

Characteristics of healthy and androgenetic alopecia scalp microbiome: Effect of *Lindera strychnifolia* roots extract as a natural solution for its modulation

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Abstract

OBJECTIVE: The human scalp harbours a vast community of microbial mutualists. Androgenetic alopecia (AGA), the most common form of hair loss in males, is a multifactorial condition involving genetic predisposition and hormonal changes. The role of microflora during hair loss remains to be understood. After having characterized the scalp microbiota of 12 healthy male subjects and 12 AGA male subjects (D0), the aim of this investigation was to evaluate the capacity of *Lindera strychnifolia* root extract (LsR) to restore a healthy bacterial and fungal scalp microflora after 83 days (D83) of treatment.

MATERIAL AND METHODS: The strategy used was based on high-throughput DNA sequencing targeting the encoding 16S ribosomal RNA for bacteria and *Internal Transcribed Spacer 1* ribosomal DNA for fungi.

RESULTS: Test analysis of relative abundance comparing healthy and AGA subjects showed a significant increase of *Cutibacterim acnes* ($P < 0.05$) and *Stenotrophomonas geniculata* ($P < 0.01$) in AGA subjects. AGA scalp condition was also associated with a significant ($P < 0.05$) decrease of *Staphylococcus epidermidis* relative abundance. A lower proportion of *Malassezia* genus in samples corresponding to AGA scalps and an increase of other bacterial genera (*Walleimia*, *Eurotium*) were also noted. At the species level, mean relative abundance of *Malassezia restricta* and *Malassezia globosa* were significantly lower ($P < 0.05$) in the AGA group. Eighty-three days of treatment induced a significant decrease in the relative abundance of *C. acnes* ($P < 0.05$) and *S. geniculata* ($P < 0.01$). *S. epidermidis* increased significantly ($P < 0.05$). At the same time, LsR treatment induced a significant increase in the proportion of *M. restricta* and *M. globosa* ($P < 0.05$).

CONCLUSION: Data from sequencing profiling of the scalp microbiota strongly support a different microbial composition of scalp between control and AGA populations. Findings suggest that LsR extract may be a potential remedy for scalp microbiota re-equilibrium.

Resume Francais

OBJECTIF: Le cuir chevelu humain abrite une vaste communauté microbienne. L'alopecie androgénétique (AGA), la forme la plus

courante de perte de cheveux chez l'homme, est une pathologie multifactorielle impliquant une prédisposition génétique et des changements hormonaux. Le rôle de la microflore lors de la chute des cheveux reste à comprendre. Après avoir caractérisé le microbiote du cuir chevelu de 12 hommes sans alopecie et 12 hommes porteur d'une alopecie, (J0), l'objectif de cette étude était d'évaluer la capacité de l'extrait de racine de *Lindera strychnifolia* (LsR) à restaurer une microflore bactérienne et fongique saine du cuir chevelu après 83 jours (D83) de traitement.

MATÉRIEL ET MÉTHODES: La stratégie utilisée était basée sur un séquençage d'ADN à haut débit ciblant l'ARN ribosomal 16S codant pour les bactéries et l'ADN ribosomal de l'espaceur transcrit interne 1 pour les champignons.

RÉSULTATS: Une augmentation significative de *Cutibacterim acnes* ($P < 0.05$) et *Stenotrophomonas geniculata* ($P < 0.01$) chez les sujets AGA a été notée à J0 comparativement aux sujets non alopecie. L'état du cuir chevelu AGA était également associé à une diminution significative ($P < 0.05$) de l'abondance relative de *Staphylococcus epidermidis*. Une plus faible proportion du genre *Malassezia* dans les échantillons correspondant aux cuirs chevelus AGA et une augmentation d'autres genres bactériens (*Walleimia*, *Eurotium*) ont également été notées. Au niveau des espèces, l'abondance relative moyenne de *Malassezia restricta* et *Malassezia globosa* était significativement plus faible ($P < 0.05$) dans le groupe AGA. Quarante-trois jours de traitement ont induit une diminution significative de l'abondance relative de *C. acnes* ($P < 0.05$) et *S. geniculata* ($P < 0.01$). *S. epidermidis* a augmenté de manière significative ($P < 0.05$). Dans le même temps, le traitement LsR a induit une augmentation significative de la proportion de *M. restricta* et *M. globosa* ($P < 0.05$).

