



## Review

# Clinical Translation of Microbiome Research in Alopecia Areata: A New Perspective?

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**Abstract:** The continuous research advances in the microbiome field is changing clinicians' points of view about the involvement of the microbiome in human health and disease, including autoimmune diseases such as alopecia areata (AA). Both gut and cutaneous dysbiosis have been considered to play roles in alopecia areata. A new approach is currently possible owing also to the use of omic techniques for studying the role of the microbiome in the disease by the deep understanding of microorganisms involved in the dysbiosis as well as of the pathways involved. These findings suggest the possibility to adopt a topical approach using either cosmetics or medical devices, to modulate or control, for example, the growth of overexpressed species using specific bacteriocins or postbiotics or with pH control. This will favour at the same time the growth of beneficial bacteria which, in turn, can impact positively both the structure of the scalp ecosystem on the host's response to internal and external offenders. This approach, together with a "systemic" one, via oral supplementation, diet, or faecal transplantation, makes a reliable translation of microbiome research in clinical practice and should be taken into consideration every time alopecia areata is considered by a clinician.

**Keywords:** alopecia areata; microbiota; omics; postbiotics



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## 1. Alopecia Areata and Microbiota—A Short History

Alopecia areata (AA) is the second most common type of hair loss disorder in humans, with a reported lifetime incidence risk of higher than 2% [1,2].

It is classified as a non-scarring autoimmune hair disorder that manifests itself as a consequence of many etiological drivers mainly genetic, environmental, and immunological [3,4]. In particular, immunity is reported to play a pivotal role [5,6].

Immune privilege (IP) collapse of the hair follicle (HF) was first described by Billingham and Silvers in 1971 [7], by the suggestion that human HFs represent an IP site. Following this, Kang et al. [8] demonstrated the downregulation of the expression of several genes important for the immunosuppressive environment in AA subjects.

Currently, the central role of IP collapse in AA pathobiology has become widely accepted in the field, with current evidence suggesting that IP in the anagen HF can sequester antigens that are produced in hair bulbs from immune recognition [9,10].

The IP collapse is a recognised prerequisite for the development of AA [11], and the mechanisms behind have been highlighted [12]. One important key factor is the upregulation of MICA or ULBP3 which are NKG2D-activating ligands [13–16].

The continuous research advances in the microbiome field are changing clinicians' points of view about the involvement of the microbiome in human health and disease, including autoimmune diseases such as AA.

Recently, Scharschmidt et al. [17] suggested an involvement also of the HF's microbiome on IP given the modulatory activity of the microbiome itself on the balance between chemokine secreted by keratinocytes and IP guardian secretion.

Thousands of microorganisms inhabit the skin; they represent the so-called microbiota or “skin microflora” [18]. An even more new concept is the one represented by the “microbiome”, that to say the genome of all the microorganisms, symbiotic, and pathogenic, that interact with all cell types of the body [18].

The microbiome can be considered as a “meta-organism” whose symbiosis with the human host and dynamics in terms of both species and functions can contribute to maintaining homeostasis or causing disease [19]. Most importantly, recent studies are focusing on the pathogenic role of microorganisms as well as on their importance as regulators of metabolism, immunity, and environmental adaptation of the host [19].

The host immune system, in particular, is in a strictly symbiotic relationship with the microbiome [20–22]. Microbiota and the host immune system exert bidirectional control on each other [23].

On the one hand, the microbiome can control the development, training, and function of the host immune system. Additionally, the gut microbiota composition and functionality have been reported to have roles in controlling the immunomodulation in autoimmune conditions, including alopecia areata [24,25].

On the other hand, as a result of evolution, the host immune system, in turn, acts as a kind of “pacemaker” in the maintenance of the symbiotic relationship between the host and the microbiota [25]. A new, ever-expanding field is exploring the influence of the local microbiome (e.g., in the gut) on the immunity of a different and distant site such as skin, and this is per the existence of the “gut–skin axis” [4,26].

Regardless of the gut microbiome, several factors may lead to dysbiosis in the skin microbiome and, in the end, to the development of skin disease [18].

Primarily, each different skin site harbours a different microbiome, and this depends on several factors (e.g., water content, sebaceous glands, temperature, exposure to the environment, etc.) [27]. Increasing evidence demonstrates that the human microbiome plays a key role in human health and diseases [28–30]. For example, a modification of the microbiome is implicated in chronic inflammatory conditions, including atopic dermatitis in which the enhanced skin colonisation by *Staphylococcus aureus* has been reported, as well as the role of specific *S. aureus* strains [31]. Other studies reported higher colonisation by *Firmicutes* with a contextual reduction in *Actinobacteria* in psoriatic lesions [32].

Historically, the involvement of *Cutibacterium acnes* (formerly, *Propionibacterium acnes*) in acne development is well-established [33–35]; however, more recently, a shift in this paradigm has been reported, and the involvement of other microorganisms such as *S. aureus* and *S. epidermidis* has also been highlighted [36,37].

Although it shares some characteristics with that of the skin, the microbiome of the scalp possesses some unique characteristics, such as primarily the presence of terminal HF and the higher number of sebaceous glands and blood vessels and thickness [38].

Despite other body sites (e.g., gut and skin), the implication of the role of the microbiome in the pathogenesis of AA is still considered to be little explored.

Indeed, only in the last three years have authors begun to provide evidence of the additional role of the microbiome in the pathogenesis of AA [20,39–41]. Undoubtedly, the discovery of the possible contribution of the gut and skin microbiota to AA represents a new field to be further explored and open to a new therapeutic approach.

Over the past three years, there has been an increasing number of scientific reports investigating the microbial population, especially bacteria and fungi, inhabiting the scalp [39–44].

These studies aimed mainly at describing these populations in terms of compositions, and some researchers have also focused their attention on characterising how the microbiome differentiates in the different layers of the skin and perifollicular region, and how the microbiome changes because of a disease affecting the scalp or the follicular unit [38–43].

Primarily, the micro geography of the scalp of subjects affected by dandruff and seborrheic dermatitis has been reported [43,44] and has also been characterised in terms of functionality [43]. Using a large-scale amplicon and shotgun metagenome-based study

on 140 women from India, Saxena et al. reported the correlation between clinical and physiological parameters and microbial populations in subjects with dandruff [43]. In line with previous studies [45–48], *P. acnes* and *S. epidermidis* were found in the major bacterial communities. In particular, their ratio (*P. acnes*/*S. epidermidis*) was lower in dandruff scalps than their ratio in healthy scalps. *Malassezia restricta* and *Malassezia globosa* were the major fungal colonisers on the scalp, and a lower *M. restricta*/*M. globosa* ratio was associated with a healthy scalp. Therefore, a higher abundance of uncultured *Malassezia* sp. was reported in the dandruff scalp microbiome compared with that in the healthy scalp, and this positively correlates with dandruff-associated parameters.

Most interestingly, they also showed a significant correlation between the microbial population and some associated functional pathways, mainly those related to nutrient homeostasis. Amino acid and lipoic acid metabolic pathways were found to be more abundant in healthy scalps than in dandruff scalps. On the other hand, pathways implicated in cell–host interaction such as that for N-glycan biosynthesis were higher in the dandruff scalp [43].

These findings were further confirmed by the work of Grimshaw et al. on the Chinese population [44].

The role of the microbiome has also been reported in hair growth disorders such as non-cicatricial and cicatricial alopecia [39–41,49–51].

In our first study [40], preliminary results obtained from relatively small-sized samples highlighted, for the first time, the presence of microbial dysbiosis on the scalp of subjects affected by hair growth disorders such as AA, Lichen planopilaris, and, to a lesser extent, androgenetic alopecia.

