAR, China
12Twitter Siew C Ng @Siew_C_Ng, Ye Peng @YePeng21, Lin Zhang @linzhang8335, Kenneth KY Wong @tk_kennethwong, Francis KL Chan @FrancisKLChan and Hein M Tun @theunlab

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Contributors
SCN, LCZ, CKPM, FKLC and HMT conceived and designed the study. CKPM and CC carried out serology testing and analysis. AVL, SZ, YP, SZ and DLS recruited participants, JJLCL executed clinical protocols. HMT, YP, SZ and IJ performed bioinformatic and statistical analyses. SCN, YP, LCZ, FKLC, CKPM and HMT wrote the manuscript with input from all co-authors. HMT acts as the guarantor for this study and publication.

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Competing interests
The Chinese University of Hong Kong and The University of Hong Kong have filed a provisional patent application in connection with this work on which SCN, FKLC and HMT are inventors (US patent application no. 63/273,088). SCN, FKLC and SCN are the scientific co-founders and sit in the board of Directors of GenieBiome Ltd.

Patient consent for publication
Not applicable.

Ethics approval
The study was approved by The Joint Chinese University of Hong Kong – New Territories East Cluster Clinical Research Ethics Committee (The Joint CUHK-UST-RECREC) (2021.260) and The Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster (HKU/HA HKW) (UW 21–203). The study was conducted in accordance with the Declaration of Helsinki (1975) and Good Clinical Practice.

Provenance and peer review
Not commissioned; externally peer reviewed.

Data availability statement
Data are available in a public, open access repository. Quality-controlled and human DNA-removed sequence data are deposited in the European Nucleotide Archive under BioProject PRJEB48269. Additional datasets generated and/or analysed in this study are available from the corresponding author on reasonable request.

Supplemental material
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REFERENCES
Gut microbiota


