IMPACT OF THE MAINTENANCE IMMUNOSUPPRESSIVE THERAPY ON THE FECAL MICROBIOME OF RENAL TRANSPLANT RECIPIENTS: COMPARISON BETWEEN AN EVEROLIMUS- VERSUS A STANDARD TACROLIMUS-BASED REGIMEN

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Introduction and Objective

In the last years, gut microbiome, the complete genetic material of all the microbes living in our intestine, is emerging as an important factor influencing drug response [1]. However, at the moment, its role and characteristic in renal transplant recipients taking different immunosuppressant drugs is still not completely defined [2].

To clarify this issue, we employed an innovative Whole Metagenomic Functional Profiling to assess the composition and differences at taxonomic, functional, genetic and pathway levels of the gut microbiome in a group of renal transplant recipients in maintenance treatment with calcineurin inhibitors (TAC) and mTOR inhibitors (EVE).

Methods

Twenty stable adult deceased-donor renal transplant recipients at least 6 months post-transplant were included in this study after signing an informed consent form. Based on the maintenance immunosuppressive treatment, 9 patients were treated with EVE (Certican, Novartis, levels 3–6 ng/ml) and 11 with TAC (Advagraft, Astellas, levels 4–8 ng/ml) in combination with mycophenolate mofetil (MMF, Cell-Cept, Roche) 1000 mg b.i.d. and methylprednisolone 4 mg/day.

Nucleic acid isolation was performed with the MoBio PowerMag® Microbiome kit (Carlsbad, CA) according to manufacturer's guidelines and optimized for high-throughput processing. Samples were prepared for sequencing with the Illumina Nextera kit and quantified with Quant-iT dsDNA High Sensitivity assays. Libraries were pooled and run with 100 bp paired-end sequencing protocols on the Illumina HiSeq 2500 platform.

MetaPhlAn2 (Metagenomic Phylogenetic Analysis, version 2.0 [3]) was used for the taxonomic profiling of the metagenomic samples.

For functional analysis filtered DNA sequences were mapped against a reference database of all proteins within the KEGG database (version 75.0).

To evaluate the degree of variation of microbial community structure within a sample, we measure the alpha-diversity by employing the Shannon diversity index [4].

Results

Bionformatics revealed a poor intra- and inter-patients variability of gut microbiome in our enrolled renal transplant patients and only consumption of sweets contributed to differences in taxonomy, functional genes and pathways (PERMANOVA using distance metrics that contributed to beta diversity, p.value: 0.01) (Figures 1-3).

Additionally, 3 genes, involved in bacterial functional activities/response to antibiotics, discriminated CNI *versus* mTOR-I. Particularly, samples from CNI-treated patients were enriched in genes encoding for FliNY and Pilm, while those treated with mTOR-I demonstrated an up-regulation of MSRA (Figure 4).

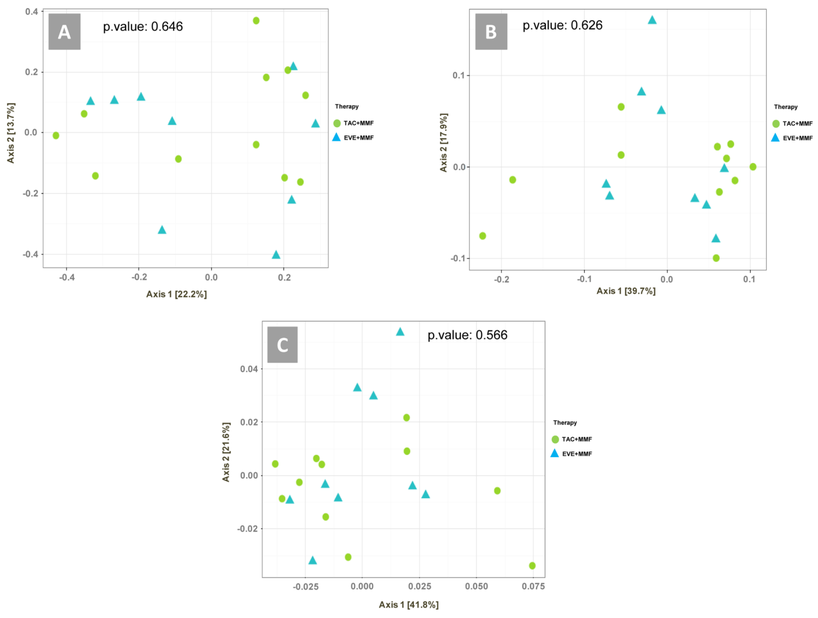

Figure 3. Beta diversity representation. The diagrams show the diversity between the two groups relatively to taxonomic distribution (A), functional genes (B) and pathways (C).