We further investigated the role of the microbiome in AA and showed, for the first time, the alteration of the “equilibrium” of the composition of the scalp microbiota in subjects affected by AA both at the superficial epidermis level [39] and in the subepidermal compartment of the scalp [39]. Using 16S taxonomic analysis, we revealed that the dysbiosis of the bacterial population on the scalp of subjects affected by AA is mainly due to an increase in Actinobacteria and a decrease in Firmicutes [39], and *Propionibacterium* and *Staphylococcus* have been reported as the main genera involved [39]. The main differences were found in the subcutaneous and especially in the dermis layer [39].

At the same time, using an oximeter we also found a reduction in tissue oxygen in correspondence to the patches of AA, and this is in line with previous evidence [52]. Lack of oxygen would seem to favour the growth of extremely anaerobic species such as *Akkermansia muciniphila* and *Prevotella copri*. The first one, *A. muciniphila*, has been reported as a gut microbiota signature in psoriasis [53]. Recently, studies have reported the Th17 cell-mediated role of the gut microbiota in host homeostasis and immune response [53].

*P. copri* is a typical intestinal microbe, found as relevant in the pathogenesis of other chronic inflammatory autoimmune disorders such as rheumatoid arthritis, which can also affect the skin [54]. The identification of the above-mentioned species in the subepidermal compartment of the scalp of AA subjects opens new therapeutic approaches to the management of AA.

Indeed, the microbiota of the scalp may initiate or amplify AA via different mechanisms, including the metabolism of nutrients, but also by exacerbating inflammation and tissue damage. In turn, enhanced inflammation or the loss of the immune privilege, typical in AA, can alter microbial communities [55].

Therefore, microbial populations inhabiting the scalp are fundamental both concerning their numerical composition as well as in terms of their functionality. Indeed, the complex theatre of metabolites and small molecules produced by the resident microbiota can mediate the relationship with the host and can no longer be neglected.

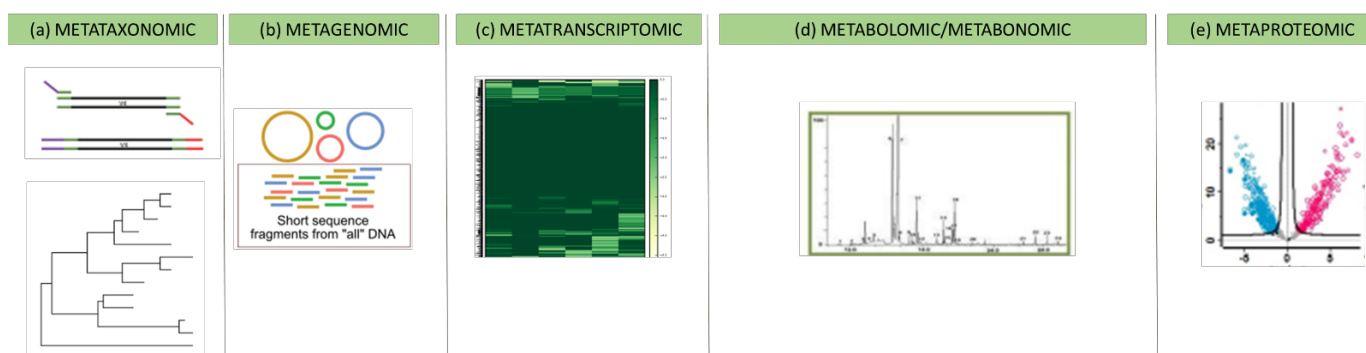
## 2. Studying the Microbiota by Omics Techniques

The study of the interaction between the host and the microbiota in terms of the specific genes, metabolites, and proteins they produced is presently feasible owing to the advent of “omics” techniques that allow delineating novel roles for microbes in health [56].

For many decades, the knowledge regarding microbiota was limited to culture-based techniques. Despite their limitations, however, they were fundamental for microbiota characterisation in the past and are still used today as the starting point for microbiome studies. The main limitations are that they are labour-intensive and not high-throughput, and they are unable to detect the virome and archaea.

“Omics” techniques fall under the great hat of systems biology techniques; they include metataxonomic, metagenomic, metabolomic, metabonomic, transcriptomic, and proteomic approaches and allow the comprehension of the microbiota inhabiting a given ecosystem, not only in terms of populations but also in terms of its functionality.

The metataxonomic approach is a high-throughput technology used to characterise the entire microbiota and create a metataxonomic tree, aiming to describe the relationships between all sequences obtained [57] (Figure 1a).



**Figure 1.** Schematic representation of omics techniques: (a) metataxonomic; (b) metagenomic; (c) metatranscriptomic; (d) metabolomic and metabonomic; (e) metaproteomic.

In the metagenomic technique, first used by Handelsman et al. [58], shotgun sequencing of DNA is used, followed by mapping to a reference database, to characterise the metagenome, to provide information on the potential function of the microbiota in a given ecosystem (Figure 1b).

The metabolomic approach, first introduced in 1998, refers to the determination of the metabolic profile in any given ecosystem [59] (Figure 1c). Further advancement of metabolomic analyses led to the development of the metabonomic approach [60], which is the analysis of metabolites in a more complex system (e.g., faecal samples, urines, plasma, etc.) (Figure 1c).

The metatranscriptomic method is the analysis of ribonucleic acid (RNA) using high-throughput sequencing. This technique helps provide information on the transcriptomic profile of the microbiota of a given ecosystem [57] (Figure 1d).

The last one, the metaproteomic approach, first described by Rodriguez-Valera in 2004 [61], refers to the characterisation of the entire protein content of a clinical sample at a given point in time without discriminating proteins from microbiota and the host (Figure 1e).

The advantages of the above-cited omic technologies, compared with more traditional methods, are higher sensitivity and resolution [62].

Indeed, the use of all these techniques found application, for example, in diseases such as inflammatory bowel disease (IBS) [63], general gut dysbiosis [64], and cancer [65], but its field of application was also recently extended to skin and hair diseases [41,66–68].

To understand the function of microbial communities in a given ecosystem, including skin and scalp, it is necessary to determine which genes and/or metabolites are expressed.

Metabolites and small molecules produced by the microbial population act as signal molecules for the communication between the host and microbiota [69]. Consequently, a change in the microbial population, for example, in the case of a disease, reflects changes in this system of communication and the pathways involved [70]. For this reason, the use of omics is becoming no longer a negligible issue in microbiome studies. This is particularly true in the study of the microbiome in AA considering the impact of macro and micronutrients on hair physiology as the involvement of inflammatory and immunological pathways.

Resident scalp microorganisms encounter epidermal cells, such as keratinocytes and immune system cells, by interacting with them and also changing their metabolic activity and transcriptomic framework.

These changes can be deeply investigated using the Kyoto Encyclopaedia of Genes and Genomes (KEGG) [71], a metagenomic-based analysis, allowing the representation of cellular functions in a given ecosystem at a high level of resolution. This analysis has been previously used by other authors in studies about skin microbiome [72–74].

KEGG analysis integrates current knowledge on molecular interaction networks and consists of three types of databases: pathways (PATHWAY database), genomic from genome projects (GENES/SSDB/KO databases), and biochemical compounds and reactions (COMPOUND/GLYCAN/REACTION databases).

Pathways related to the cellular response to external stress, membrane transport, vitamins, amino acid metabolism, carbohydrate metabolism, energy metabolism, replication and repair, and immunological response can be investigated by KEGG analysis [75].

### 3. Recent Advances in Microbiota in Alopecia Areata

Through an omics analysis, we recently compared microbial populations in dermis and hypodermis from biopsy samples of subjects affected by the AA with healthy subjects, and we identified a subset of 10 pathways that are significantly differentially regulated by the resident microbiota [41] (Table 1).

