Virulence Gene Expression in *Malassezia* spp from Individuals with Seborrheic Dermatitis

Journal of Investigative Dermatology (2011) **131,** 2134–2136; doi:10.1038/jid.2011.178; published online 23 June 2011

TO THE EDITOR

Seborrheic dermatitis (SD) is a chronic inflammatory disease that compromises skin areas rich in sebaceous glands, such as the face, scalp, and upper trunk. The condition is frequently observed in acquired immune deficiency syndrome patients (30–85% compared with 3–5% of immunocompetent adults). Its appearance is considered to be an early marker of the evolutionary trend of HIV infection (Gupta *et al.*, 2001; Gupta and Bluhm, 2004; Ashbee, 2007; Naldi and Rebora, 2009).

The cause or causes of SD and the pathogenic role of *Malassezia* spp are, at present, not completely understood (Gupta *et al.*, 2000; DeAngelis *et al.*, 2005; Tajima *et al.*, 2008). At this time, we can only rely on hypotheses of the pathogenicity mechanisms and the pathogenic determinants (Ran *et al.*, 1993; Brunke and Hube, 2006; Xu *et al.*, 2007).

Analyses of the complete genome of *Malassezia globosa* and the partial genome of *M. restricta* (Xu *et al.*, 2007) have presented gene-encoding enzymes of the lipase and phospholipase families that could explain the lipid dependency of the genus. The secretion of enzymes by human pathogenic fungi has been considered an important factor in the invasion and dissemination in the host (Staib *et al.*, 1999; Descamps *et al.*, 2002) and, thus, it is suggested that lipases and phospholipases are involved in the mechanisms of pathogenicity of *Malassezia* spp. However, except for an early approximation of *M. globosa* lipase gene expression on human scalp, the expression of these genes has never been tested during disease development (Xu *et al.*, 2007).

In order to assess the induction of lipases and phospholipases in SD, we analyzed the expression profiles of candidate virulence genes of *Malassezia* spp during host infection.

A total of 40 *Malassezia* isolates were obtained from four groups of individuals (non-*Malassezia* lesion (NML), SD, NML + HIV, and SD + HIV) (Table 1). The selection of SD and SD + HIV patients was based on dermatologist evaluation of the presence of erythema and peeling in areas such as the face and scalp. We assessed isolates for morphological and physiological features, and we confirmed their identification by amplification and sequencing of 5.8S rDNA-ITS2 regions (Supplementary Materials and Methods online). Six *Malassezia* spp strains were used as a reference (*M. furfur* CBS 1878, *M. sympodialis* CBS 7222, *M. globosa* CBS 7966, *M. restricta* CBS 7877, *M. slooffiae* CBS 7956, and *M. pachydematis* CBS 1879). All of the *Malassezia* species isolated from NML, SD, NML + HIV, and SD + HIV individuals exhibited typical morphological, physiological, and molecular features (Supplementary Table S1 online), with the exception of one: *M. Furfur*, which presented atypical Tween assimilation pattern (Gonzalez *et al.*, 2009).

We found *M. restricta* to be the predominant species in all groups of individuals, and *M. furfur* was found in all groups except the SD group. *M. furfur* with an atypical Tween assimilation pattern was present only in SD + HIV individuals, and *M. globosa* was present in SD and SD + HIV individuals. These results coincide with previous reports (Tajima *et al.*, 2008; Oh *et al.*, 2010).

For the expression assays, four genes, *Mgl0797*/*M. globosa LIP1*, *Mgl0798*/*M. globosa* hypothetical secretory lipase, *Mgl3326*/*M. globosa* hypothetical phospholipase, and *Mflip 1*/*M. furfur MfLIP1* were used for a quantitative analysis using real-time PCR (Supplementary Table S2 online). All the genes were detected in all samples.

