INFLUENCE OF THE GUT MICROBIOME IN ISCHAEMIC STROKE RISK AND ISCHAEMIC STROKE OUTCOME

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Background and Aims

Gut microbiota composition is a modifiable factor associated with stroke risk and neurological outcome after stroke. However, most studies have been performed in animal models using 16S rRNA gene sequencing[1,2]. Our aim is to identify those bacteria and metabolic pathways associated with neurological outcome in the acute phase (onset and 24 hours after stroke) and functional outcome at 3 months after ischaemic stroke (IS).

Methods

Observational study in a tertiary stroke centre (January 2020 - April 2022). We analysed the first faecal sample after IS in 156 patients and 19 controls by using shotgun metagenomic sequencing. Gut microbiota diversity was assessed using α- and β-diversity. Linear discriminant analysis (LDA) Effect Size (LEfSe) was used to discriminate the significantly differential taxa in different comparisons: case/control, mild (NIHSS < 5)/moderate (NIHSS = 5-15)/severe (NIHSS > 15) stroke, neurological deterioration during the first 24h (worsening if ΔNIHSS $\geq$ 4), and functional outcome at 3 months (poor if mRS $\geq$ 3). $p < 0.05$ and logarithmic LDA score $\geq$ 2 was considered statistically significant. Analyses were adjusted for age, sex, baseline NIHSS and stroke subtype (TOAST classification). We performed Mendelian Randomisation (MR) analyses to assess causality between different bacteria and stroke risk and outcome.

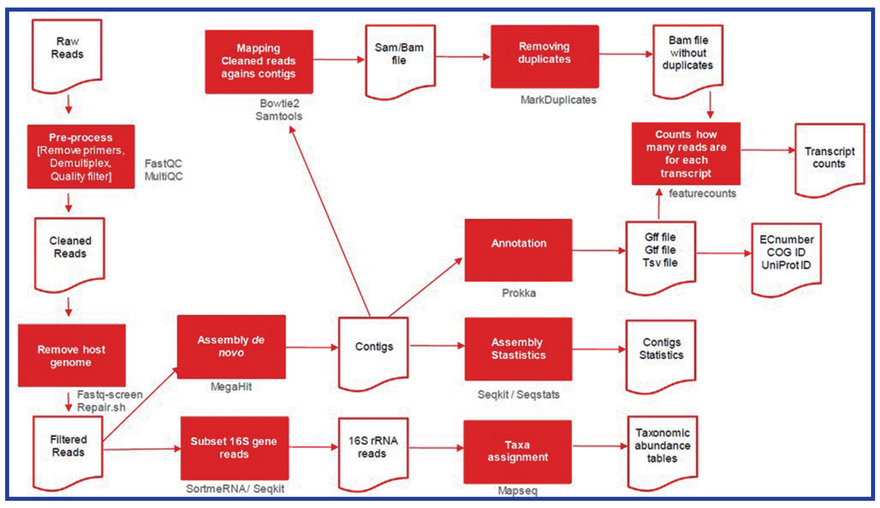

Figure 1. Sequence processing and analysis pipeline

Results

We have identified new bacteria associated with IS risk (*Fusobacterium*, $p=1.4x10^{-6}$), as well as confirmed some previously identified bacteria (*Lactobacillus*, $p=4.3x10^{-11}$)[3]. MR analyses have confirmed the causality between *Fusobacteriaceae* abundance and an increased risk of IS (IVW p=0.02). In addition, we identified new bacteria associated with increased stroke severity at the time of infarction (*Negativibacillus*, $p=5.3x10^{-5}$) and at 24h (*Lentisphaeria*, $p=1.9x10^{-5}$). We also identified multiple bacteria (*Pseudomonas*, $p=7.5x10^{-6}$; *Enterococcus*, $p=1.9x10^{-4}$) and metabolic pathways (16S rRNA (uracil1498-N3)-methyltransferase, $p=7.8x10^{-5}$; ethylbenzene degradation pathway, $p=1.1x10^{-3}$) associated with functional outcome 3 months after stroke.

