

Supplementary Material

Altered Gut Microbiota in Older Adults with Mild Cognitive Impairment: a Case-Control study

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1 Supplementary Figures and Tables

1.1 Supplementary Tables

| | Age | MCI n=31 | | Normal n=65 | |
|-------|-----|-------------|--------|----------------|--------|
| | | Male | Female | Male | Female |
| Urban | <75 | 7 | 5 | 12 | 12 |
| | ≥75 | 5 | 3 | 7 | 12 |
| Rural | <75 | 3 | 5 | 5 | 6 |
| | ≥75 | 1 | 2 | 7 | 4 |

Supplementary Table 1. The exact numbers of cases and controls in each stratum.

| Reads | Input | Filtered % | Denoised Forward % | Denoised Reverse % | Merged % | Nonchimeric % |
|--------|----------|---------------|--------------------|--------------------|---------------|---------------|
| MCI | 155109.8 | 117792.3 77.5 | 116701.8 76.7 | 116793.4 76.8 | 111374.5 73.1 | 99771.4 65.5 |
| Normal | 122941.7 | 96982.6 80.5 | 95980.8 79.6 | 96087.4 79.7 | 91025.8 75.4 | 81194.4 67.4 |
| Total | 133329.3 | 103702.4 79.5 | 102671.9 78.7 | 102773.7 78.8 | 97596.7 74.7 | 87193.2 66.8 |

Supplementary Table 2. DADA2 denoising process of 16S rRNA reads.

| | MCI | | | | | |
|----------------------------|----------------|---------|----------------|---------|-----------------|---------|
| | Model 1* | | Model 2† | | Model 3‡ | |
| | β (SE) | p-value | β (SE) | p-value | β (SE) | p-value |
| <i>Richness indices</i> | | | | | | |
| Observed | -6.1 (16.6) | 0.71 | -7.5 (15.3) | 0.62 | -2.2 (16.4) | 0.89 |
| Chao1 | -5.8 (16.7) | 0.73 | -7.3 (15.4) | 0.64 | -2.2 (16.6) | 0.89 |
| <i>α-diversity indices</i> | | | | | | |
| Shannon | -0.1 (0.1) | 0.33 | -0.1 (0.1) | 0.30 | -0.05 (0.1) | 0.65 |
| Simpson | -0.003 (0.007) | 0.62 | -0.003 (0.007) | 0.65 | 0.0004 (0.0009) | 0.95 |
| InvSimpson | -3.3 (2.9) | 0.26 | -3.3 (2.8) | 0.24 | -2.7 (3.0) | 0.36 |

* Model 1: unadjusted

† Model 2: adjusted for age, gender, community (urban and rural)

‡ Model 3: adjusted for age, gender, community (urban and rural), education level (≤ 9 and > 9 years), hypertension

Supplementary Table 3. The association between MCI and richness / α -diversity indices.