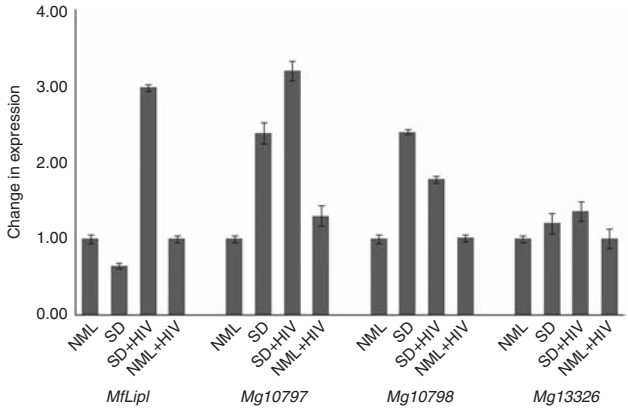

Figure 1. Changes in expression of lipase and phospholipase genes in non-*Malassezia* lesion (NML), seborrheic dermatitis (SD), SD + HIV, and NML + HIV individuals quantified by relative *qRT-PCR*. *Mgl0797*: NML ($P = 0.253$), SD ($P < 0.001$), SD + HIV ($P = 0.001$), and NML + HIV ($P = 0.201$). *Mgl0798*: NML ($P = 0.187$), SD ($P = 0.002$), SD + HIV ($P = 0.001$), and NML + HIV ($P = 0.113$). *Mflip1*: NML ($P = 0.182$), SD ($P < 0.001$), SD + HIV ($P = 0.002$), and NML + HIV ($P = 0.173$). *Mgl3326*: NML ($P = 0.116$), SD ($P = 0.115$), SD + HIV ($P = 0.119$), and NML + HIV ($P = 0.121$).

*Abbreviations: NML, non-*Malassezia *lesion; SD, seborrheic dermatitis*

Table 1. Sources and origins of strains of *Malassezia* species used in this study

Malassezia species	Strain	Origin	Accession no./5.8 rDNA-ITS2
M. sympodialis (*n=4*)	1SD	SD patient	HQ710829
	5SD	SD patient	HQ710821
	8SD	SD patient	HQ710803
	9SD	SD patient	HQ710811
M. restricta (*n=4*)	2SD	SD patient	HQ710806
	3SD	SD patient	HQ710822
	6SD	SD patient	HQ710819
	7SD	SD patient	HQ710809
M. slooffiae	4SD	SD patient	HQ710802
M. globosa (*n=2*)	10SD	SD patient	HQ710807
	11SD	SD patient	HQ710812
M. sympodialis (*n=2*)	1NML	NML	HQ710808
	2NML	NML	HQ710810
M. furfur (*n=2*)	1NML+HIV	NML and HIV patient	HQ710816
	3NML+HIV	NML and HIV patient	HQ710827
M. globosa	2NML+HIV	NML and HIV patient	HQ710814
M. restricta	4NML+HIV	NML and HIV patient	HQ710817
M. restricta	1SD+HIV	SD and HIV patient	HQ710805
M. sympodialis (*n=3*)	2SD+HIV	SD and HIV patient	HQ710804
	5SD+HIV	SD and HIV patient	HQ710813
	10SD+HIV	SD and HIV patient	HQ710823
M. furfur (*n=4*)			
	3SD+HIV	SD and HIV patient	HQ710825
	4SD+HIV	SD and HIV patient	HQ710828
	7SD+HIV	SD and HIV patient	HQ710824
	8SD+HIV	SD and HIV patient	HQ710826
M. globosa (*n=3*)			
	9SD+HIV	SD and HIV patient	HQ710820
	11SD+HIV	SD and HIV patient	HQ710815
	12SD+HIV	SD and HIV patient	HQ710818
M. furfur[1]	13SD+HIV	SD and HIV patient	HQ710830

Abbreviations: NML, non-*Malassezia* lesion; SD, seborrheic dermatitis.
[1]Isolates with atypical pattern of Tween assimilation.

To determine the differential expression between individual groups, normalized expression was compared between them using NML as the calibrator sample. A significant higher expression of *Mgl0797*, *Mgl0798*, and *Mflip1* was found in SD + HIV patients ($P<0.005$). The same pattern was observed in SD individuals with the exception of *Mflip1* expression that presented a downregulation ($P<0.005$). There were no significant differences for the *Mgl3326* gene (Figure 1).

Our results point to the possibility that lipases may be related to the development of SD and could be considered as virulence factors. However, some authors support the idea that these enzymes provide the ability to metabolize lipids and to integrate the fatty acids into the fungal cell wall, and thus are very important for growth and survival in a host environment and could be the result of human skin adaptation (Gupta and Bluhm, 2004; DeAngelis *et al.*, 2005; Tajima *et al.*, 2008).

In this study, we demonstrated that *M. restricta* is the predominant *Malassezia* species in SD and SD + HIV individuals and we evidenced potential virulence genes expression during infection. To our knowledge this is previously unreported. Our data prove that lipases *Mgl0797*, *Mgl0798*, and *Mflip1* are highly expressed in SD and SD + HIV individuals and it suggests that they may be involved in the *Malassezia* spp pathogenesis mechanism in SD.

