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# 'Omics' approaches for studying the microbiome in Alopecia areata

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## ABSTRACT

Nowadays, the involvement of the microbiome in human health and many human diseases, including that strictly related to the scalp has been brought to the light. Indeed, more recently, authors highlighted the presence of a significant microbial shift both in nonscarring (Androgenetic alopecia and Alopecia areata) and scarring Alopecias. The advent of novel technologies together with the effort of many scientists in the microbiome field could provide in the nearest future a clearest framework about the strict relationship between human healthiness and symbiotic microorganism resident on different ecosystem of our body. In this view, the use of Omics approaches has to be considered as no longer negligible when studying the microbiome implication in human health and disease.

## INTRODUCTION

Hair is a fast-growing element, which needs a healthy ecosystem and a balanced supply of nutrients to grow correctly.<sup>1 2</sup> Hair growth occurs through different stages within which the hair follicle experiences first strong developments (anagen), followed by metabolic rest periods (catagen) as well as quite regressive or shedding processes (telogen).<sup>3</sup>

A disorder in hair follicle cycling evolves in hair loss and hair diameter and length modification.<sup>4</sup> Both exogenous and endogenous factors<sup>5</sup> may unbalance the cycle. Hormonal, blood, metabolic, or psychological changes as well as an imbalance in nutrients intake may exert a negative influence on the lifespan of hair.<sup>6</sup>

The most common disorders involving hair loss related to an unbalancing in hair follicle cycle are Alopecia androgenetica<sup>7</sup> and Alopecia areata (AA).<sup>8</sup>

AA is the second most common type of nonscarring alopecia with an incidence higher than 2% and a lifetime risk of 1.7% both in men and women.<sup>9</sup> AA is characterized by a complex and not completely understood etiology<sup>10 11</sup> and, among many recognized etiological factors,<sup>11 12</sup> immunity is reported playing an important role in its development.<sup>13–15</sup>

Gut microbiota has been recently reported playing a pivotal role in controlling immunomodulation in autoimmune conditions including AA.<sup>16</sup>

More recently, a role for the microbial population inhabiting the scalp has also been hypothesized in hair growth disorders.<sup>17</sup> Therefore, microbial dysbiosis has been deeply investigated in subjects affected by AA.<sup>18</sup> Propionibacterium and Staphylococcus have been reported as the main genera involved in microbial shift and a different microbial composition between healthy and AA affected subjects have also been reported at the different area surrounding hair follicle.<sup>18</sup>

Understanding the role of the microbial population inhabiting the human body, including the scalp, remain a challenge but the advent of novel technologies as the effort of many research groups on microbiome field could provide in the nearest future a clearest framework about the strict relationship between human healthiness and symbiotic microorganism resident on the different ecosystem of our body. Indeed, the use of omics techniques, especially metatranscriptomic, can help in the deep understanding the changes in microbiota composition related to hair growth disease such as AA and how these changes impact on different pathways and biological mechanisms related or not related to the disease pathophysiology.

## ILLUSTRATING THE PERSPECTIVE: STUDYING THE MICROBIOME BY OMICS APPROACH

Recently, the implementation of "omics" techniques is helping in overcoming the gap between the compositional and functional characterization of the human microbiome. Use of these techniques allows the study of interactions between the host and the microbiota and the role of specific genes, metabolites, and proteins produced by the microbial community and by which it plays a key role in healthiness or disease conditions.<sup>19</sup>

Microorganisms inhabiting a given ecosystem on our body, including the scalp, constitute a peculiar community in terms of populations but also in terms of functionality. In this view, omics techniques, in particular, metabolomics and proteomics can represent a useful tool for understanding the functional implications of possessing a defined microbial community on a specific site of the body.

This community is changing depending on the state of health or illness of the host. Changes



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in the microbial community have as a consequence on the functionality of the microbial populations and metabolites produced or in the pathways network activated by microorganisms.

The usefulness of omics techniques has been, recently, reported for a disease like cancer,<sup>20</sup> inflammatory bowel disease,<sup>21</sup> and gut dysbiosis in general.<sup>22</sup>

Used techniques are mainly metagenomics shotgun and metabolomics. The first allows the characterization of potential metabolites produced by a defined microbial population by mean of the analysis of the metagenome. The latter is the study of the real-time response to changes and is related to a quantitative measurement of metabolites produced.

Limitations for both techniques are mainly related to confounder factors such as, for example, interindividual variation, the analytical platform that can be used, and the complexity of computational analysis and their interpretation.<sup>23 24</sup>

Metabolites and small molecules are key mediators of the relationship between host and microbiota and, due to their involvement in the symbiotic cross-talk, quantifying them can help, for example, understanding the changes induced in host physiology or by a disease or a treatment.