CONCLUSION: Les données de séquençage soutiennent fortement une composition microbienne différente du cuir chevelu entre les populations témoin et AGA. Les résultats suggèrent que l'extrait de LsR peut être un remède potentiel pour le rééquilibrage du microbiote du cuir chevelu.

Introduction

Androgenetic alopecia (AGA) or simply baldness is the most common form of permanent hair loss in both men and women with an increasing prevalence with age. It is a hereditary pattern affecting 80% of Caucasian men [1]. Although there is racial variation in

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the incidence of androgenetic alopecia, it affects at least 50% of men by 50 years of age and up to 80% of men in later life [2]. Prevalence in women varies widely, ranging from 6% in women under 50 to 30-40% in women over the age of 70 [2]. The occurrence of AGA has been considered to have a significant negative impact on patient psychology and quality of life [3].

Androgenetic alopecia is characterized by a progressive loss of hair length, diameter and pigmentation. The hair growth cycle comprises of anagen, catagen and telogen phases. Shortening of the hair growth phase (anagen phase) and a slow progressing miniaturization of the hair follicle occur over time. During the transition between these phases, a group of specialized fibroblasts known as dermal papillae cells (DPCs) present in the hair follicle bulb plays an essential role in the regulation of the hair growth cycle, and factors affecting the functions of DPCs are of great importance for the development of therapy for the treatment and/or prevention of hair loss [4]. These factors include, but are not limited to, multiple signalling molecules, such as Wnts and Sonic hedgehog (Shh), which contribute to the anagen initiation of multipotent epithelial stem cells [5]. Targeting these biochemical signalling pathways of hair growth regulation would be a rational approach for the treatment of alopecia.

The genetic inheritance of AGA is well known. However, its ethology is multifactorial including micronutrients, stress and alterations of hormonal secretion [6]. Infiltration of mononuclear cells and lymphocytes is also detected in skin samples of AGA. This micro-inflammation takes place in the upper third of the hair follicle, where a large number of microorganisms are harboured. Besides these factors, the strongest evidence supporting correlations with microorganisms colonizing the scalp has been found in seborrhoeic dermatitis and dandruff [7]. The most abundant bacteria found in scalp swabs of healthy individuals are *Cutibacterium* spp. (formerly *Propionibacterium*) (with the vast majority of *C. acnes*) and *Staphylococcus* spp. (with the predominance of *S. epidermidis*), comprising approximately 90% of the total gene sequences.

Corynebacterium spp., *Streptococcus* spp., *Acinetobacter* spp. and *Prevotella* spp. are listed among other significantly less numerous species [8]. Among fungi, *Malassezia* spp. is largely predominate on the scalp. *Malassezia globosa* and *M. restricta* are the most abundant species. Ascomycota (*Acremonium* spp., *Didymella bryoniae*), Basidiomycota (*Cryptococcus liquefaciens* and *C. diffluens*), *Coniochaeta* spp. and *Rhodotorula* spp. were also identified on healthy scalp. It appears that fungal invasion and prevalence of *C. acnes* result in increased hair shedding [9]. Nevertheless, the data are scarce.

Management of alopecia is an essential aspect of clinical dermatology given the prevalence of hair loss and its significant impact on patients' quality of life. Over the centuries, a wide range of remedies has been suggested for androgenetic alopecia. Current treatments include surgery, hormone action modifiers and non-hormonal therapy. Pharmacological therapies are based on the understanding of androgen action mechanisms in hair follicles. Use of natural products is quite common in the hair care industry, and the search for natural products is continuously promoted [10]. It seems that polyphenols and terpenes have positive effects on hair growth cellular pathways. Indeed, polyphenols have been shown to enhance proliferation of human dermal papilla cells, to increase growth factors concentrations such as IGF-1 and VEGF and to reduce oxidative stress, resulting in an improved hair growth [11]. Lignans were also shown to exert hair growth-promoting effects by increasing the Wnt/ β -catenin signalling pathway in human dermal papilla cells [12]. Terpenes such as linderane were also able to

inhibit the cAMP/PKA/CREB pathway [13], whereas agents that increase cAMP levels were identified as potent inhibitors of human hair follicle growth. These molecules can be found in some plants such as *Lindera strychnifolia* roots (LsR) [14]. This plant is distributed in several Asian countries and is considered a longevity-promoting 'elixir of life'. Extracts of roots are used as traditional medicine and recent studies reported that these extracts antioxidant and anti-inflammatory effects [15].

