

Cutaneous *Malassezia* Microbiota in Atopic Dermatitis Patients Differ by Gender and Body Part

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Key Words

Malassezia • Cutaneous resident microbiota •
Atopic dermatitis • *Staphylococcus aureus*

Abstract

Background: *Malassezia* is a particularly important factor in the occurrence of atopic dermatitis (AD). **Aim:** The aim of this study was to quantitatively clarify the *Malassezia* species isolated from AD patients by gender, body part and analytical method in detail. **Methods:** The subjects were 20 AD males and 47 AD females. Samples were collected from lesion and nonlesion areas on the face and upper trunk of AD patients. *Malassezia* DNA was analyzed using a real-time PCR system. **Results:** The cutaneous *Malassezia* microbiota in AD patients differed by gender, body part and analytical method. **Conclusions:** The present results indicate the possibility that the influence of *Malassezia* antigens is different according to gender and body part.

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Introduction

Atopic dermatitis (AD) is a multiple-factor-related chronic inflammatory disorder marked by pruritus (often intense) and characteristic eczematous lesions with erythema, fine scaling and thickening of the epidermis. *Malassezia* yeasts appear to be a particularly important factor in the occurrence of AD in adults [1]. It has been suggested that *Malassezia* yeasts act as allergens in AD patients who are susceptible, rather than as an infectious agent [2, 3]. This hypothesis has been supported by the report that AD patients have positive reactions to *Malassezia* yeasts in patch tests [4]. In addition, specific *Malassezia* IgE antibodies were found in AD patients but not in healthy individuals [5–10].

Genus *Malassezia* are basidiomycetous yeasts associated with *Malassezia* folliculitis, pityriasis versicolor, seborrheic dermatitis, dandruff, AD and psoriasis, although they are also members of the normal resident microbiota on human skin [11]. This genus consists of 13 species [12–19]. The taxonomy of genus *Malassezia* was revised in 1996 [12], and further new species have been reported in recent years [15–19]. There have been several studies concerning the frequency of isolation of each species and its

correlation with the clinical manifestations of AD [20–25]. These previous studies used either nonculture or culture methods, and the differences by gender and body part of *Malassezia* species isolated from AD patients have not been thoroughly considered. However, it has been reported that there are differences in the *Malassezia* species detected on human skin by gender, body part and analytical method (nonculture method or culture method) [26, 27]. Moreover, although these previous reports contain data about the detection rate of each *Malassezia* species, there are few reports where each *Malassezia* species was analyzed quantitatively. The aim of this study was to quantitatively clarify the *Malassezia* species isolated from AD patients by gender and body part by both nonculture and culture methods. At the same time, *Staphylococcus aureus*, coagulase-negative staphylococci (CNS), *Propionibacterium* species and non-*Malassezia* (nonlipophilic) fungi were also analyzed.

Subjects and Methods

Subjects

The study design was reviewed and approved by the institutional review board of Fujita Health University. The authors gained the informed consent from all the subjects. The subjects were 67 Japanese AD outpatients (20 males of age 35 ± 20 years, range 14–80 years, and 47 females of age 33 ± 9 years, range 11–54 years) treated continuously at the Fujita Health University Hospital from June to September 2007. Severe AD patients comprised 29 subjects (10 males and 19 females), moderate AD patients 24 subjects (6 males and 18 females), and mild AD patients 14 subjects (4 males and 10 females). The severity of AD cases was judged according to the standard guidelines from the Japanese Dermatological Association [28].

Sample Collection

Samples were collected from lesion and nonlesion areas of 10 cm² of skin surface on the face (forehead or cheek) and upper trunk (upper chest or upper back) of the AD patients by a swab method using 5 ml of phosphate buffer containing 2% polysorbate 80 (pH 7.0) in the morning from 9 to 12 a.m. We collected the samples without make-up from the skin of the female subjects.

Quantitative Analysis of *Malassezia* Species

Malassezia species were identified using both culture and nonculture methods, as in the previous study [26, 27]. For analysis by the nonculture method, *Malassezia* DNA was extracted directly from 3 ml of the collected samples. For analysis by the culture method, 0.1 ml of the collected samples were cultivated at 32°C on Leeming & Notman agar medium for 14 days [29]. DNA of *Malassezia* species was analyzed using a real-time PCR system (7300 Real Time PCR system; Applied Biosystems, Foster City, Calif., USA) and a quantitative PCR reagent (Platinum SYBR Green qPCR SuperMix-UDG with ROX; Invitrogen, Carlsbad, Calif., USA). Nine *Malassezia* species isolated from human skin were tar-

geted in this study. The standard curves of each *Malassezia* species using quantitative analysis of the skin surface *Malassezia* microbiota were generated with *Malassezia globosa* CBS 7966, *Malassezia restricta* CBS 7877, *Malassezia sympodialis* CBS 7222, *Malassezia furfur* NBRC 0656, *Malassezia obtusa* CBS 7876, *Malassezia slooffiae* CBS 7956, *Malassezia dermatis* JCM 11348, *Malassezia japonica* JCM 11963 and *Malassezia yamatoensis* CBS 9725 counted beforehand using a bacterium counting chamber. The detection limit of the culture method was 5 colony-forming units (CFU)/cm², and that of the nonculture method was 50 cells/cm² [26].