**Table 1.** PiCRUST-predicted pathways that significantly differed between AA and healthy samples.

KEGG Pathways	Diff. between Means	<i>p</i> -Value	Corrected <i>p</i> -Value
Flagellar assembly	0.164	$1.74 \times 10^{-4}$	0.014
Mineral absorption	−0.015	$2.77 \times 10^{-4}$	0.018
ABC transporters	−0.645	$6.60 \times 10^{-5}$	0.022
Bacterial chemotaxis	0.152	$4.04 \times 10^{-4}$	0.022
Cellular antigens	0.026	$6.58 \times 10^{-4}$	0.027
Glycosaminoglycan degradation	0.06	$6.02 \times 10^{-4}$	0.028
Lysosome	0.085	$1.15 \times 10^{-3}$	0.042
Sphingolipid metabolism	0.1	$1.35 \times 10^{-3}$	0.044
Cell division	0.024	$1.68 \times 10^{-4}$	0.045
Protein digestion and absorption	0.019	$1.44 \times 10^{-4}$	0.047

It was not possible to distinguish biopsy layer samples based on total pathway prediction abundances [43]. However, interestingly, among all the predicted pathways, we identified a subset of 10 pathways differently and significantly expressed in the dermis and hypodermis of AA subjects, compared with those in the healthy group.

The results of this previous study revealed eight pathways significantly increased in the AA group—namely, bacterial chemotaxis, flagellar assembly, glycosaminoglycan degradation, cellular antigens, lysosome and sphingolipid metabolism, cell division, protein digestion and absorption, and energy metabolism [41].

The only two pathways we found with a lower expression in the AA group were related to ABC transporter and mineral absorption. These findings are in line with previous studies showing the impact of the modifications in the metabolism of micro and macronutrients on a further risk associated with the development of AA, through the



dysregulation of immune cells and coenzyme-dependent enzyme function and imbalance in redox potential [76–78].

Most interestingly, in the AA group, a significant increase was found in the cellular antigenic pathway. This is important in the clinical study of AA since AA is a cell-mediated autoimmune hair loss disease for which autoantigen epitopes have been recently identified [79,80]. Additionally, microbial-derived antigens were reported to be responsible for the susceptibility of the follicle to an autoimmune attack, acting via the modulation microbial ecosystem of the scalp [79,80].

Using KEGG analysis, we also found a higher expression of the glycosaminoglycan (GAG) degradation pathway in AA samples [41], and this is in line with previous findings on the role of GAGs in HF integrity [81–85].

HF is a self-renewing organ, in which epithelial and dermal compartments interact with each other. In particular, the dermal part consists of the dermal papilla and connective tissue sheath rich in extracellular matrix compounds, including GAGs [81].

Several studies on HF revealed growth cycle reported a reduction in GAGs in the connective tissue around HF, in subjects with hair loss [81,82]. GAG reduction is clinically reflected in abnormalities in the structure and density of hair HFs, probably linked to keratin degradation [83,84].

Interestingly, the increase in GAG degradation pathways in the AA group corresponds to a significant decrease in ATP-binding cassette (ABC) transporters, the system deputy for the import of host mammalian GAGs [85], suggesting that GAGs are highly degraded but also less available for HF.

Therefore, pathways related to energy metabolism, mineral absorption, protein digestion, and absorption in the AA group, compared with the healthy group [28]. This confirms that the resident microbiota of the scalp can also affect its metabolic activity and macro and micronutrient supply. Many published studies reported the role of micronutrients in hair loss, including AA [76–78]. The impact of the microbiome on host metabolism is also pivotal because of the strict relation between metabolic and immunomodulatory pathways. Indeed, the metabolic intermediates in the cholesterol biosynthetic pathway can directly affect immunomodulatory pathways such as Janus kinase (JAK) signalling and peroxisome proliferator-activated receptor pathways [26]. Not least, metabolites from the resident microbiota can also impact HF's response to external and internal stress [26,86] and inflammatory cytokines released [87].

All these findings added important knowledge to the role of the microbiome in hair growth disorders, especially AA. However, to substantiate the robustness of these findings, studies with larger sample sizes and longer follow-up periods are required.

#### 4. Modulation of Microbiota in Alopecia Areata

A link between dysbiosis of the microbiome and AA has been previously reported but was first related to gut comorbidities such as celiac disease [88] and irritable bowel disease (IBS) [89].

A link between AA and gut microbiome could be hypothesised following recent evidence on the involvement of short-chain fatty acids, mainly butyric acid, derived from microbial metabolism in the gut and the differentiation of peripheral Treg lymphocytes [90]. These last are the important immunological mediators of AA [91].

Nair et al. suggested, for the first time, a role for the gut microbiome in the pathogenesis of AA in 2017, in C3H/HeJ mouse model [92]. Before grafting unaffected C3H mice with skin from AA-affected C3H mice with AA, a group of mice was treated with a broad-spectrum antibiotic cocktail, and one group was left untreated. Interestingly, only mice treated with antibiotics did not develop AA, and this suggests a role of the gut microbiome in AA development. In a clinical trial on 30 subjects affected by *alopecia universalis*, Moreno-Arrones et al. [93] reported specific bacterial biomarkers associated with the disease.

Two published case reports—one from Rebello et al. [94] and the other from Xie et al. [95]—reported hair growth in response to faecal microbiota transplantation (FMT) in subjects with AA, raising the option that an FMT may have an additional effect on the autoimmune reaction against the hair follicle in AA. Given the close link between gut dysbiosis and AA and the widespread efficacy of FMT, it might serve as a potential therapy for AA via the restoration of gut microbiota balance.

FMT consists of the infusion of stool from a healthy subject to another with presumed gut dysbiosis [96]. This treatment is gaining popularity, given its success in treating several gastrointestinal and extraintestinal diseases.

One of the main drivers of the gut microbiome is diet [97], and much research focused on establishing the role of diet in shaping the microbiome; this was also reported as regards the skin microbiome [98,99]. Indeed, diets have been reported to affect skin physiology and microbiome. Therefore, the existence of a gut–skin microbiome axis has been well-established for many dermatological diseases including atopic dermatitis [100].

The role of diet in AA can also be hypothesised [101,102]. Firstly, an unbalanced diet can lead to a lower intake of some macronutrients and micronutrients, and this can have an impact on gut microbial composition and functionality as well as the microbiome inhabiting the scalp up to the perifollicular region [101]. Hair is a fast-growing element, which needs a balanced supply of nutrients to grow correctly [9,102].

Under this assumption, targeted dietary approaches could represent a further therapeutic option or adjuvant therapy for AA subjects.

Even though there is presently no scientific basis for the hypothesis that, for example, the syndrome of “leaky gut” may be one of the etiological factors of AA, the latter shares some genetic characteristics with other autoimmune diseases (rheumatoid arthritis, diabetes I, celiac disease, systemic lupus erythematosus (SLE), psoriasis, multiple sclerosis, etc.) in which the association of the disease with an altered intestinal permeability has been demonstrated [103]. Suggested therapies include, among others, diet, additional nutritional supplementation with probiotics or botanical extract, a gluten-free or low-FODMAP diet, a low-sugar diet, or an antifungal diet.

Additionally, restoring the unbalanced gut microbiota with a healthy one via FMT could represent useful therapeutic options, as reported above [101].

The rationale behind the usefulness of “rebalancing” the gut microbiome is linked to the improvement of the absorption and synthesis of nutrients (amino acid/proteins, biotin, SCFAs, and vitamin D), which are also essential for hair follicle tropism and immunomodulation, and this, ultimately, results in hair regrowth [104]. However, current legislative limitations and the scarcity of clinical trials pose the need for larger studies before implementing FMT in the panel of the treatments currently available and approved for AA.

Recent advances in the microbiome field lead us to consider the intestine as not the only one responsible for the dysbiosis we found in subjects with AA.

Indeed, gut dysbiosis should not be considered a localised phenomenon. Alteration in the gut microecology as in the microbiome functionality may have consequences on the general inflammatory and immunological state of the host up to involving the scalp ecosystem and physiology [98].

The evidence of the modification of fundamental pathways of the immune and inflammatory responses and pathways involved in the transport of micronutrients, such as vitamin D (VitD) on the scalp of subjects affected by AA, could be the first stage for an evaluation of etiological agents important in the knowledge of AA.

