Exploring the impact of gut microbial metabolites on inactivated SARS-CoV-2 vaccine efficacy during pregnancy and mother-to-infant antibody transfer

The recent publication by Ng *et al* titled 'Gut microbiota composition is associated with SARS-CoV-2 vaccine immunogenicity and adverse events' provides valuable insights on gut microbiota in modulating immune responses to both inactivated (CoronaVac) and mRNA (BNT162b2) SARS-CoV-2 vaccines. The authors identified specific gut microbiota markers influenced immunity and vaccine efficacy, suggesting that microbiota-targeted interventions could potentially enhance vaccine effectiveness in adults.[1]

Expanding on Ng *et al*'s research, our study further investigates the associations between gut microbial taxa, metabolites and immune response to inactivated vaccines. Our findings establish a connection between maternal gut microbiota/metabolites and the efficacy of mother-to-infant antibody transfer, providing a potential protective strategy for infants against COVID-19 symptoms.

We conducted a prospective, observational investigation involving 97 vaccinated pregnant women in Guangdong, China, and profiled gut microbiota and metabolome using shotgun metagenomics and non-targeted metabolomics. All participants received two doses of inactivated SARS-CoV-2 vaccine, including CoronaVac and Sinopharm. Of these, 29 (29.9%) had negative neutralising antibody (NAb) levels, while 68 (70.1%) had positive NAb levels (online supplemental tables S1 and S2). Within the positive group, the median NAb level was 16, with a range from 8 to 192. Given the large variation in NAb levels within the positive group, we further divided it into the weak positive group (NAb median: 12, Q1–Q3: 8–12) and the strong positive group (NAb median: 32, Q1–Q3: 24–48). Subsequent analyses were mainly conducted using three-group comparisons. However, results from two-group comparisons are also provided in online supplemental materials.

In total, 2304 gut microbial species and 1 432 165 functional genes were characterised. *Anaeromyces*, *Bacteroides plebeius*, *Collinsella tanakaei*, *Actinomyces*, *Parabacteroides distasonis*, *Coprobacillus* and *Anaeromassilibacillus*, *Bifidobacterium longum*, *Erysipelatoclostridium ramosum, Streptococcus parasanguinis, Streptococcus salivarius* and several *Ruminococcus* species were more abundant in the NAb positive groups (linear discriminant analysis (LDA)>2, $p<0.05$; figure 1A,B and online supplemental figure S1). Interestingly, these microbiota are predominantly associated with short-chain fatty acids (SCFAs) production.[2] Notably, the most enriched microbial functional pathway in the NAb strong positive group was fatty acid degradation, which breaks down lipid triglycerides into glycerol and free fatty acids (figure 1C, online supplemental figure S2). This finding aligns with previous research that reported an enhanced immune response to respiratory influenza infection due to SCFAs produced by gut microbiota, further supporting our results.[3]

A total of 1292 metabolites were characterised by non-targeted metabolomics. Among them four prenol lipids/triterpenoids (oleanolic acid, lupenone, 4α-carboxy-4β-methyl-5α-cholesta-8,24-dien-3β-Ol and alpha-tocotrienol) exhibited significantly higher levels in faecal samples of the NAb strong positive group ($p<0.05$, false discovery rate (FDR)<0.1, table 1). Triterpenes are secondary metabolites derived from medicinal plants and micro-organisms with potential immunomodulatory effects.[4] Previous molecular-docking and animal studies revealed that certain triterpenoids could potentially inhibit the main protease of SARS-CoV-2 (S-CoV-2 Mpro) and hinder ACE2 inhibitory activity, thereby preventing SARS-CoV-2 infection.[5–7]

Through neural network analysis, we aimed to identify potential microbial sources of these triterpenes. Notably, *Actinomyces*, *Collinsella tanakaei*, *Bacteroides plebeius*, *Parabacteroides distasonis*, *Streptococcus* and *Ruminococcus* species that were enriched in the NAb positive group, demonstrated strong co-occurrence with triterpenes (figure 1D,E). This suggests that these gut bacteria may be responsible for triterpenoid production. Previous studies also reported a relationship between gut microbiota and triterpenoids, including the relation of *Bacteroides* to ginsenoside and *Ruminococcus* spp *PO1-3* to glycyrrhetic acid.[8 9] Collectively, triterpenoids or probiotics capable of producing triterpenoids may be used as potential inhibitors or vaccine adjuvant for COVID-19 treatment in the future.