After having characterized the scalp microbiota of 12 healthy males and 12 AGA male subjects (D0), the aim of this investigation was to evaluate the capacity of LsR extract to restore a healthy bacterial and fungal scalp microflora after 83 days (D83) of treatment. The methodology used was based on high-throughput DNA sequencing targeting the encoding 16S ribosomal RNA for bacteria and ITS1 (*Internal Transcribed Spacer 1*) ribosomal DNA for fungi.

Materials and methods

Preparation of extract

The dried roots of *Lindera strychnifolia* were collected in the Zhejiang Province of China, where they were cultivated at an altitude ranging from 200 to 1000 m. They were harvested in September, after 3 to 4 years of development. The same batch was used for the whole study.

Dried roots of *Lindera strychnifolia* were first washed by alkaline water and then extracted by 70% alcohol at 65°C over 12 h. After 10 μ m clarification, the extracted solution was concentrated, under vacuum at 55°C, up to 6% of dry matter in propan-1,3-diol (<10% of residual water). The decontamination was realized by filtration under 2 μ m.

Lindera strychnifolia root extract (LsR) contains polyphenols (29.4% per dry matter), linderane (1.2% per dry matter) and linderalactone (1.8% per dry matter). The polyphenol content is mainly constituted by tannins (24% per dry matter) and catechin derivatives (5% per dry matter).

Subject recruitment

The AGA group (twelve male AGA subjects aged 50.5 ± 3.2 years) with chronic alopecia of androgenetic origin were recruited. These AGA subjects had stage III or IV alopecia based on the Norwood Hamilton classification.

All enrolled AGA subjects had to meet the following criteria: (i) no antibiotics taken in the 30 days leading up to the sampling; (ii) no probiotics taken in the last 15 days; (iii) no shampoo applied to scalp within 48 h prior to sampling; (iv) no diagnosis of other dermatological diseases; (v) no diagnosis of an inflammatory skin disease or progressive skin lesion on the scalp (psoriasis, seborrhoeic dermatitis, severe erythema, severe excoriation, severe sunburn, etc.); (vi) no anti-tumour, immunosuppressant, or radiation therapy received in the last 3 months; (vii) no topical or hormonal therapy applied to the scalp in the last 3 months; (viii) no local or general treatment that may affect hair growth or hair loss received in the last few months prior to the investigation; (ix) no similar hair loss treatment received (e.g. minoxidil or finasteride received within 6 months prior to the start of the study) or any another product for oral or topical hair loss treatment received within 3 months prior to the selection visit.

The control group (twelve male healthy subjects aged 48.6 ± 2.1 years) had to meet the following criteria: (i) the age and sex of

healthy volunteers were required to approximately match with those who participated in the AGA group; (ii) no perms or hair dyes applied within 2 months prior the treatment (anti-hair loss shampoo included); (iii) no oral or topical antifungal preparations received within 1 month prior to treatment; (iv) no diagnosis of scalp-related diseases such as scalp folliculitis, head lice or alopecia areata.

Treatment

After having characterized the scalp microbiota of 12 healthy subjects and 12 AGA subjects, AGA subjects were asked to apply either a lotion with LsR extract at 1% and a placebo lotion twice daily for 83 days on a randomised half-head (one product per half-head).

Swab sample collection

The scalp surface was sampled by means of swab procedure according to previously reported methods [16] with minor modifications. Sterile cotton swabs were soaked for at least 30 s in ST solution (NaCl 0.15 M and 0.1% Tween-20) before sampling. A comb was used to separate hair fibres and collect samples from a total area of 16 cm² from a different area of the scalp. After collection, the head of each swab was cut and stored in ST solution. Samples from the same subjects were collected together and stored at 4°C until DNA extraction. Sterile cotton swabs placed in ST solution were used as negative controls.

