Gut Microbiota Impact on Angolan Children with Sickle Cell Disease

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INTRODUCTION

Clinical manifestations of Sickle cell disease (SCD) are very heterogeneous, and the intestinal microbiome appears to be crucial in the modulation of inflammation, cell adhesion and induction of aged neutrophils, which are the main interveners of recurrent vaso-occlusive crisis.

Enterocyte injury, increased permeability, altered microbial composition, and bacterial overgrowth have all been documented as microbial and pathophysiologic changes in the gut microbiome of SCD patients in recent research studies.

Microbiota analysis in SCD populations will be essential to demonstrate the importance of specific bacteria and their function in this disease and provide new insights for attenuating symptoms and new drug targets.

AIM

Given this, our aim is to sequence by NGS bacterial 16S RNA gene in order to characterize the gut microbiome of SCD children and healthy siblings, as a control.

METHODS

Cross-sectional study included 72 Angolan children (36 SCD patients and 36 healthy siblings) aged between 3–14 years old.

Stool samples were collected using the DNA/RNA Shield Fecal Collection tubes (Zymo Research). Metagenomic DNA was extracted using ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research).

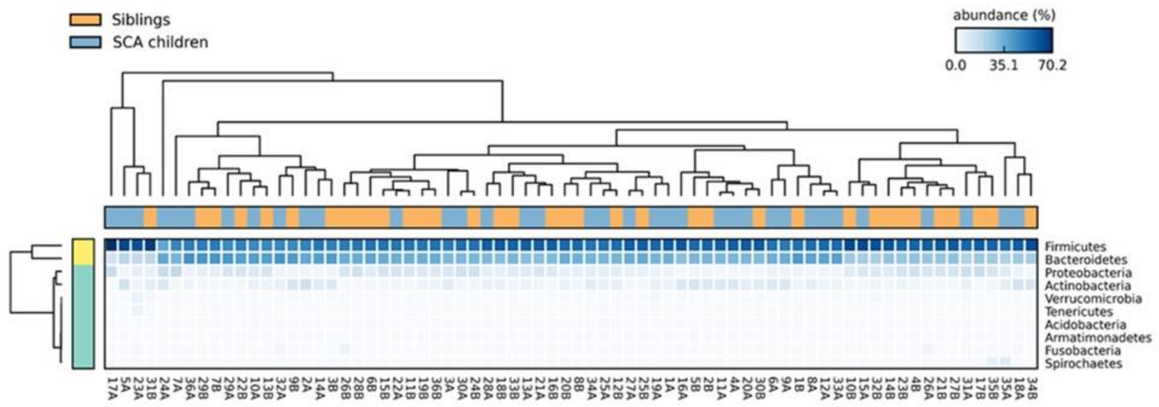
The V3-V4 hypervariable regions of the bacterial 16S rRNA gene sequences were amplified by following the Amplicon PCR protocol of the 16S Metagenomic Sequencing Library Preparation Illumina document and paired-end reads was performed on the NextSeq 550 instrument (Illumina).

The 16S Metagenomics app v1.1.0 within BaseSpace (Illumina) was used to perform taxonomic classification, which uses the RefSeq RDP 16S v3 database and the RDP Naïve Bayes taxonomic classification algorithm. Shannon indices for alpha diversity were calculated from OTU data and checked for statistical differences using Mann–Whitney U-test.

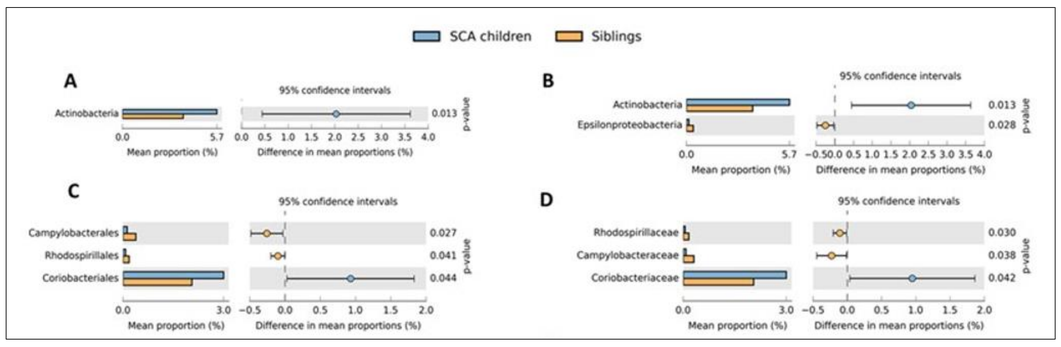
Clinical data were analyzed with SPSS version 27 (IBM) and significant differences between the patient's microbiota at various taxonomic levels were assessed using the Statistical Analysis of Metagenomic Profiles (STAMP) software package v2.1.3. The statistical significance was tested using Welch's test. P-values < 0.05 were considered as statistically significant.

RESULTS

In total, 5337 operational taxonomic units (OTUs) were identified and classified into 50 phyla, 123 classes, and 235 orders. A mean of 82.8% reads were classified for the genus level and an average of 1076 species were identified in our samples. Proteobacteria, Firmicutes, Bacteroidetes and Actinobacteria were the most abundant phyla of the total reads.



The SCA and control samples show some notable differences in microbiota relative abundance, as a percent of reads assigned, at different levels of classification.



Genus *Blautia* had significant differences in the proportion of sequences between the two groups (p=0.041). *Clostridium* cluster XI bacteria was more prevalent in the SCA children, whereas the siblings had higher numbers of *Aestuariispira*, *Campylobacter, Helicobacter*, all from Proteobacteria phylum, and *Polaribacter, Anaerorhabdus* both from Bacteroidetes phylum.

CONCLUSIONS

- No significant differences were found in alpha-diversity (Shannon Index).
- The SCD group had a higher prevalence of the genus *Clostridium XI*, a pathogenic bacteria associated with intestinal dysbiosis.
- Lower abundance of *Blautia* in the SCD children than in the sibling group. *Blautia* is known for its contribution in alleviating metabolic and inflammatory diseases.
- *Ruminococcus* abundance was higher in the group of patients with higher levels of HbF (better prognosis).

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