A total of 63 women and their infants were followed up. COVID-19 infection was observed in 80.95% of women, with 64.71% experiencing symptoms for <1 week and an average maximum temperature of 38.4°C. Among infants, the prevalence of COVID-19 infection was 82.54%, and the average highest body temperature reached 38.8°C. We observed a negative correlation between the maternal NAb levels after vaccination and the highest body temperature of infants infected with COVID-19 ($p=0.030$). Additionally, maternal IgG antibody levels during pregnancy correlated positively with IgG antibody levels in umbilical cord blood (online supplemental table S7), suggesting a maternal–infant antibody transfer potentially through breast milk or the placenta.[10] This underscores the protective value of COVID-19 vaccination during pregnancy for newborns. Additionally, potential protective faecal metabolites homo-L-arginine ($p=0.026$) and vomilenine ($p=0.010$) in mother were positively correlated with maternal antibody levels and negatively correlated with the highest body temperature of newborns infected with SARS-CoV-2 (online supplemental table S4), suggesting a potential role for the mother's gut metabolites in modulating infant immune responses.

In conclusion, our multiomics study provides insights into the potential interplay between gut microbiota, metabolites and the efficacy of inactivated SARS-CoV-2 vaccine in pregnant women. We identified maternal gut microbiota species and their metabolites, particularly triterpenoids, which may be associated with vaccine efficacy and the overall immune response in both mothers and infants. However, it should be noted that these findings are observational, and further research is needed to confirm any causal relationships and to explore their potential implications for clinical practice and pharmaceutical development. Additionally, we acknowledge the limitation of our study being conducted at a single