Various autoimmune diseases have been associated with a deficiency in VitD [105]. Indeed, VitD is strictly linked with skin immunity since it can regulate lymphocyte functionality, dendritic cell maturation, and cytokine secretion [105]. In particular, it suppresses T-helper 1 and T-helper 17 cell formation, and this leads to a decrease in inflammatory cytokines [105].

A deficiency of this vitamin has also been reported in AA [106]. Therefore, topical calcipotriol has been successfully used for treating AA [107–109].

Using a meta-analysis, Lee et al. [110] reported a higher prevalence of VitD deficiency in AA subjects than in the control group. Most interestingly, according to several lines of evidence, the decrease in serum VitD levels significantly and inversely correlates with AA severity [111,112], also in children [113]. The production of IFN- $\gamma$  by human peripheral blood mononuclear cells (PBMCs) and CD4+ T cells was significantly decreased by Vit D [114–116]. This suggested that VitD might probably counteract the IP collapse in AA by modulating the production of IFN- $\gamma$  [106].

Therefore, the evidence of VitD deficiency in the AA could be a consequence of the decreased expression of some bacterial-related pathways. Indeed, human studies have reported significant associations between vitamin D and microbiome composition [117]. Therefore, as stated by Thompson et al. [76], a deficiency in micronutrients might also contribute to AA through dysregulation of immune cell function, DNA synthesis, and oxidative stress induction.

Our prediction concerned the significantly lower expression of pathways related to mineral absorption and ABC transporters in AA subjects, compared with the healthy group [41].

AA has been taken as a model of skin autoimmune pathology, but the same mechanism could involve other diseases such as LPP, psoriasis, etc.

We are also conducting specific clinical studies on bacterial overexpression of Jak-signal receptors and pattern recognition receptors (PRRs) to verify whether the control of dysbiosis can somehow reduce pathway alteration and in some way have a therapeutic effect, as indicated by the efficacy of the Jak antagonists. The data we currently have do not allow us to make any predictions; therefore, further research is necessary to understand if there is a more physiological way to control this pathological pathway.

Similarly, these data can represent a starting point for the study of a therapeutic approach with all those substances (topical or systemic) useful for controlling cutaneous and intestinal dysbiosis, whether they are prebiotics, specific probiotics, or postbiotics [118].

Indeed, the use of topically applied probiotics may be a natural, targeted treatment approach to several skin disorders in which a dysbiosis of the microbiome could be hypothesised, including AA. There is a growing amount of research reporting evidence of the health-promoting effect of probiotics on skin health [119]. Probiotics act primarily by increasing levels of beneficial bacteria or, indirectly, by influencing the immune system which, in turn, influences the host microbiome. However, the use of live bacteria on skin poses several challenges. For this reason, they are usually used in topical formulation in the form of non-viable microorganisms with the same probiotic activity and health benefits as viable microorganisms but safer than live probiotics [120]. They are the so-called “paraprobiotics” [120]. A new open perspective in the field is that represented by “postbiotics”. The term refers to molecules released by beneficial bacteria that are responsible for the beneficial effects of probiotics themselves [120,121]. They include peptides, enzymes, short-chain fatty acids (SCFAs), antimicrobial peptides (AMPs), polysaccharides, cell-surface proteins, vitamins, plasmalogens, and organic acids [121]. The mechanisms implicated in their health benefits are not fully elucidated, but a recent study reported different functional properties (e.g., antimicrobial, antioxidant, and immunomodulatory) [121].

In summary, paraprobiotics, and especially postbiotics, can represent novel frontiers in dermatology due to their high specificity of action on resident microbiota as of interaction with host cells, and their health-promoting effects can be helpful in many dermatological conditions. including alopecia areata, in which a role of the microbiome has been hypothesised.

## 5. Conclusions

From a clinical point of view, all these findings suggest the possibility to use a topical approach with either cosmetics or medical devices, to modulate or control, for example,



the growth of overexpressed species using specific bacteriocins or postbiotics or with pH control. This will favour, at the same time, the growth of beneficial bacteria which, in turn, can impact positively both the structure of the scalp's ecosystem and the host's response to internal and external offenders.

This approach, together with a more “systemic” one via oral supplementation, diet, or FMT, makes a reliable translation of microbiome research in clinical practice and should be taken into consideration every time AA is considered by a clinician.

**Author Contributions:** D.P., A.P. and F.R. conceived, designed, and wrote the original draft. D.P. and F.R. contributed to the conceptualisation and revision of the study. A.T. and G.G. contributed to the conceptualisation. F.R. finally approved the version to be published. All authors have read and agreed to the published version of the manuscript.

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## References

1. Odom, R.B.; Davidsohn, I.J.; William, D.; Henry, J.B.; Berger, T.G. Clinical diagnosis by laboratory methods. In *Andrews' Diseases of the Skin: Clinical Dermatology*; Elston, D.M., Ed.; Elsevier: Amsterdam, The Netherlands, 2006.
2. Dawber, R. Alopecia areata. *Monogr. Dermatol.* **1989**, *2*, 89–102.
3. Syed, S.A.; Sandeep, S. Alopecia areata: A review. *J. Saudi Soc. Dermatol. Dermatol. Surg.* **2013**, *17*, 37–45.
4. Borde, A.; Åstrand, A. Alopecia areata and the gut—the link opens up for novel therapeutic interventions. *Expert Opin. Ther. Targets* **2018**, *22*, 503–511. [[CrossRef](#)] [[PubMed](#)]
5. Brenner, W.; Diem, E.; Gschnait, F. Coincidence of vitiligo, alopecia areata, onychodystrophy, localized scleroderma and lichen planus. *Dermatologica* **1979**, *159*, 356–360. [[CrossRef](#)]
6. Hordinsky, M.; Ericson, M. Autoimmunity: Alopecia areata. *J. Investig. Dermatol. Symp. Proc.* **2004**, *9*, 73–78. [[CrossRef](#)]
7. Billingham, R.E.; Silvers, W.K. A biologist's reflections on dermatology. *J. Investig. Dermatol.* **1971**, *57*, 227–240. [[CrossRef](#)]
8. Kang, H.; Wu, W.Y.; Lo, B.K.; Yu, M.; Leung, G.; Shapiro, J.; McElwee, K.J. Hair follicles from alopecia areata patients exhibit alterations in immune privilege-associated gene expression in advance of hair loss. *J. Investig. Dermatol.* **2010**, *130*, 2677–2680. [[CrossRef](#)]
9. Leung, M.C.; Sutton, C.W.; Fenton, D.A.; Tobin, D.J. Trichohyalin is a potential major autoantigen in human alopecia areata. *J. Proteome Res.* **2010**, *9*, 5153–5163. [[CrossRef](#)]
10. Wang, E.H.C.; Yu, M.; Breitkopf, T.; Akhoundsadegh, N.; Wang, X.; Shi, F.T.; Leung, G.; Dutz, J.P.; Shapiro, J.; McElwee, K.J. Identification of Autoantigen Epitopes in Alopecia Areata. *J. Investig. Dermatol.* **2016**, *136*, 1617–1626. [[CrossRef](#)]
11. Bertolini, M.; McElwee, K.; Gilhar, A.; Bulfone-Paus, S.; Paus, R. Hair follicle immune privilege and its collapse in alopecia areata. *Exp. Dermatol.* **2020**, *29*, 703–725. [[CrossRef](#)]
12. Elsner, L.; Flügge, P.F.; Lozano, J.; Muppala, V.; Eiz-Vesper, B.; Demiroglu, S.Y.; Malzahn, D.; Herrmann, T.; Brunner, E.; Bickeböller, H.; et al. The endogenous danger signals HSP70 and MICA cooperate in the activation of cytotoxic effector functions of NK cells. *J. Cell. Mol. Med.* **2010**, *14*, 992–1002. [[CrossRef](#)] [[PubMed](#)]
13. Ito, T.; Ito, N.; Saato, M.; Hashizume, H.; Fukamizu, H.; Nickoloff, B.J.; Takigawa, M.; Paus, R. Maintenance of hair follicle immune privilege is linked to prevention of NK cell attack. *J. Investig. Dermatol.* **2008**, *128*, 1196–1206. [[CrossRef](#)] [[PubMed](#)]
14. Ito, T.; Meyer, K.C.; Ito, N.; Paus, R. Immune privilege and the skin. *Curr. Dir. Autoimmun.* **2008**, *10*, 27–52. [[PubMed](#)]
15. Petukhova, L.; Duvic, M.; Hordinsky, M.; Norris, D.; Price, V.; Shimomura, Y.; Kim, H.; Singh, P.; Lee, A.; Chen, W.V.; et al. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. *Nature* **2010**, *466*, 113–117. [[CrossRef](#)]
16. Xing, L.; Dai, Z.; Jabbari, A.; Cerise, J.E.; Higgins, C.; Gong, W.; de Jong, A.; Harel, S.; DeStefano, G.M.; Rothman, L.; et al. Alopecia areata is driven by cytotoxic T lymphocytes and is reversed by JAK inhibition. *Nat. Med.* **2014**, *20*, 1043–1049. [[CrossRef](#)]
17. Scharschmidt, T.C.; Vasquez, K.S.; Pauli, M.L.; Leitner, E.G.; Chu, K.; Truong, H.A.; Lowe, M.M.; Rodriguez, R.S.; Ali, N.; Laszik, Z.G.; et al. Commensal Microbes and Hair Follicle Morphogenesis Coordinately Drive Treg Migration into Neonatal Skin. *Cell Host Microbe* **2017**, *21*, 467–477.e5. [[CrossRef](#)]
18. Byrd, A.L.; Belkaid, Y.; Segre, J.A. The human skin microbiome. *Nat. Rev. Microbiol.* **2018**, *16*, 143–155. [[CrossRef](#)]