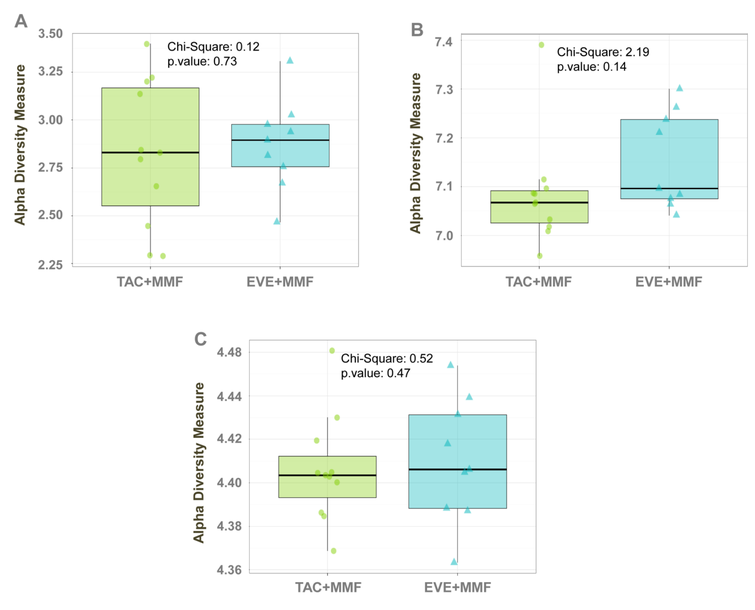

Figure 1. Alpha-diversity estimates. Each point shows a sample's pathway diversity calculated with the Shannon Diversity Index. Shannon index was used to analyze alpha-diversity within the sample. P-value calculated with Kruskal-Wallis rank sum test.

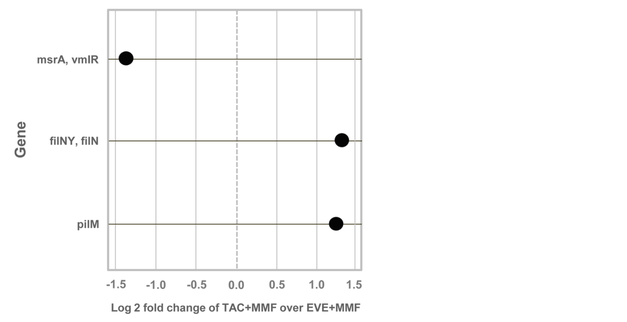

Figure 4. Differentially expressed genes between the two groups of patients. Each point represents a functional gene with FDR correct p value ≤0.05 and log 2 fold change ≥ 1.

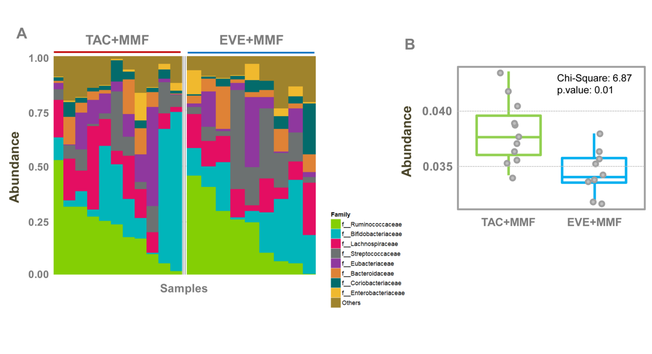

Figure 2. Proportional abundance. A) Plot shows the most abundant taxa at the Family level. B) Box plot represent the level of abundance of the Starch and surcrose metabolism pathway in the 2 study groups. P-value calculated with Kruskal-Wallis rank sum test.

Conclusions

Our study, revealed, for the first time, that transplant patients should carefully manage their diet (e.g., by reducing sweet aliments and employing probiotics) and it underlined that, in future, a larger employment of metagenomics could help clinicians to better customize antibiotic therapy minimizing resistance.

References

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DOI: 10.3252/pso.eu.54ERA.2017
ePosters supported by F. Hoffmann- La Roche Ltd.
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