Swab collection was taken at the beginning of the experimentation for both groups and after the treatment for the AGA group.

DNA extraction

Genomic DNA was extracted with the 'DNeasy PowerSoil kit' following supplier's recommendations. In order to ensure that any observed differences in differential abundance analysis were not due to the presence of contaminating DNA from the products used, extractions were performed from LsR extract and placebo. Genomic DNA samples were stored at -80°C and then sent to an external firm for next-generation sequencing.

Amplification and sequencing of 16S RNA gene

Microbiota composition analysis of samples was performed by amplifying the hypervariable regions V1-V3 of the 16S RNA gene. Sequences were processed using Mothur (version 1.36.1) according to the MiSeq SOP pipeline (Schloss, 2009). Barcodes, primers, and sequences showing homopolymers of more than 8 bp were discarded. Sequences showing 100% homology were grouped in unique sequences and then in operational taxonomic units (OTUs) based on 97% homology. Sequences were then assigned to match to a sequence in Greengenes (August 2013 release of gg_13_8_99, containing 202,421 bacterial and archaeal sequences) to identify the genus level.

Amplification and sequencing of ITS1 RNA gene

Fungal composition analysis of samples was performed by amplifying the ITS1 regions of the RNA gene. This amplification was done using universal primers ITS5 (GGAAGTAAAAGTCGTAA-CAAGG) and 5.8S-1R (GTTCAAAGAYTCGATGATTAC) which target the conserved regions of this gene common to all fungi. The products of this amplification were sequenced by MiSeq

technology. Sequences were processed using Mothur (version 1.36.1) according to the pipeline developed by our provider. Barcodes, primers and sequences showing homopolymers of more than 8 bp were discarded. Sequences showing 100% homology were grouped in unique sequences, then in OTUs. Sequences were then assigned to match to a sequence in Findley [17] to identify the genus level.

Statistical analysis

Data were expressed as relative abundance. Statistically significant differences in bacterial communities, comparing AGA and healthy control samples, were detected by *t* test. The level of significance was set at 5%.

Concerning the scalp microbiome, alpha diversity (Shannon diversity index) was evaluated. Alpha diversity is a measure of the biodiversity of samples and is characterized by the observation of the taxonomic richness and distribution of OTUs.

It was calculated using the following formula:

$$H = \sum_{i=1}^n p_i \log 2 p_i$$

n: number of OTU.

p_i: Fraction of reads that belong to the OTU.

Results

Identification of bacteria communities: Taxonomic abundance analysis

Cloning and sequencing of the conserved ribosomal unit regions (16S for bacterial and 28S-ITS for fungal) lead to the identification of the major bacterial and fungal scalp colonizers.

Alpha diversity did not differ between the groups studied, regardless of the period of investigation.

Table 1 Mean relative abundance at the phylum, genus and species level at D0 (beginning of the study) for healthy and AGA group and after the LsR treatment for AGA group (D83)

	Relative abundance healthy group	Relative abundance DO-AGA group	Relative abundance D83-AGA group
Phylum			
<i>Actinobacteria</i>	83%	80%	81%
<i>Firmicutes</i>	13%	14%	13%
<i>Proteobacteria</i>	2%	5%	3%
Other	2%	1%	3%
Genus			
<i>Cutibacterium</i>	79%	76.5%	78%
<i>Staphylococcus</i>	12%	14%	12%
<i>Corynebacterium</i>	2%	2%	2%
Other	7%	1.5%	8%
<i>Stenotrophomonas</i>	0%	6%	0%
Species			
<i>Cutibacterium acnes</i>	79%	84%	78%
<i>Staphylococcus epidermidis</i>	12%	10%	15.4%
<i>Corynebacterium unclassified</i>	2.5%	1.3%	1.7%
<i>Cutibacterium granulosum</i>	2.1%	1.7%	2.5%
<i>Stenotrophomonas geniculata</i>	0%	1.6%	0%