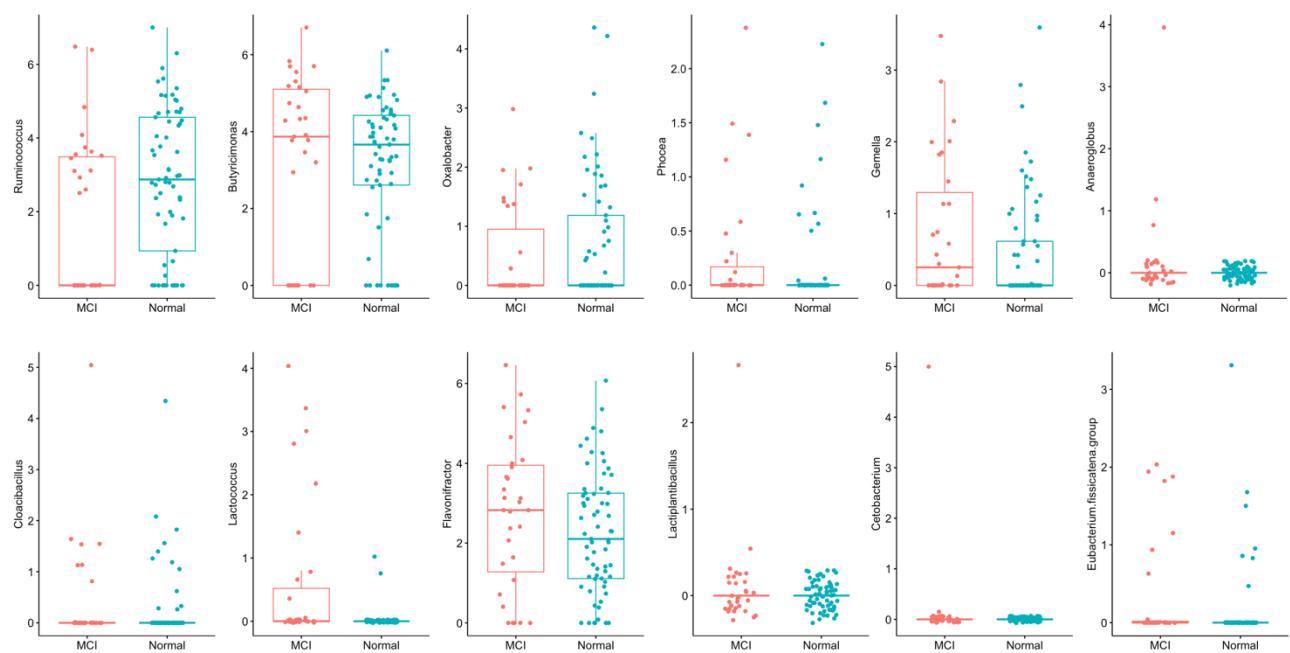
| PERMANOVA | | | | | | | Wd* | |
|-----------|----|------------------|--------|----------------|----------|--------------|-----------|-------------|
| | Df | Distance Method | Sum Sq | R ² | Pseudo-F | p-value | Statistic | p-value |
| MCI | 1 | Bray-Curtis | 0.317 | 0.011 | 1.089 | 0.25 | 1.07 | 0.25 |
| | | Jaccard | 0.390 | 0.011 | 1.046 | 0.23 | 1.04 | 0.28 |
| | | UniFrac | 0.414 | 0.018 | 1.709 | 0.006 | 1.57 | 0.02 |
| | | Weighted UniFrac | 0.020 | 0.010 | 0.921 | 0.47 | 0.79 | 0.68 |

Supplementary Table 4. PERMANOVA and Wd* test of beta-diversity

| Volume, cm ³ , mean ± SD | MCI n = 26 | Normal n = 64 | p-value |
|-------------------------------------|---------------|------------------|---------|
| Total brain | 999.2 ± 131.1 | 1035.0 ± 98.3 | 0.16 |
| Ventricle | 35.5 ± 11.8 | 32.5 ± 12.4 | 0.29 |
| Gray matter | 407.9 ± 48.2 | 422.8 ± 39.9 | 0.13 |
| White matter | 411.0 ± 66.7 | 431.5 ± 53.9 | 0.13 |
| Hippocampus | 7.5 ± 1.0 | 7.8 ± 0.8 | 0.15 |
| Entorhinal | 3.5 ± 0.9 | 3.7 ± 0.6 | 0.36 |
| Amygdala | 2.9 ± 0.5 | 3.0 ± 0.4 | 0.38 |

| Thickness, cm, mean ± SD | MCI n = 26 | Normal n = 64 | p-value |
|--------------------------|---------------|------------------|---------|
| AD-score | 2.5 ± 0.15 | 2.5 ± 0.11 | 0.53 |

Supplementary Table 5. Characteristics of brain structure between MCIs and cognitively normal adults.



Supplementary Figure 1. Centred-log ratio (CLR) transformed relative abundances of genera different between MCIs and cognitively normal adults, which were identified by linear discriminant analysis (LDA) effect size (LEfSe).