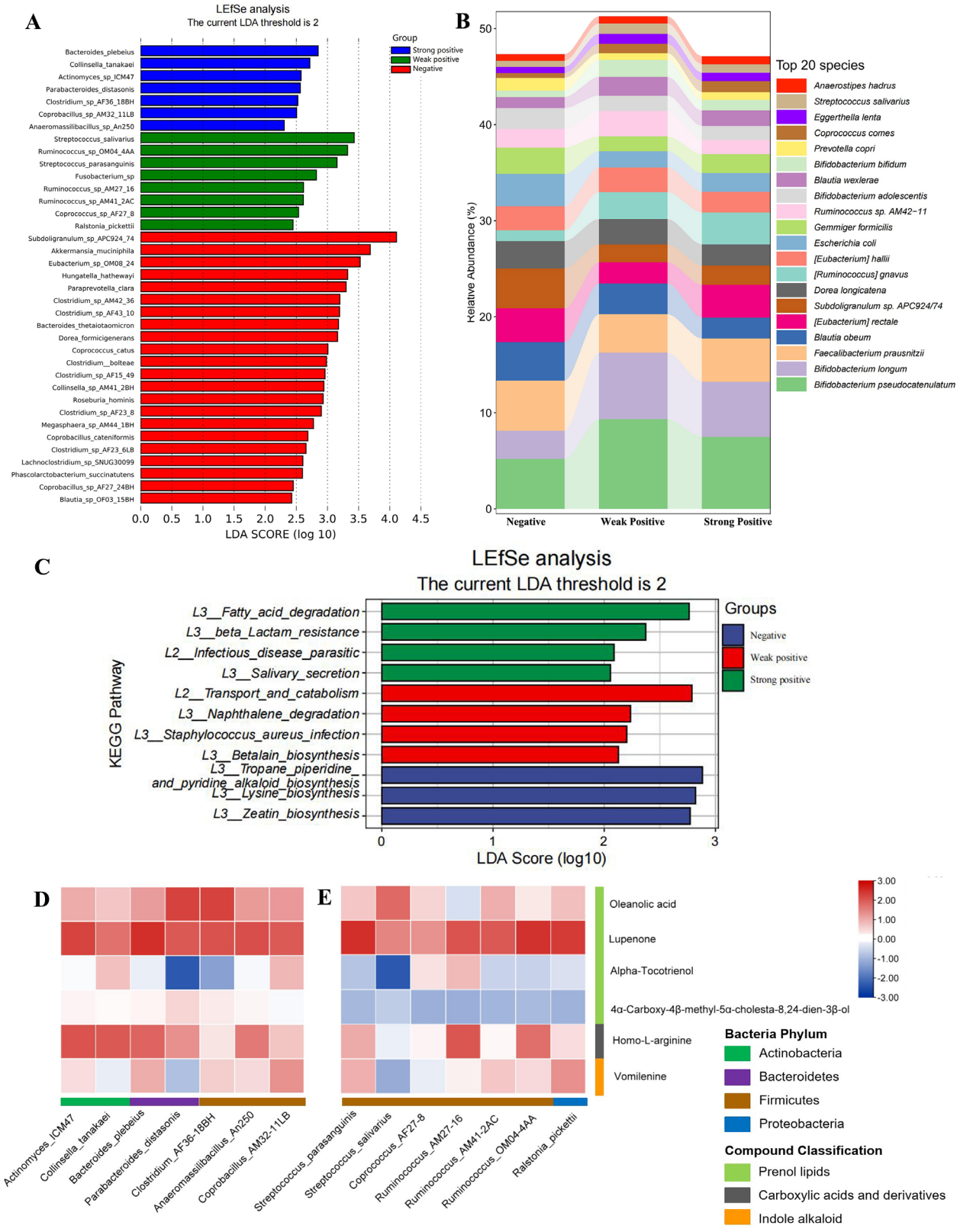

Figure 1 Enriched maternal gut microbial taxa, functional pathways and metabolites in three groups: negative neutralising antibody (NAb), weak positive NAb and strong positive NAb. (A) Enriched maternal gut microbial taxa were identified by LEfSe (linear discriminant analysis effect size), with only taxa with linear discriminant analysis (LDA) score>2 were presented in the figure. (B) The relative abundance of major gut microbial species. (C) Enriched gut microbial functional pathways. This analysis was also conducted using LEfSe, with only pathways exhibiting an LDA score >2 presented. Co-occurrence probability of protective metabolite enriched in the positive group and the featured microbial taxa in the strong (D) and weak (E) positive groups.

Table 1 Linear regression models for stool metabolites associated with neutralising antibodies or IgG

Metabolites	Compound classification	NAb			IgG		
		Coefficient	P value	95% CI	Coefficient	P value	95% CI
Alpha-Tocotrienol	Prenol lipids	1.87	**0.000**	(0.97 to 2.77)	2.28	**0.005**	(0.72 to 3.85)
4α-Carboxy-4β-methyl-5α-cholesta-8,24-dien-3β-ol	Prenol lipids/triterpenoids	1.29	**0.003**	(0.46 to 2.11)	0.95	0.19	(−0.48 to 2.37)
Lupenone	Prenol lipids/triterpenoids	1.12	**0.003**	(0.38 to 1.86)	1.21	0.06	(−0.05 to 2.48)
Oleanolic acid	Prenol lipids/triterpenoids	1.02	**0.001**	(0.43 to 1.62)	0.61	0.24	(−0.43 to 1.65)
Vomilenine	Indole alkaloid	0.60	**0.007**	(0.17 to 1.03)	0.24	0.51	(−0.50 to 0.99)
Homo-L-arginine	Carboxylic acids and derivatives	0.44	**0.002**	(0.16 to 0.71)	0.39	0.11	(−0.91 to 0.87)
Asymmetric dimethylarginine	Carboxylic acids and derivatives	0.64	**0.007**	(−1.11 to −0.18)	1.27	**0.001**	(−2.03 to −0.51)
12-OPDA	Fatty Acyls	1.01	**0.001**	(−1.60 to −0.41)	1.55	**0.003**	(−2.55 to −0.55)
(S)-Scoulerine	Protoberberine alkaloids and derivatives	1.03	**0.007**	(−1.78 to −0.28)	1.37	**0.033**	(−2.63 to −0.11)
1-Methylxanthine	Imidazopyrimidines	1.24	**0.009**	(−2.17 to −0.32)	1.59	**0.048**	(−3.15 to −0.17)
Prostaglandin E3	Fatty acyls	1.76	**0.017**	(−3.20 to −0.33)	1.67	**0.18**	(−4.11 to 0.77)
Imidazol-5-yl-pyruvate	Azoles	2.37	**0.010**	(−4.15 to −0.59)	3.59	**0.019**	(−0.66 to −0.60)