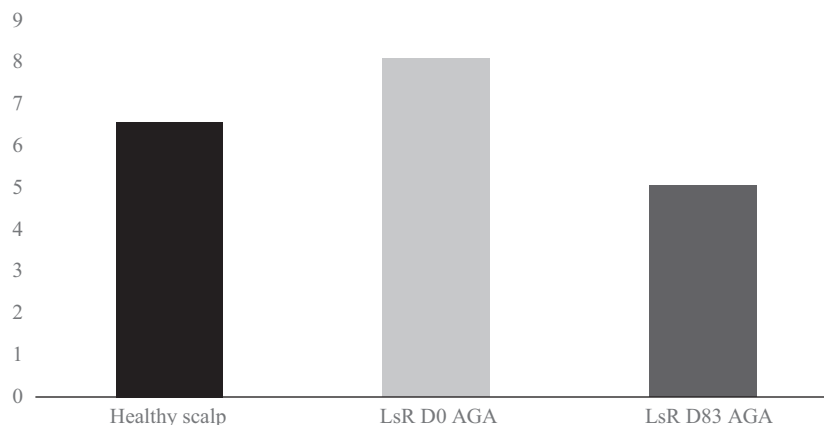


Figure 1 Effect of LsR extract on the ratio *Cutibacterium acnes*/*Staphylococcus epidermidis* in Healthy Group AGA Group at D0 (beginning of the study) and after the LsR treatment for AGA group (D83).

At D0, about 83% of sequences from the healthy group were assigned to *Actinobacteria* phylum and 13% of sequences were assigned to *Firmicutes*. The bacterial landscape corresponding to pathogenic scalps was not statistically different to that observed for healthy scalps (Table 1).

The most abundant genera observed across all samples were *Cutibacterium* and *Staphylococcus* in both groups. The changes in relative abundance for these genera between healthy and AGA groups were not significant. However, the analysis of bacterial distribution at the genus level, interestingly, highlighted the presence of *Stenotrophomanas* only in AGA subjects (Table 1).

As previously reported by other authors [18], *C. acnes* and *S. epidermidis* are the both major microbial species found on the scalp. However, significant increase of *C. acnes* ($P < 0.05$) and *Stenotrophomanas geniculata* ($P < 0.01$) in AGA subjects compared to control subjects (Table 1) was noted. AGA scalp condition was also associated with a significant ($P < 0.05$) decrease of *S. epidermidis* relative abundance. Thus, the ratio *C. acnes*: *S. epidermidis* was significantly higher ($P < 0.05$) in AGA subjects compared to control subjects (Fig. 1).

Table 2 Mean relative abundance at the phylum and genus level at D0 (beginning of the study) for healthy and AGA group, and after the LsR treatment for AGA group (D83)

	Relative abundance healthy group	Relative abundance DO-AGA group	Relative abundance D83-AGA group
Phylum			
<i>Basidiomycota</i>	99%	91%	95%
<i>Ascomycota</i>	1%	8%	5%
Other	0%	1%	0%
Genus			
<i>Malassezia</i>	99%	87%	93%
<i>Walleimia</i>	0%	2%	0%
<i>Eurotium</i>	0%	1%	0%
Other	1%	10%	7%

Table 3 Relative abundance at the species belonging to *Malassezia* species at D0 (beginning of the study) for healthy and AGA group and after the LsR treatment for AGA group (D83)

	Relative abundance healthy group	Relative abundance DO-AGA group	Relative abundance D83-AGA group
Species of <i>Malassezia</i>			
<i>Uncultured fungus</i>	25%	40%	31%
<i>Malassezia globosa</i>	20%	10%	14%
<i>Uncultured Basidiomycota</i>	0%	0%	0%
<i>Malassezia restricta</i>	55%	50%	55%
<i>Malassezia sympodialis</i>	0%	0%	0%
<i>Malassezia dermatis</i>	0%	0%	0%
<i>Malassezia slooffiae</i>	0%	0%	0%
<i>Malassezia obtusa</i>	0%	0%	0%
<i>Malassezia furfur</i>	0%	0%	0%
<i>Malassezia sympodialis</i>	0%	0%	0%

Identification of fungal communities

The alpha diversity for the fungal population was not significantly different in the scalp of healthy subjects compared to the scalp of AGA subjects, regardless of the period of investigation.

Taxonomic fungal composition of the scalp of healthy subjects and AGA subjects at the beginning of the study (D0) is presented in Tables 2 and 3.