The Ethics Committee of the Universidad de los Andes approved the protocol. Written informed consent was obtained from each subject before the sampling. The investigations were conducted according to the Declaration of Helsinki Principles.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

We thank the Departamento Administrativo de Ciencia, Tecnología e Innovación (Colciencias; contract no. 659-2009) and the Department of Biological Science, Universidad de los Andes, for their financial support for this project.

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SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/jid

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Lamellar Lipid Organization and Ceramide Composition in the Stratum Corneum of Patients with Atopic Eczema

Journal of Investigative Dermatology (2011) **131,** 2136–2138; doi:10.1038/jid.2011.175; published online 30 June 2011

TO THE EDITOR

The existence of an impaired skin-barrier function in atopic eczema (AE, also referred to as atopic dermatitis) has been demonstrated previously, revealing the importance of the skin barrier in the pathophysiology of AE (Jakasa *et al.*, 2011). Loss-of-function mutations in the filaggrin (*FLG*) gene are known to be major risk factors for developing AE (Palmer *et al.*, 2006). However, recent findings report a reduced skin-barrier function irrespective of filaggrin genotype, suggesting the importance of additional factors for an impaired skin barrier in AE (Jakasa *et al.*, 2011).

The skin barrier is primarily provided by the stratum corneum (SC), consisting of enucleated cells surrounded by lipid regions. SC lipids (primarily ceramides (CERs), cholesterol, and free fatty acids) form two lamellar phases: the short- and long-periodicity phase (LPP), with periodicities of ~6 and ~13 nm, respectively. These periodicities were determined by small-angle X-ray diffraction (Bouwstra *et al.*, 1991). Barrier properties of the skin may also depend on the lipid organization of the SC lipids, and an altered lipid composition or organization may cause a reduced skin barrier in AE. Several studies have reported on the CER composition in AE patients (Ishikawa *et al.*, 2010; Jungersted *et al.*, 2010). In the present study, the CER composition and lamellar lipid organization in the SC of AE skin have been simultaneously examined. For AE patients, we observe drastic changes in lipid organization, which correlate with changes in CER composition, as compared with healthy subjects.

The study was conducted in accordance with the Declaration of Helsinki Principles and approved by the Ethical Committee of the Leiden University Medical Center. Subjects gave written informed consent. Six Caucasian AE patients (23.3 ± 5.2 years; two males) and six Caucasian subjects without (history of) dermatological disorders (24.7 ± 7.6 years; one male) were included. The subjects did not apply any dermatological products to their forearms for at least 1 week before the studies. To study the lipid properties irrespective of filaggrin mutations, we excluded subjects with any of the four most prevalent mutations found in European Caucasians: 2282del4, R501X, S3247X, and R2447X, analyzed by genotyping (Sandilands *et al.*, 2007).

The lamellar lipid organization was studied using 4-mm biopsies harvested from the ventral forearm of uninvolved skin. The local severity of uninvolved AE skin was evaluated by local SCORing Atopic Dermatitis (Stalder and Taieb, 1993) and was 0 for all patients. SC was isolated by trypsin digestion (Tanojo *et al.*, 1997) and analyzed by small-angle X-ray diffraction using a microfocus beam (European Synchrotron Radiation Facility, Grenoble, France), similarly as described elsewhere (Bras *et al.*, 2003). The CER composition was examined in SC harvested by tape stripping uninvolved regions from the ventral forearm (PPS tape, Nichiban, Tokyo, Japan) close to the region of the biopsy. Tape strip numbers 6–9 were extracted, pooled, and analyzed by liquid chromatography (Alliance 2695, Waters, Milford, MA, USA) coupled to mass spectrometry (TSQ-Quantum, Thermo Finnigan, San Jose, CA) (Thakoersing *et al.*, 2010; van Smeden *et al.*, 2011). The nomenclature of used CERs is described previously (Motta *et al.*, 1993). Briefly, CERs contain a fatty-acid chain (an esterified ω-hydroxy (EO), α-hydroxy (A), or non-hydroxy (N) fatty acid) linked via an amide to a sphingosine chain (a sphingosine (S), dihydrosphingosine (dS), phytosphingosine (P),

Abbreviations: AE, atopic eczema; CER, ceramide; EO, esterified ω-hydroxy; LPP, long-periodicity phase; SC, stratum corneum