Potential protective gut metabolites were defined as those significantly enriched in the NAb positive group (p<0.05, false discovery rate (FDR)<0.1 and fold change >2). Four prenol lipids/triterpenoids (oleanolic acid, lupenone, 4α-carboxy-4β-methyl-5α-cholesta-8,24-dien-3β-OI and alpha-tocotrienol), one carboxylic acids (homo-L-arginine) and one indole alkaloid (vomilenine) were characterised as protective gut metabolites in the positive group. Previous studies reported that triterpenoids can potentially act as inhibitors of the SARS-CoV-2 main protease (S-CoV-2 Mpro), and therefore, may inhibit SARS-CoV-2 infection. Two fatty acyls (12-oxophytodienoic acid and prostaglandin E3), one protoberberine alkaloids ((s)-scoulerine), one carboxylic acids (asymmetric dimethylarginine), one imidazopyrimidines (1-methylxanthine) and one azoles (imidazol-5-yl-pyruvate) were enriched in the NAb negative group.
Bold indicates the siginificance level is p<0.05.

FDR, false discovery rate; LDA, linear discriminant analysis; NAb, neutralising antibody.

centre with a relatively small participant size.

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Acknowledgements We gratefully acknowledge the contribution of all pregnant women participating in the study, doctors and nurse of Guangzhou Baiyun District Maternal and Child Health Hospital and Shenzhen Longhua District Maternal and Child Health Hospital, and all the investigators of Guangdong Pharmaceutical University.

Contributors XF, BD, P-AC and AS drafted the manuscript and conducted statistical analyses. BC, XZeng, XH, YXia, CS and YG involved in the acquisition of data, analysis and interpretation. XZhang, WY, XX, ZL, YF, DK, NW, YXu, YS and QC provided critical review of the manuscript. All authors participated in the conceptualisation and conduction of the study. All authors read and approved the final version of the article.

Funding Guangdong Provincial Medical Science and Technology Research Fund (20201125123734390).

Competing interests None declared.

Patient consent for publication Consent obtained directly from patient(s).

Ethics approval This study involves human participants and the study protocol was approved by the Medical Ethics Review Board of the School of Public Health, Guangdong Pharmaceutical University (School of Public Health, Guangdong Pharmaceutical University, Medical Ethics (2021) No.01). Participants gave informed consent to participate in the study before taking part.

Provenance and peer review Not commissioned; externally peer reviewed.

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► Additional supplemental material is published online only. To view, please visit the journal online (http://dx.doi.org/10.1136/gutjnl-2023-330497).

XF and BD contributed equally.

To cite Fu X, Du B, Chen P-A, *et al*. *Gut* Epub ahead of print: [*please include* Day Month Year]. doi:10.1136/gutjnl-2023-330497

Received 15 June 2023
Accepted 30 August 2023

Gut 2023;**0**:1–4. doi:10.1136/gutjnl-2023-330497

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