Healthy and AGA scalps are mostly composed of one major phyla: *Basidiomycota* (predominant fungus being *Malassezia*). However, the bacterial landscape corresponding to pathogenic scalps differed from that of healthy scalps by a lower abundance of *Basidiomycota* and a higher proportion of *Ascomycota*.

A lower proportion of *Malassezia* genus and an increase of other bacterial genera (*Walleimia*, *Eurotium*) in samples corresponding to AGA subjects were noted (Table 2).

At the species level, results showed that the samples were mainly composed of fungi belonging to *M. restricta* and *M. globosa*, mean relative abundance of *M. restricta* and *M. globosa* being

significantly lower ($P < 0.05$) in the AGA group as compared to the healthy group. *Malassezia slooffiae*, *Malassezia furfur*, *Malassezia dermatis*, *Malassezia sympodialis* were not found on the scalp of either group (Table 3).

Effect of LsR extract treatment on microbiome

At the phylum and genus level, LsR extract maintained the biodiversity of bacteria (Table 1). Relative abundance of *Stenotrophomonas* returned to values observed in the healthy group.

Results also showed a significant decrease in the relative abundance of *C. acnes* (−15%, ($P < 0.05$) and *Stenotrophomanas geniculata* ($P < 0.01$).

Staphylococcus epidermidis increased significantly ($P < 0.05$) by 33% between D0 and D83. Thus, the ratio of *C. acnes*: *S. epidermidis* decreased by 37.8% as compared to the ratio noted at D0.

Concerning fungi, at the phylum level, LsR extract tended to restore the 'normal' fungal landscape for the *Basidiomycota* phylum and the three fungal genera studied. In fact, LsR extract allowed an increase in the abundance of *Malassezia* (+6%). A decrease of *Eurotium* and *Wallemia* were also observed.

A significant increase in the proportion of *M. restricta* ($P < 0.05$) and *M. globosa* ($P < 0.05$) was noted.

Discussion

The role of microbial dysbiosis in scalp disease has been recently hypothesized. However, little information is available with regard to the association between microbial populations on the scalp and hair diseases related to hair growth [19,20]. We focused our attention on the main bacterial and fungal populations of the scalp of healthy and AGA subjects. Our results agree with Pinto's work [20], showing an increase in *C. acnes* and a decrease in *S. epidermidis*, suggesting the role of the *Cutibacterium*: *Staphylococcus* balance in AGA. Wang *et al.* [9] have already noted the relationship between *C. acnes* and alopecia. In fact, *C. acnes* is able to synthesize many enzymes involved in the metabolism of porphyrins that, once activated, may contribute to oxidation and follicular inflammation. Virulence in the hair follicle is noted to cause hair loss as a consequence [9]. Therefore, the role of the hypoxic condition of the follicular region may be speculated in AGA and this may encourage *C. acnes* overgrowth [9]. *C. acnes* predominance is also identified in non-lesional scalp of patients with seborrhoeic dermatitis, providing support for the development in the sebaceous gland in AGA, which may attract the proliferation of *C. acnes* for lipids and fatty acids [21].

Our data also suggested a higher diversity of bacterial species inhabiting the scalp of AGA subjects, such as *S. geniculata*. Currently, the genus *Stenotrophomonas* comprises 12 validated species, with many taxonomical revisions in the genus. Moreover, there are three species of genus *Pseudomonas* (i.e. *P. beteli*, *P. hibiscicola* and *P. geniculata*) which are transferred to the genus *Stenotrophomonas* considered as synonyms of the *S. maltophilia* [22]. *S. geniculata* is an opportunistic human pathogen, characterized by high keratinase activities [23]. Keratinases are proteolytic enzymes capable of catalysing the hydrolysis of highly stable keratin proteins that compose hair.

Even if further investigations are needed, we can put forward the hypothesis that the increase of *S. geniculata* may induce inner root sheat disintegration due to the presence of a keratinolytic environment.