19. Jaspers, C.; Fraune, S.; Arnold, E.A.; Miller, D.J.; Bosch, T.C.G.; Voolstra, C.R. Resolving structure and function of metaorganisms through a holistic framework combining reductionist and integrative approaches. *Zoology* **2019**, *133*, 81–87. [CrossRef]
20. Migacz-Gruszka, K.; Branicki, W.; Obtulowicz, A.; Pirowska, M.; Gruszka, K.; Wojas-Pelc, A. What's New in the Pathophysiology of Alopecia Areata? The Possible Contribution of Skin and Gut Microbiome in the Pathogenesis of Alopecia—Big Opportunities, Big Challenges, and Novel Perspectives. *Int. J. Trichology* **2020**, *11*, 185–188. [CrossRef]
21. Belkaid, Y.; Hand, T.W. Role of the microbiota in immunity and inflammation. *Cell* **2014**, *157*, 121–141. [CrossRef]
22. Malys, M.K.; Campbell, L.; Malys, N. Symbiotic and antibiotic interactions between gut commensal microbiota and host immune system. *Medicina* **2015**, *51*, 69–75. [CrossRef] [PubMed]
23. Belkaid, Y.; Harrison, O.J. Homeostatic Immunity and the Microbiota. *Immunity* **2017**, *46*, 562–576. [CrossRef] [PubMed]
24. Wu, H.J.; Wu, E. The role of gut microbiota in immune homeostasis and autoimmunity. *Gut Microbes* **2012**, *3*, 4–14. [CrossRef] [PubMed]
25. Gallo, R.L.; Nakatsuji, T. Microbial symbiosis with the innate immune defense system of the skin. *J. Investig. Dermatol.* **2011**, *131*, 1974–1980. [CrossRef] [PubMed]
26. Naik, S.; Bouladoux, N.; Wilhelm, C.; Molloy, M.J.; Salcedo, R.; Kastenmuller, W.; Deming, C.; Quinones, M.; Koo, L.; Conlan, S.; et al. Compartmentalized control of skin immunity by resident commensals. *Science* **2012**, *337*, 1115–1119. [CrossRef]
27. Lee, Y.B.; Byun, E.J.; Kim, H.S. Potential Role of the Microbiome in Acne: A Comprehensive Review. *J. Clin. Med.* **2019**, *8*, 987. [CrossRef]
28. Huttenhower, C.; Gevers, D.; Knight, R.; Abubucker, S.; Badger, J.H.; Chinwalla, A.T.; Creasy, H.H.; Earl, A.M.; FitzGerald, M.G.; Fulton, R.S.; et al. Structure, function and diversity of the healthy human microbiome. *Nature* **2012**, *486*, 207–214.
29. Cogen, A.L.; Nizet, V.; Gallo, R.L. Skin microbiota: A source of disease or defence? *Br. J. Dermatol.* **2009**, *158*, 442–455. [CrossRef]
30. Zeeuwen, P.L.; Kleerebezem, M.; Timmerman, H.M.; Schalkwijk, J. Microbiome and skin diseases. *Curr. Opin. Allergy Clin. Immunol.* **2013**, *13*, 514–520. [CrossRef]
31. Edslev, S.M.; Agner, T.; Andersen, P.S. Skin Microbiome in Atopic Dermatitis. *Acta Derm. Venereol.* **2020**, *100*, adv00164. [CrossRef]
32. Thio, H.B. The Microbiome in Psoriasis and Psoriatic Arthritis: The Skin Perspective. *J. Rheumatol. Suppl.* **2018**, *94*, 30–31. [CrossRef] [PubMed]
33. Rocha, M.A.; Bagatin, E. Skin barrier and microbiome in acne. *Arch. Dermatol. Res.* **2018**, *310*, 181–185. [CrossRef] [PubMed]
34. Dréno, B.; Dagnelie, M.A.; Khammari, A.; Corvec, S. The Skin Microbiome: A New Actor in Inflammatory Acne. *Am. J. Clin. Dermatol.* **2020**, *21* (Suppl. 1), 18–24. [CrossRef] [PubMed]
35. Fitz-Gibbon, S.; Tomida, S.; Chiu, B.H.; Nguyen, L.; Du, C.; Liu, M.; Elashoff, D.; Erfe, M.C.; Loncaric, A.; Kim, J.; et al. Propionibacterium acnes strain populations in the human skin microbiome associated with acne. *J. Investig. Dermatol.* **2013**, *133*, 2152–2160. [CrossRef]
36. Dreno, B.; Martin, R.; Moyal, D.; Henley, J.B.; Khammari, A.; Seité, S. Skin microbiome and acne vulgaris: Staphylococcus, a new actor in acne. *Exp. Dermatol.* **2017**, *26*, 798–803. [CrossRef]
37. Claudel, J.P.; Auffret, N.; Leccia, M.T.; Poli, F.; Corvec, S.; Dréno, B. Staphylococcus epidermidis: A Potential New Player in the Physiopathology of Acne? *Dermatology* **2019**, *235*, 287–294. [CrossRef]
38. Tajran, J.; Gosman, A.A. Anatomy, Head and Neck, Scalp. [Updated 2021 Jul 26]. In *StatPearls [Internet]*; StatPearls Publishing: Treasure Island, FL, USA, 2022. Available online: <https://www.ncbi.nlm.nih.gov/books/NBK551565/> (accessed on 31 March 2022).
39. Rinaldi, F.; Pinto, D.; Marzani, B.; Rucco, M.; Sorbellini, E.; Giuliani, G. Human microbiome: What's new in scalp diseases. *J. Transl. Sci.* **2018**, *4*, 1–4.
40. Pinto, D.; Sorbellini, E.; Marzani, B.; Rucco, M.; Giuliani, G.; Rinaldi, F. Scalp bacterial shift in Alopecia areata. *PLoS ONE* **2019**, *14*, e0215206. [CrossRef]
41. Pinto, D.; Calabrese, F.M.; De Angelis, M.; Celano, G.; Giuliani, G.; Gobbetti, M.; Rinaldi, F. Predictive Metagenomic Profiling, Urine Metabolomics, and Human Marker Gene Expression as an Integrated Approach to Study Alopecia Areata. *Front. Cell. Infect. Microbiol.* **2020**, *10*, 146. [CrossRef]
42. Nakatsuji, T.; Chiang, H.I.; Jiang, S.; Nagarajan, H.; Zengler, K.; Gallo, R.L. The microbiome extends to subepidermal compartments of normal skin. *Nat. Commun.* **2013**, *4*, 1431. [CrossRef]
43. Saxena, R.; Mittal, P.; Clavaud, C.; Dhakan, D.B.; Hegde, P.; Veeranagaiah, M.M.; Saha, S.; Souverain, L.; Roy, N.; Breton, L.; et al. Comparison of Healthy and Dandruff Scalp Microbiome Reveals the Role of Commensals in Scalp Health. *Front. Cell. Infect. Microbiol.* **2018**, *8*, 346. [CrossRef] [PubMed]
44. Grimshaw, S.G.; Smith, A.M.; Arnold, D.S.; Xu, E.; Hoptroff, M.; Murphy, B. The diversity and abundance of fungi and bacteria on the healthy and dandruff affected human scalp. *PLoS ONE* **2019**, *14*, e0225796. [CrossRef] [PubMed]
45. Clavaud, C.; Jourdain, R.; Bar-Hen, A.; Tichit, M.; Bouchier, C.; Pouradier, F.; El Rawadi, C.; Guillot, J.; Ménard-Szczebara, F.; Breton, L.; et al. Dandruff is associated with disequilibrium in the proportion of the major bacterial and fungal populations colonizing the scalp. *PLoS ONE* **2013**, *8*, e58203.
46. Wang, L.; Clavaud, C.; Bar-Hen, A.; Cui, M.; Gao, J.; Liu, Y.; Liu, C.; Shibagaki, N.; Guéniche, A.; Jourdain, R.; et al. Characterization of the major bacterial-fungal populations colonizing dandruff scalps in Shanghai, China, shows microbial disequilibrium. *Exp. Dermatol.* **2015**, *24*, 398–400. [CrossRef]

