SHORT COMMUNICATION

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Shotgun Metagenomic Analysis of Cutaneous Microbiome in Patients with Atopic Dermatitis

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Abstract

Atopic dermatitis (AD) is a chronic complex inflammatory skin disorder. AD characterized by multiple contributing factors, such as impairment in skin barrier integrity, participation of either innate or adaptive immunity arms, in addition to the pivotal roles of cutaneous microbiome. The analysis of cutaneous microbiota was performed using shotgun metagenomic sequencing on an Illumina HiSeq platform in parallel with 16s rRNA genes using MiSeq sequencing. Two samples for each sequencing technique were collected from one child and one adult subjects with AD. Metagenomic data from 16srRNA was in accordance with those from shotgun sequencing. Taxonomic profile of samples at genus level showed overrepresentation of *Streptococcus* in child and *Staphylococcus* in adults. Species level analysis of reads revealed monoclonality of *Staphylococcus aureus* community in comparison to heterogeneity of *Staphylococcus epidermidis*. 16S rRNA gene based analysis was unable to provide comprehensive description of bacterial communities, while shotgun metagenomic analyses described the composition of microbiota with high resolution at different taxonomic levels especially subspecies and single nucleotide variants levels. Finally, further studies using shotgun metagenomic approaches will be required for characterization of microbiome in healthy and diseased subjects.

Keywords: Doping, Atopic dermatitis, Shotgun metagenomic analysis, Microbiome, 16srRNA.

1. Introduction

Atopic dermatitis is an allergic disease that impacts the life of about 20% of children and 2.3-4.9% between adults worldwide (Barbarot et al., 2018). Understanding the role of cutaneous microbiota in development as well as worsening of disease severity is essential for developing new treatment polices for elimination and prophylaxis against AD (Nakatsuji and Gallo, 2018).

Although discovery and exploration of human microbiome mainly depend on annotation of 16S rRNA hypervariable regions, these approaches in addition to traditional culture based unable to provide full description of underlying microbial communities (Zhang et al., 2011) . On the other hand, shotgun metagenomic tools enable imaging of entire members of human associated microbial communities including bacteria, fungi and viruses (Ranjan et al., 2016). The current study was

launched to identify the association between the composition of bacterial communities and AD in Egyptian patients.

2. Results

2.1 Taxonomic profiling of cutaneous microbiota

Bioinformatics sequence processing and annotation of 16s rRNA reads from two sequencing approaches were in agreement to each other at higher taxonomic levels from phylum to genus levels (Fig. 1).

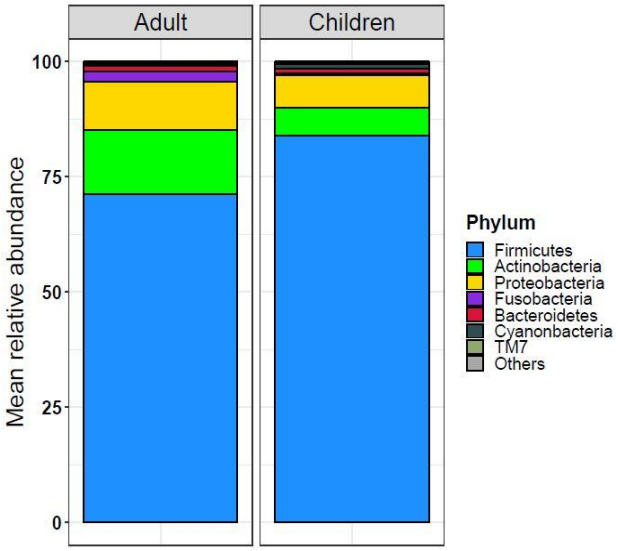

Figure 1. Mean relative abundance of most predominant phylum in cutaneous microbiota in patients with AD

At genus level, genera belonging to *Firmicutes* included *Staphylococcus*, *Streptococcus* and *Bacillus*, were detected with comparable abundance where *Staphylococcus* was enriched in both children and adults but with overrepresentation in adults. On the other hand *Streptococcus* was more significantly abundant in children compared to adults. Moreover, the representation of *Cutibacterium* and *Corynebacterium* significantly enriched in adults and diminished in children.

