Deconfounding the intestinal microbiome signature of responders versus non-responders to immune checkpoint inhibitor therapies

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INTRODUCTION

- In recent years, the **intestinal microbiome** has arisen as a potential target for **biomarker discovery**. A good example is the use of the fecal microbiome as a predictor of response to immune checkpoint inhibitor (ICI) cancer therapy.
- Yet, **biological, and technical cofounders** introduced by variation among publicly available studies **may hinder biomarker discovery** and **introduce biases** that affect the detection of response-specific signatures.
- **The aim of this study was to investigate the impact of study and host covariates in downstream analysis.**

METHODS

- A total of **489 baseline stool samples** from **nine 16S rRNA studies** investigating ICI response was merged in phyloseq v.1.38.0 after analysis with a standardized bioinformatics workflow.
- The dataset containing **species-level** information was clr-transformed and Aitchison distance matrix was determined. Permutational analysis of variance (PERMANOVA) was calculated with the vegan v.2.6.2 package and metadeconfoundR v.0.2.8 was used to investigate which microbial features associated with ICI response were being confounded.
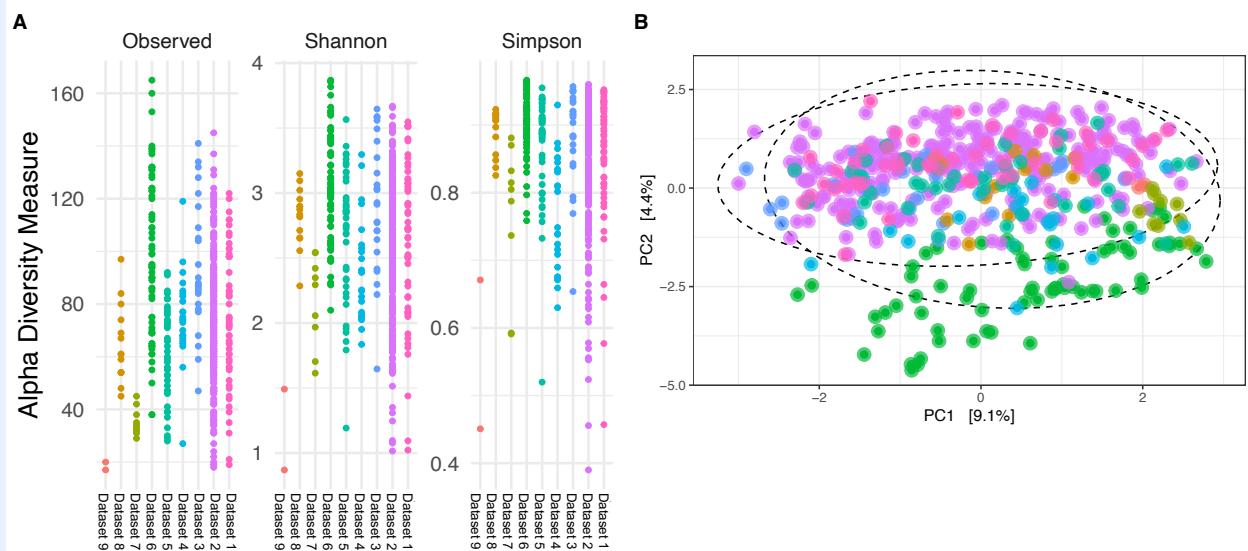- Differential abundance analysis was conducted with MaAsLin2 v.1.8.0.

RESULTS

- This analysis identified significant differences at the level of the structure of the intestinal microbiome between **responders (R) and non-responders (NR)** to ICI ($P = 0.001$).
- From the 959 species identified, *Bifidobacterium longum* and *Escherichia-Shigella* group were found to be confounded by **study, cancer type, prior therapy, hypervariable region, and country**. *Eubacterium hallii* was also found to be confounded by some of these variables, while *Faecalibacterium* was found to have a non-confounded signal.
- Interestingly, all these bacterial species were found to be differentially abundant between R and NR.

Figure 1. Alpha- and beta-diversity of stool microbiome samples from the 9 studies included in our analysis. (A) Number of observed ASVs, Shannon diversity and Simpson diversity indices did not reveal any differences between R and NR (P > 0.30). (B) Principal Component Analysis (PCA) of the CLR-normalized dataset at species level coloured by study. A significant difference between R and NR was found at the level of the microbial community structure ($P = 0.01$).

Table 1. Differentially abundant bacteria identified with MaAsLin2 as discriminating between R and NR. Results from metadeconfoundR show which of these are also biased by other variables in the metadata, and which of these covariates should be included in a multivariate analysis. [1]Reference level: Responders.

Taxonomy	MaAsLin2			MetadeconfoundR
	coef[1]	P-value	Q-value	Confounded?
g__Intestinimonas.s__massiliensis	0.27	0.01	0.23	x
g__Hydrogenoanaerobacterium.s__nan	0.24	0.01	0.10	x
g__Actinomyces.s__nan	-0.27	< 0.01	0.08	x
f__UCG.010.g__nan.s__nan	0.37	< 0.01	0.08	x
c__Clostridia.o__nan.f__nan.g__nan.s__nan	0.52	< 0.01	0.07	x
g__Lachnospiraceae.NC2004.group.s__nan	-0.45	< 0.01	0.03	x
f__Ruminococcaceae.g__nan.s__nan	0.53	< 0.01	0.03	x
g__Faecalibacterium.s__nan	0.83	< 0.01	0.03	Ok_sd
g__Bacteroides.s__thetaiotaomicron	-0.58	< 0.01	0.02	x
g__Streptococcus.s__nan	-0.75	< 0.01	0.01	x
g__Bifidobacterium.s__longum	-0.59	< 0.01	0.01	C: Dataset, Cancer, PriorTherapy, Hypervariable, Country
g__.Eubacterium..hallii.group.s__nan	-0.71	< 0.01	0.01	C: Stage, Dataset, Cancer, PriorTherapy
g__Escherichia.Shigella.s__nan	-0.71	< 0.01	0.01	C: Dataset, Cancer, PriorTherapy, Hypervariable, Country

DISCUSSION

These results highlight the need for caution when finding significant associations of e.g., disease with the intestinal microbiome, and reinforce the need for standardization of human gut microbiome studies and appropriate curation of metadata in public repositories.

CONCLUSION

Further studies will highly profit from the deconfounding of other host-associated variables, such as diet and other lifestyle factors. By accounting for covariates that can obscure microbial signatures associated with health and disease, bringing microbiome-based diagnostics into standard clinical practice may become a reality very soon.

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