47. Soares, R.C.; Camargo-Penna, P.H.; de Moraes, V.C.S.; De Vecchi, R.; Clavaud, C.; Breton, L.; Braz, A.S.K.; Paulino, L.C. Dysbiotic bacterial and fungal communities not restricted to clinically affected skin sites in dandruff. *Front. Cell. Infect. Microbiol.* **2020**, *6*, 157. [\[CrossRef\]](#)
48. Xu, Z.; Wang, Z.; Yuan, C.; Liu, X.; Yang, F.; Wang, T.; Wang, J.; Manabe, K.; Qin, O.; Wang, X.; et al. Dandruff is associated with the conjoined interactions between host and microorganisms. *Sci. Rep.* **2016**, *6*, 24877. [\[CrossRef\]](#)
49. Ho, B.S.; Ho, E.X.P.; Chu, C.W.; Ramasamy, S.; Bigliardi-Qi, M.; de Sessions, P.F.; Bigliardi, P.L. Microbiome in the hair follicle of androgenetic alopecia patients. *PLoS ONE* **2019**, *14*, e0216330. [\[CrossRef\]](#)
50. Huang, J.; Ran, Y.; Pradhan, S.; Yan, W.; Dai, Y. Investigation on Microecology of Hair Root Fungi in Androgenetic Alopecia Patients. *Mycopathologia* **2019**, *184*, 505–515. [\[CrossRef\]](#)
51. Filaire, E.; Dreux, A.; Boutot, C.; Ranouille, E.; Berthon, J.Y. Characteristics of healthy and androgenetic alopecia scalp microbiome: Effect of *Lindera strychnifolia* roots extract as a natural solution for its modulation. *Int. J. Cosmet. Sci.* **2020**. Epub ahead of print. [\[CrossRef\]](#)
52. Kato, H.; Kinoshita, K.; Saito, N.; Kanayama, K.; Mori, M.; Asahi, N.; Sunaga, A.; Yoshizato, K.; Itami, S.; Yoshimura, K. The Effects of Ischemia and Hyperoxygenation on Hair Growth and Cycle. *Organogenesis* **2020**, *16*, 83–94. [\[CrossRef\]](#)
53. Tan, L.; Zhao, S.; Zhu, W.; Wu, L.; Li, J.; Shen, M.; Lei, L.; Chen, X.; Peng, C. The *Akkermansia muciniphila* is a gut microbiota signature in psoriasis. *Exp. Dermatol.* **2017**, *27*, 144–149. [\[CrossRef\]](#) [\[PubMed\]](#)
54. Pianta, A.; Arvikar, S.; Strle, K.; Drouin, E.E.; Wang, Q.; Costello, C.E.; Steere, A.C. Evidence of the Immune Relevance of *Prevotella copri*, a Gut Microbe, in Patients with Rheumatoid Arthritis. *Arthritis Rheumatol.* **2017**, *69*, 964–975. [\[CrossRef\]](#) [\[PubMed\]](#)
55. Azzawi, S.; Penzi, L.R.; Senna, M.M. Immune Privilege Collapse and Alopecia Development: Is Stress a Factor. *Skin Appendage Disord.* **2018**, *4*, 236–244. [\[CrossRef\]](#) [\[PubMed\]](#)
56. Sharon, G.; Garg, N.; Debelius, J.; Knight, R.; Dorrestein, P.C.; Mazmanian, S.K. Specialized metabolites from the microbiome in health and disease. *Cell Metab.* **2014**, *20*, 719–730. [\[CrossRef\]](#) [\[PubMed\]](#)
57. Marchesi, J.R.; Ravel, J. The vocabulary of microbiome research: A proposal. *Microbiome* **2015**, *3*, 31. [\[CrossRef\]](#)
58. Handelsman, J.; Rondon, M.R.; Brady, S.F.; Clardy, J.; Goodman, R.M. Molecular biological access to the chemistry of unknown soil microbes: A new frontier for natural products. *Chem. Biol.* **1998**, *5*, R245–R249. [\[CrossRef\]](#)
59. Oliver, S.G.; Winson, M.K.; Kell, D.B.; Bagan, F. Systematic functional analysis of the yeast genome. *Trends Biotechnol.* **1998**, *16*, 373–378. [\[CrossRef\]](#)
60. Nicholson, J.K. Global systems biology, personalized medicine and molecular epidemiology. *Mol. Syst. Biol.* **2006**, *2*, 52. [\[CrossRef\]](#)
61. Rodriguez-Valera, F. Environmental genomics, the big picture? *FEMS Microbiol. Lett.* **2004**, *231*, 153–158. [\[CrossRef\]](#)
62. Kitano, H. Systems biology: A brief overview. *Science* **2002**, *295*, 1662–1664. [\[CrossRef\]](#)
63. Liu, S.; Si, C.; Yu, Y.; Zhao, G.; Chen, L.; Zhao, Y.; Zhang, Z.; Li, H.; Chen, Y.; Min, L.; et al. Multi-omics Analysis of Gut Microbiota and Metabolites in Rats with Irritable Bowel Syndrome. *Front. Cell. Infect. Microbiol.* **2019**, *9*, 178. [\[CrossRef\]](#) [\[PubMed\]](#)
64. Malla, M.A.; Dubey, A.; Kumar, A.; Yadav, S.; Hashem, A.; Abd Allah, E.F. Exploring the Human Microbiome: The Potential Future Role of Next-Generation Sequencing in Disease Diagnosis and Treatment. *Front. Immunol.* **2019**, *9*, 2868. [\[CrossRef\]](#) [\[PubMed\]](#)
65. Mullish, B.H.; Osborne, L.S.; Marchesi, J.R.; McDonald, J.A. The implementation of omics technologies in cancer microbiome research. *Ecanermedicalscience* **2018**, *12*, 864. [\[CrossRef\]](#) [\[PubMed\]](#)
66. Blumenberg, M. SKINOMICS: Transcriptional Profiling in Dermatology and Skin Biology. *Curr. Genom.* **2012**, *13*, 363–368. [\[CrossRef\]](#) [\[PubMed\]](#)
67. Oliva, M.; Renert-Yuval, Y.; Guttman-Yassky, E. The ‘omics’ revolution: Redefining the understanding and treatment of allergic skin diseases. *Curr. Opin. Allergy Clin. Immunol.* **2016**, *16*, 469–476. [\[CrossRef\]](#)
68. He, J.; Jia, Y. Application of omics technologies in dermatological research and skin management. *J. Cosmet. Dermatol.* **2022**, *21*, 451–460. [\[CrossRef\]](#)
69. Jansma, J.; El Aidy, S. Understanding the host-microbe interactions using metabolic modeling. *Microbiome* **2021**, *9*, 16. [\[CrossRef\]](#)
70. Cho, I.; Blaser, M.J. The human microbiome: At the interface of health and disease. *Nat. Rev. Genet.* **2012**, *13*, 260–270. [\[CrossRef\]](#)
71. Oh, J.; Byrd, A.L.; Park, M.; NISC Comparative Sequencing Program; Kong, H.H.; Segre, J.A. Temporal Stability of the Human Skin Microbiome. *Cell* **2016**, *165*, 854–866. [\[CrossRef\]](#)
72. Li, Z.; Xia, J.; Jiang, L.; Tan, Y.; An, Y.; Zhu, X.; Ruan, J.; Chen, Z.; Zhen, H.; Ma, Y.; et al. Characterization of the human skin resistome and identification of two microbiota cutotypes. *Microbiome* **2021**, *9*, 47. [\[CrossRef\]](#)
73. Barnard, E.; Shi, B.; Kang, D.; Craft, N.; Li, H. The balance of metagenomic elements shapes the skin microbiome in acne and health. *Sci. Rep.* **2016**, *6*, 39491. [\[CrossRef\]](#) [\[PubMed\]](#)
74. Kanehisa, M.; Goto, S.; Kawashima, S.; Okuno, Y.; Hattori, M. The KEGG resource for deciphering the genome. *Nucleic Acids Res.* **2004**, *32* (Suppl. 1), D277–D280. [\[CrossRef\]](#) [\[PubMed\]](#)
75. Langille, M.; Zaneveld, J.; Caporaso, J.G.; McDonald, D.; Knights, D.; Reyes, J.A.; Clemente, J.C.; Burkepile, D.E.; Vega Thurber, R.L.; Knight, R.; et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat. Biotechnol.* **2013**, *31*, 814–821. [\[CrossRef\]](#) [\[PubMed\]](#)
76. Thompson, J.M.; Mirza, M.A.; Park, M.K.; Qureshi, A.A.; Cho, E. The Role of Micronutrients in Alopecia Areata: A Review. *Am. J. Clin. Dermatol.* **2017**, *18*, 663–679. [\[CrossRef\]](#) [\[PubMed\]](#)