For this reason, the use of the metabolomics approach to study the human microbiome is gaining increasing attention and becoming a no longer negligible issue when studying the link between the human microbiome and health or disease status in the host.

## OMICS IN ALOPECIA AREATA

Due to the poor information currently available as regards microbial communities inhabiting the scalp, the first aim of the research on this field is the taxonomic assignment of metagenomic reads obtained by 16S sequencing, to characterize the microbial composition of scalp ecosystem. Although it is essential to have this kind of information, the development of novel “omics” techniques opens to novel research approaches in which also the role of a microbial community inhabiting a given ecosystem could be deeply investigated.

Investigating the impact of the inhabiting microbial population on the scalp in terms of metabolites produced and biosynthetic pathways involved is of particular importance when considering the impact of macronutrients and micronutrients on hair physiology as the involvement of immunity and inflammation in scalp conditions related to hair growth, including AA.

In this optics, when approaching the study of scalp microbiome and related changes in presence of autoimmune diseases such as AA, researchers can no longer ignore changes also in the functionality of the microbial resident community.

This could be obtained, as widely described above, using metabolomics and metagenomics. In this sense, one useful metagenomic-based analysis is KEGG (Kyoto Encyclopedia of Genes and Genomes), a database resource for understanding high-level functions and utilities of the biological system, such as the cell, the organism, and the ecosystem, from molecular-level information, especially large-scale molecular datasets generated by genome sequencing and

other high-throughput experimental technologies.<sup>25</sup> Integrating genomic, chemical, and network information with cross-references to external deposited databases, KEGG analysis allows the representation of cellular functions in a given ecosystem at a high level of resolution.<sup>25</sup> KEGG categories that can be studied include, among others, pathways related to vitamins and amino acids metabolism, cellular response to external stress, membrane transport, replication and repair, carbohydrate metabolism, energy metabolism, and immunological response.

This integrated approach allows also the study of microbial community response to external environmental insults by studying bacterial chemotaxis, motility, and xenobiotics degradation.

In the presence of AA, the hair follicle ecosystem is characterized, for example, by strong hypoxia and collapse of immune-privilege.<sup>26</sup> Other modifications related to nutrients metabolism occur.<sup>1 27</sup> Most in general, the role of vitamins (vitamin A, B, C, D, E) and micronutrients such as iron, zinc, and selenium has been reported.<sup>1</sup> Vitamin D and Biotin deficiency have, for example, been reported in patients with AA<sup>2 28</sup> and are considered as ones of an etiological factor of this disorder. Given the role exerted by such vitamins and micronutrients in the hair cycle and immune defense, balancing a deep understanding of the association between their deficiency and hair loss is needed. This can be reached by large double-blind placebo-controlled trials as by means of omics techniques coupled to microbiome analysis.

AA of the scalp reflects in a changing in microbial population and as a consequence, in changes in metabolites, small molecules enzymes, that, once produced by bacteria, strongly interact with human cells and might exacerbate the disease. Microorganisms use enzymes to break down food and produce macronutrients and micronutrients for human cells.

For example, some of the human gut bacteria can produce biotin<sup>29</sup> and the balance between biotin-producing and biotin-eating bacteria could determine how much biotin is available also for the hair follicle. We can also assume a role for bacteria of the scalp as regards biotin metabolism and our preliminary findings<sup>30</sup> strongly suggest a strict correlation between microbial population inhabiting the scalp of AA subjects and biotin biosynthesis, as predicted by KEGG analysis. This is also true as regards other important pathways related to the immunomodulatory, inflammatory response, and other cellular pathways (eg: ion channels, pores ion channels, inorganic ion transport and metabolism, and so on).

## FUTURE DIRECTIONS

Nowadays, the relationship between metabolic pathways and the microbial community resident on the human scalp has not been deeply studied and studies have been mainly aimed at investigating the bacterial shift between normal resident scalp microbiome and scalp in pathological conditions in terms of taxonomic composition.

Given the strict symbiotic relationship existing between the microbiome and human host and its involvement in health as in disease, and ever deeper knowledge may be a weapon in the development of novel therapeutic approaches

in human disease involving microbiome, including that strictly related to the scalp.

In this view, omics techniques can certainly represent one of the most promising tools available for scientists of the field and the knowledge of how a different microbial community on the scalp can be related to hair growth conditions such as AA also in terms of functionality reveals itself as a useful tool also for the development of targeted and increasingly specific therapies, including “postbiotic” drugs.

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