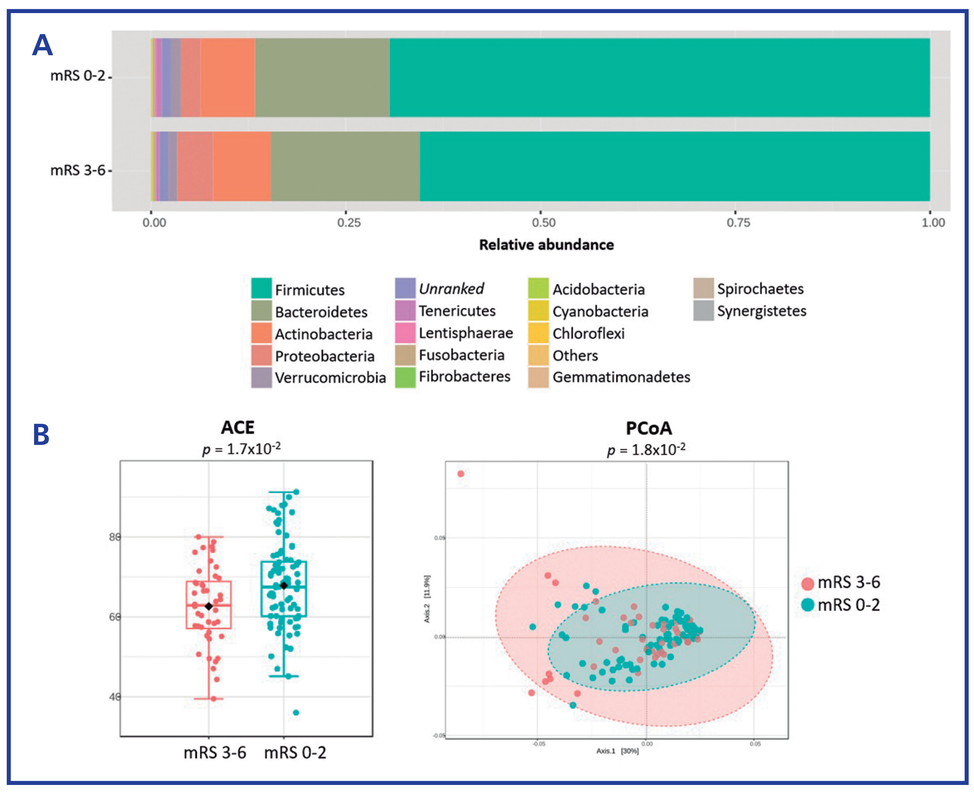

Figure 2. (A) Relative abundance of the main bacterial phyla in favorable and unfavorable outcome patients. (B) Differences between mRS 3-6 and mRS 0-2 patients in α- and β-diversity measured with the Abundance-based coverage estimator (ACE) index and the Principal Coordinates Analysis (PCoA), respectively.

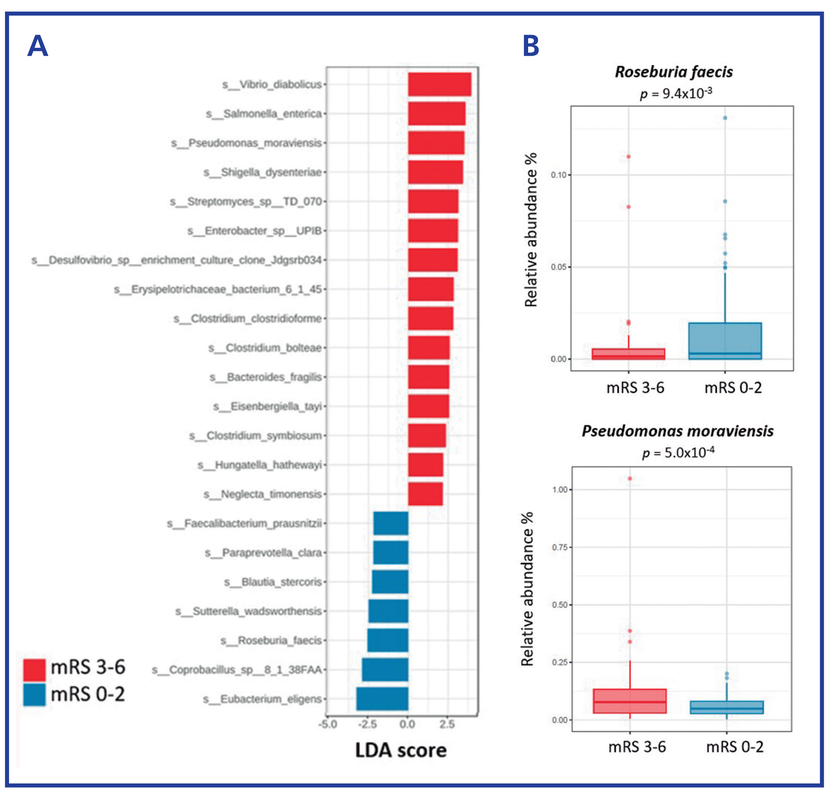

Figure 3. (A) Significantly discriminant taxa between the favorable and unfavorable outcome patients determined using LEfSe. (B) Relative abundances of *Roseburia faecis* and *Pseudomonas moraviensis*.

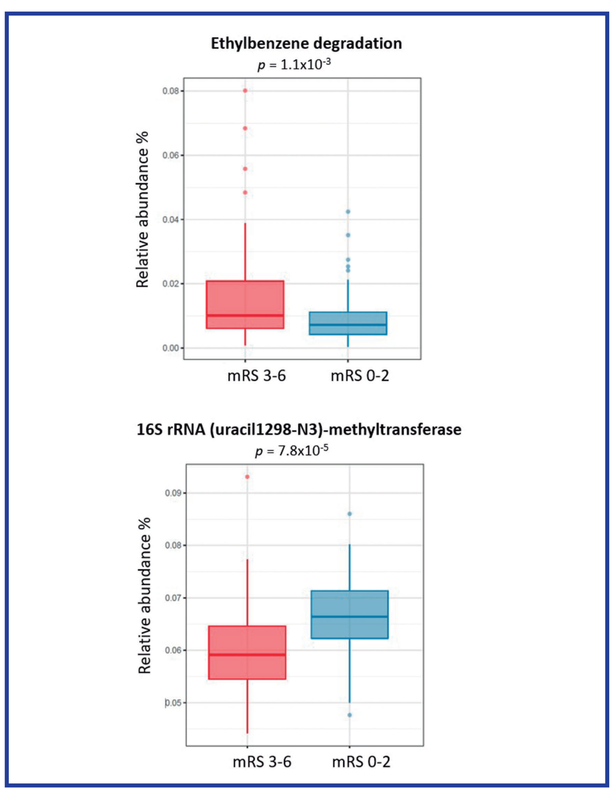

Figure 4. Relative abundances of ethylbenzene degradation and 16S rRNA (uracil1298-N3)-methyltransferase enzyme activity.

Conclusions

We have found multiple new bacteria and metabolic pathways associated with IS and its evolution in the acute phase and at 3 months post-stroke.

Disclosure and Funding

Nothing to disclose. This study has been funded by ISCIII (PI18/01338, PI20/00925), ERA-NET NEURON (AC19/00106), RICORS-ICTUS (RD21/0006/0006), FEDER, and NextGeneration EU.

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