77. Ruiz-Tagle, S.A.; Figueira, M.M.; Vial, V.; Espinoza-Benavides, L.; Miteva, M. Micronutrients in hair loss. *Our Dermatol. Online* **2018**, *9*, 320–328. [[CrossRef](#)]
78. Almohanna, H.M.; Ahmed, A.A.; Tsatalis, J.P.; Tosti, A. The Role of Vitamins and Minerals in Hair Loss: A Review. *Dermatol. Ther.* **2018**, *9*, 51–70. [[CrossRef](#)]
79. Tobin, D.J.; Orentreich, N.; Fenton, D.A.; Bystry, J.C. Antibodies to hair follicles in alopecia areata. *J. Investig. Dermatol.* **1994**, *102*, 721–724. [[CrossRef](#)]
80. Tobin, D.J.; Hann, S.K.; Song, M.S.; Bystry, J.C. Hair follicle structures targeted by antibodies in patients with alopecia areata. *Arch. Dermatol.* **1997**, *133*, 57–61. [[CrossRef](#)]
81. Maniatopoulou, E.; Bonovas, S.; Sitaras, N. Isolation and quantification of glycosaminoglycans from human hair shaft. *Ann. Dermatol.* **2016**, *28*, 533–539. [[CrossRef](#)]
82. Malinowska, M.; Jakóbkiewicz-Banecka, J.; Kloska, A.; Tylki-Szymańska, A.; Czartoryska, B.; Piotrowska, E.; Wegrzyn, A.; Wegrzyn, G. Abnormalities in the hair morphology of patients with some but not all types of mucopolysaccharidoses. *Eur. J. Pediatr.* **2008**, *167*, 203–209. [[CrossRef](#)]
83. Moretti, G.; Cipriani, C.; Rebora, A.; Rampini, E.; Crovato, F. Correlation of tissue mucopolysaccharides with the hair cycle. *J. Investig. Dermatol.* **1967**, *48*, 498–503. [[CrossRef](#)] [[PubMed](#)]
84. Saitoh, M.; Uzuka, M.; Sakamoto, M. Human hair cycle. *J. Investig. Dermatol.* **1970**, *54*, 65–81. [[CrossRef](#)] [[PubMed](#)]
85. Oiki, S.; Mikami, B.; Maruyama, Y.; Murata, K.; Hashimoto, W. A bacterial ABC transporter enables import of mammalian host glycosaminoglycans. *Sci. Rep.* **2017**, *7*, 1069. [[CrossRef](#)] [[PubMed](#)]
86. Scharschmidt, T.C. Establishing tolerance to commensal skin bacteria: Timing is everything. *Dermatol. Clin.* **2017**, *35*, 1–9. [[CrossRef](#)]
87. Watanabe, S.; Kano, R.; Sato, H.; Nakamura, Y.; Hasegawa, A. The effects of *Malassezia* yeasts on cytokine production by human keratinocytes. *J. Investig. Dermatol.* **2001**, *116*, 769–773. [[CrossRef](#)] [[PubMed](#)]
88. Corazza, G.R.; Andreani, M.L.; Venturo, N.; Bernardi, M.; Tosti, A.; Gasbarrini, G. Celiac disease and alopecia areata: Report of a new association. *Gastroenterology* **1995**, *109*, 1333–1337. [[CrossRef](#)]
89. Sobolewska-Włodarczyk, A.; Włodarczyk, M.; Fichna, J.; Wiśniewska-Jarosińska, M. Alopecia areata in patients with inflammatory bowel disease: An overview. *Folia Med. Crac.* **2016**, *56*, 5–12.
90. Furusawa, Y.; Obata, Y.; Fukuda, S.; Endo, T.A.; Nakato, G.; Takahashi, D.; Nakanishi, Y.; Uetake, C.; Kato, K.; Kato, T.; et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* **2013**, *504*, 446–450. [[CrossRef](#)]
91. Han, Y.M.; Sheng, Y.Y.; Xu, F.; Qi, S.-S.; Liu, X.-J.; Hu, R.-M.; Miao, Y.; Huang, G.-Q.; Yang, Q.-P. Imbalance of T-helper 17 and regulatory T cells in patients with alopecia areata. *J. Dermatol.* **2015**, *42*, 981–988. [[CrossRef](#)]
92. Nair, L.; Dai, Z.; Christiano, A.M. Gut microbiota plays a role in the development of alopecia areata. *J. Investig. Dermatol.* **2017**, *137*, S112. [[CrossRef](#)]
93. Moreno-Arrones, O.M.; Serrano-Villar, S.; Perez-Brocal, V.; Saceda-Corralo, D.; Morales-Raya, C.; Rodrigues-Barata, R.; Moya, A.; Jaen-Olasolo, P.; Vano-Galvan, S. Analysis of the gut microbiota in alopecia areata: Identification of bacterial biomarkers. *J. Eur. Acad. Dermatol. Venereol.* **2020**, *34*, 400–405. [[CrossRef](#)] [[PubMed](#)]
94. Rebello, D.; Wang, E.; Yen, E.; Lio, P.A.; Kelly, C.R. Hair Growth in Two Alopecia Patients after Fecal Microbiota Transplant. *ACG Case Rep. J.* **2017**, *4*, e107. [[CrossRef](#)] [[PubMed](#)]
95. Xie, W.R.; Yang, X.Y.; Xia, H.H.; Wu, L.H.; He, X.X. Hair regrowth following fecal microbiota transplantation in an elderly patient with alopecia areata: A case report and review of the literature. *World J. Clin. Cases* **2019**, *7*, 3074–3081. [[CrossRef](#)]
96. Kim, K.O.; Gluck, M. Fecal Microbiota Transplantation: An Update on Clinical Practice. *Clin. Endosc.* **2019**, *52*, 137–143. [[CrossRef](#)]
97. Singh, R.K.; Chang, H.W.; Yan, D.; Lee, K.M.; Ucmak, D.; Wong, K.; Abrouk, M.; Farahnik, B.; Nakamura, M.; Zhu, T.H.; et al. Influence of diet on the gut microbiome and implications for human health. *J. Transl. Med.* **2017**, *15*, 73. [[CrossRef](#)] [[PubMed](#)]
98. De Pessemier, B.; Grine, L.; Debaere, M.; Maes, A.; Paetzold, B.; Callewaert, C. Gut-Skin Axis: Current Knowledge of the Interrelationship between Microbial Dysbiosis and Skin Conditions. *Microorganisms* **2021**, *9*, 353. [[CrossRef](#)]
99. Bowe, W.P.; Joshi, S.S.; Shalita, A.R. Diet and acne. *J. Am. Acad. Dermatol.* **2010**, *63*, 124–141. [[CrossRef](#)]
100. Lee, S.Y.; Lee, E.; Park, Y.M.; Hong, S.J. Microbiome in the Gut-Skin Axis in Atopic Dermatitis. *Allergy Asthma Immunol. Res.* **2018**, *10*, 354–362. [[CrossRef](#)]
101. O'Neill, C.A.; Monteleone, G.; McLaughlin, J.T.; Paus, R. The gut-skin axis in health and disease: A paradigm with therapeutic implications. *Bioessays* **2016**, *38*, 1167–1176. [[CrossRef](#)]
102. Rinaldi, F.; Pinto, D.; Giuliani, G.; Sorbellini, E. Diet and Microbiome Influence on Alopecia Areata: Experience from Case Reports. *J. Nutr. Med. Diet Care* **2019**, *5*, 037.
103. Mu, Q.; Kirby, J.; Reilly, C.M.; Luo, X.M. Leaky Gut as a Danger Signal for Autoimmune Diseases. *Front. Immunol.* **2017**, *8*, 598. [[CrossRef](#)] [[PubMed](#)]
104. Yang, Q.; Liang, Q.; Balakrishnan, B.; Belobrajdic, D.P.; Feng, Q.J.; Zhang, W. Role of Dietary Nutrients in the Modulation of Gut Microbiota: A Narrative Review. *Nutrients* **2020**, *12*, 381. [[CrossRef](#)] [[PubMed](#)]
105. Samantam, S. Vitamin D and immunomodulation in the skin: A useful affirmative nexus. *Explor. Immunol.* **2021**, *1*, 90–111.
106. Lin, X.; Meng, X.; Song, Z. Vitamin D and alopecia areata: Possible roles in pathogenesis and potential implications for therapy. *Am. J. Transl. Res.* **2019**, *11*, 5285–5300.