2.2 Species level and single nucleotide variant annotation of sequencing reads

Staphylococcus species, especially *S. hominis* and *S. haemoliticus* were assigned to the same clade. As compared to 16SrRNA sequencing, taxonomic assignment of assembled shotgun sequencing gets rid of misclassification of unassigned sequences where *Staphylococcus* species were classified to *S, aureus*, *S. epidermidis*, *S. haemoliticus*, *S. cohnii*, *S. aureus*, *S. caprae* and *S. hominis* (Fig. 2). Although Sequences assigned to *S. aureus* were annotated to 16 separate clades, *S. aureus* community in adult was predominant by single clade while children were predominant by another clade.

2.3 Nonbacterial inhabitants on skin associated with AD

Nonbacterial members of skin microbial communities included fungi and viruses that were detected with lower density compared to bacterial residents. Viral community was composed of predominate phages with few viruses. On the other hand fungal residents mainly represented by *Malasseziaceae* family that dominated by *M. globosa* and *M. restricta*, in addition to Aspergillaceae family.

3. Discussion

AD is a multifaced skin disorder with increased prevalence over the world including developed industrialized countries as well as developing countries (Ng et al., 2018, Goh et al., 2018). Deep understanding of cutaneous microbiome using advanced tools seems to provide a better explanation of the potential contributions of AD associated microbial communities in the pathogenesis of AD (Chng et al., 2016).

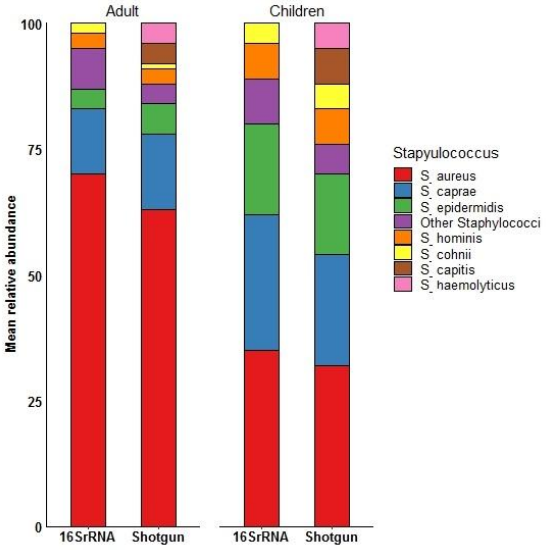

Figure 2. Mean relative abundances of *Staphylococcus* species in skin microbiota using 16S rRNA based sequencing and shotgun metagenomic analysis.

Age dependent shifts in skin microbiota previously reported and showed distinct age specific patterns in skin microbiota (Oh et al., 2012). Composition of microbiome in children and adults at higher taxonomic levels showed predominance of four major phyla *Firmicutes*, *Proteobacteria*, *Actinobacteria* and *Bacteroidetes* that previously reports with different rank where *Proteobacteria* was detected with lowered abundance, while *Cutibacterium* were found with increased proportions with progress in age as well as inverse correlation with *Staphylococcus* could be linked to maturation related physiological and endocrinologic changes (Wang et al., 2008, Abrahamsson et al., 2012, Kong et al., 2012).

S. aureus was detected with enriched abundance in adult and children. These findings are consistent with previous reports that defined *S. aureus* as a major player in pathogenesis of AD (Williams and Gallo, 2015, Totte et al., 2016).

In this study, *M. dermatis* and *M. sympodalis* were detected with enriched abundances in adult and child respectively which could be contributed to species switching in pathogenesis of AD (Han et al., 2018). The current study provides new insight about microbiota associated to AD in Egyptian patients using shotgun metagenomic approach.

4. References

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