As demonstrated previously and irrespective of the scalp condition, the major component of the fungal mycobiome of both healthy and AGA scalp is the genera *Malassezia* (Table 2). *Malassezia* is a lipophilic yeast and resident flora on the skin of humans and animals [24]. At present, 11 out of the above 17 species of *Malassezia* have been demonstrated to be involved in the human skin microecology. Due to the absence of fatty acid synthase, most *Malassezia* yeast growth depends on the presence of host lipids and the expression of an extensive number of lipases. *Malassezia* species have been associated with a number of skin conditions, including psoriasis and dandruff [25]. However, the presence of *Malassezia* is not sufficient to cause either dandruff or the more extreme skin condition, seborrhoeic dermatitis; in fact, many people harbour *Malassezia* without showing symptoms. It seems that alteration of *Malassezia* spp. levels, as well as host predisposition, is required for disease progression [25]. Honnavar *et al.* [26] showed the predominance of *M. globosa* on scalps of control subjects. Jo *et al.* [27] hypothesized a protective effect due to increased *Malassezia* colonization in adults, preventing colonization by more pathogenic species, specifically dermatophytes. In a recent study, Huang *et al.* [28] suggested that *Malassezia* species contain a small amount of proteases, inducing hair loss. However, contrary to these authors, we noted in our study, a significant higher abundance of *M. restricta* and *M. globosa* in healthy subjects as also reported by Grimshaw *et al.* [22]. In healthy scalp, it seems that *Malassezia* maintains a harmonious and balanced symbiotic relationship with the host and played an opposing role in AGA subjects because of the different scalp microecology environments. The exact causative relationship between *Malassezia* spp. and scalp disease is confounded by the high prevalence of *Malassezia* on both healthy and disease-affected skin [29]. This has led to the development of a susceptibility hypothesis where both altered levels of *Malassezia* spp. and host predisposition are required for disease progression [25].

Generally speaking, currently there are few studies focusing on the link between alopecia and scalp microbiota. As suggested by Clavaud *et al.* [18], the contradictory results may be explained by (i) uncertainty in the dermatological diagnosis and uncompleted or erroneous species identification, (ii) the identification techniques used in the different studies were different, or (iii) different origins of the sampled populations (North America, Korea or France). In fact, it is known that environment, ethnicities and food may influence the composition of a microbiome in the oral cavity, vagina and gut.

Treatment during the 83 days with LsR extract tended to restore the 'normal' fungal landscape and scalp microbiota, limiting specific species, inducing keratin alterations and microinflammation (Table 2). In fact, relative abundance of *S. geniculata* returned to values observed in the healthy group. At the same time, the ratio of *C. acnes*: *S. epidermidis* significantly decreased and no significant differences between the healthy group and AGA group were noted at D83 (Fig. 1). LsR extract also allowed an increase in the abundance of *Malassezia* (+6%). A decrease of *Eurotium* and *Wallemia* was also observed. Genus *Eurotium* and all *Eurotium* species are currently transferred to the *Aspergillus* name. The genus inhabits human skin and may occasionally cause clinical conditions. *Wallemia* has an allergological and infective potential. Together with the toxins that the majority of *Wallemia* spp. produce, they are defined by filamentous pathogenic fungi [30].

Larger studies are still needed for a more precise identification of bacterial communities on the scalp as for the analysis of fungi in AGA subjects. Nevertheless, our study highlighted the fact that a

natural extract has the possibility of modulating the alopecia scalp microbiota. The question remains—how does this extract have the ability to modulate it? *Lindera strychnifolia* is a traditional Chinese medicine, which contains sesquiterpene lactones, and has anti-inflammatory effects by inhibiting NF- κ B and mitogen-activated protein kinase activation [31]. Recently, Lou *et al.* [32] noted that *Linderae radix* ethanol extract (LREE) attenuates disturbance gut microbiota in liver disease via a prebiotic effect. Molinaro *et al.* [33] reported that many sesquiterpene lactones have strong antimicrobial activity against important human pathogens. Our data reinforced the idea that LsR extract has the capacity to also modulate the scalp microbiota.

Conclusion

Our results confirm the presence of a significant bacterial and fungal disequilibrium on the scalp of AGA subjects compared to

healthy subjects. Eighty-three days of LsR treatment induced a reversible microbiome environment. This active ingredient offers a new natural solution for formulated products aiming to manage hair loss by acting on the scalp microbiome.

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Conflicts of interest

The authors report no conflicts of interest.

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