107. Kim, D.H.; Lee, J.W.; Kim, I.S.; Choi, S.Y.; Lim, Y.Y.; Kim, H.M.; Kim, B.J.; Kim, M.N. Successful treatment of alopecia areata with topical calcipotriol. *Ann. Dermatol.* **2012**, *24*, 341–344. [[CrossRef](#)]
108. Çerman, A.A.; Solak, S.S.; Altunay, İ.; Küçükünal, N.A. Topical calcipotriol therapy for mild-to-moderate alopecia areata: A retrospective study. *J. Drugs Dermatol.* **2015**, *14*, 616–620.
109. Narang, T.; Daroach, M.; Kumaran, M.S. Efficacy and safety of topical calcipotriol in management of alopecia areata: A pilot study. *Dermatol. Ther.* **2017**, *30*, e12464. [[CrossRef](#)]
110. Lee, S.; Kim, B.J.; Lee, C.H.; Lee, W.S. Increased prevalence of vitamin D deficiency in patients with alopecia areata: A systematic review and meta-analysis. *J. Eur. Acad. Dermatol. Venereol.* **2018**, *32*, 1214–1221. [[CrossRef](#)]
111. Gade, V.K.V.; Mony, A.; Munisamy, M.; Chandrashekar, L.; Rajappa, M. An investigation of vitamin D status in alopecia areata. *Clin. Exp. Med.* **2018**, *18*, 577–584. [[CrossRef](#)]
112. Daroach, M.; Narang, T.; Saikia, U.N.; Sachdeva, N.; Sendhil Kumaran, M. Correlation of vitamin D and vitamin D receptor expression in patients with alopecia areata: A clinical paradigm. *Int. J. Dermatol.* **2018**, *57*, 217–222. [[CrossRef](#)]
113. Unal, M.; Gonulalan, G. Serum vitamin D level is related to disease severity in pediatric alopecia areata. *J. Cosmet. Dermatol.* **2018**, *17*, 101–104. [[CrossRef](#)] [[PubMed](#)]
114. Ragab, D.; Soliman, D.; Samaha, D.; Yassin, A. Vitamin D status and its modulatory effect on interferon gamma and interleukin-10 production by peripheral blood mononuclear cells in culture. *Cytokine* **2016**, *85*, 5–10. [[CrossRef](#)]
115. Fawaz, L.; Mrad, M.F.; Kazan, J.M.; Sayegh, S.; Akika, R.; Khoury, S.J. Comparative effect of 25(OH)D3 and 1,25(OH)2D3 on Th17 cell differentiation. *Clin. Immunol.* **2016**, *166–167*, 59–71. [[CrossRef](#)] [[PubMed](#)]
116. Sheikh, V.; Kasapoglu, P.; Zamani, A.; Basiri, Z.; Tahamoli-Roudsari, A.; Alahgholi-Hajibehzad, M. Vitamin D3 inhibits the proliferation of T helper cells, downregulate CD4(+) T cell cytokines and upregulate inhibitory markers. *Hum. Immunol.* **2018**, *79*, 439–445. [[CrossRef](#)] [[PubMed](#)]
117. Yamamoto, E.A.; Jørgensen, T.N. Relationships Between Vitamin D, Gut Microbiome, and Systemic Autoimmunity. *Front Immunol.* **2020**, *10*, 3141. [[CrossRef](#)]
118. Rinaldi, F.; Giuliani, G.; Pinto, D. Postbiotic Evolution in Dermatology. *Medicine* **2019**, *16*, 3.
119. Markowiak, P.; Ślizewska, K. Effects of Probiotics, Prebiotics, and Synbiotics on Human Health. *Nutrients* **2017**, *9*, 1021. [[CrossRef](#)]
120. Toalaa, J.E.; Garcia Varelab, R.; Garcias, H.S.; Mata-Harod, V. Postbiotics: An evolving term within the functional foods field. *Trends Food Sci. Technol.* **2018**, *75*, 105–114. [[CrossRef](#)]
121. Tsilingiri, K.; Rescigno, M. Postbiotics: What else? *Benef. Microbes* **2013**, *4*, 101–107. [[CrossRef](#)]