Quantitative Analysis of *Staphylococcus* Species

Each 0.05-ml aliquot of the collected samples or buffer, diluted appropriately, was cultivated on mannitol salt agar medium (Eiken Chemical) at 35°C for 3 days. The colonies that appeared were subcultured on mannitol salt agar medium for 24 h for purification and distinguished between *S. aureus* and CNS by coagulase test (Eiken Chemical). In addition, the *mecA* gene amplification was determined using the real-time PCR system for methicillin-resistant *S. aureus* (MRSA). DNA extraction and amplification were carried out by the same method as for the *Malassezia* analysis, and the primer sets used were: 5'-AAG CGA CTT CAC ATC TAT TAG GTT AT-3' and 5'-TAT ATT CTT CGT TAC TCA TGC CAT AC-3'. The *mecA* specificity of the primers was confirmed by amplification and melting temperatures using *S. aureus* JCM 8702 (MRSA), JCM 8703 (MRSA), JCM 8704 (MRSA), ATCC 6538 (methicillin-susceptible *S. aureus*), *Staphylococcus epidermidis* NBRC 12993 and 6 strains of clinically isolated methicillin-susceptible staphylococci.

Quantitative Analysis of Other Cutaneous Resident Microorganisms

For the analysis of *Propionibacterium* species, 0.05-ml aliquots of the collected samples or buffer diluted appropriately were cultivated on modified Gifu anaerobic agar medium (Nissui Pharmaceutical, Tokyo, Japan) at 35°C for 7 days under anaerobic conditions. The bacilli that did not increase under aerobic conditions were judged to be *Propionibacterium* species. The samples were also cultivated on potato dextrose agar medium (Nihon Pharmaceutical) at 32°C for 7 days for the isolation of non-*Malassezia* fungi.

Statistical Analysis

In the quantitative analysis, the number of cells lower than the detection limit (nonculture method for *Malassezia* species: 50 cells/cm²; culture method for *Malassezia* species: 5 CFU/cm²; culture method for other cutaneous resident microorganisms: 10 CFU/cm²) was assumed to be 10⁰/cm². Student's *t* test and the paired *t* test (nonlesion area vs. lesion area) were used.

Results

Cutaneous Resident Microbiota of AD Patients

The total numbers of *Malassezia* species assessed by both the nonculture method and the culture method in AD patients are shown in figure 1. For the total number of *Malassezia* species, there were no significant differences between lesion and nonlesion areas. In the com-

Fig. 1. Numbers of *Malassezia* species on the skin of AD patients. The total numbers of *Malassezia* species by the nonculture method and the culture method in AD patients are shown. The samples from 20 AD males and 47 AD females were examined. Error bars: SD. *Significant difference versus upper trunk ($p < 0.05$); †significant difference versus female ($p < 0.05$); #significant difference versus nonculture method ($p < 0.05$).

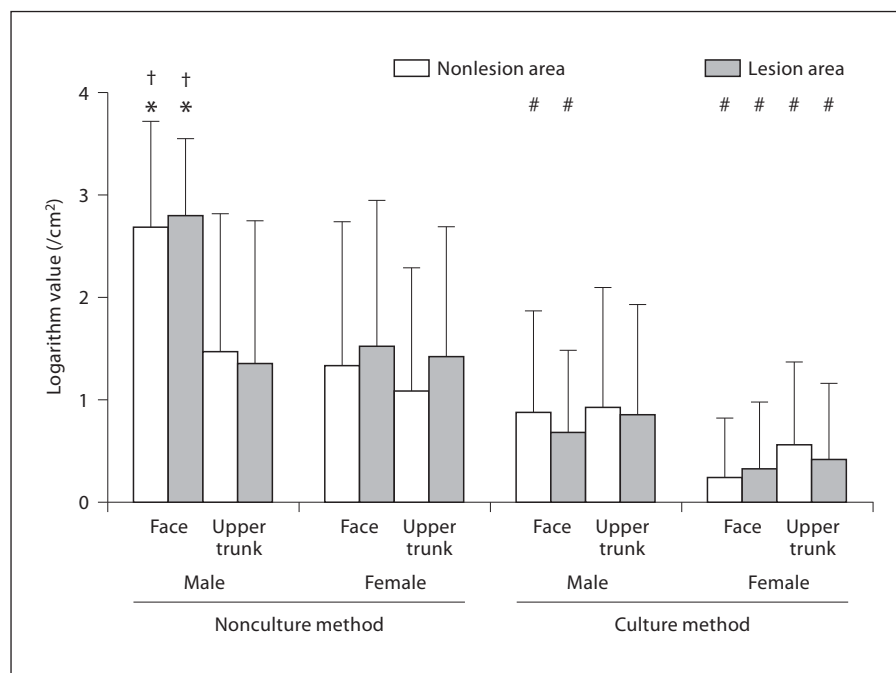
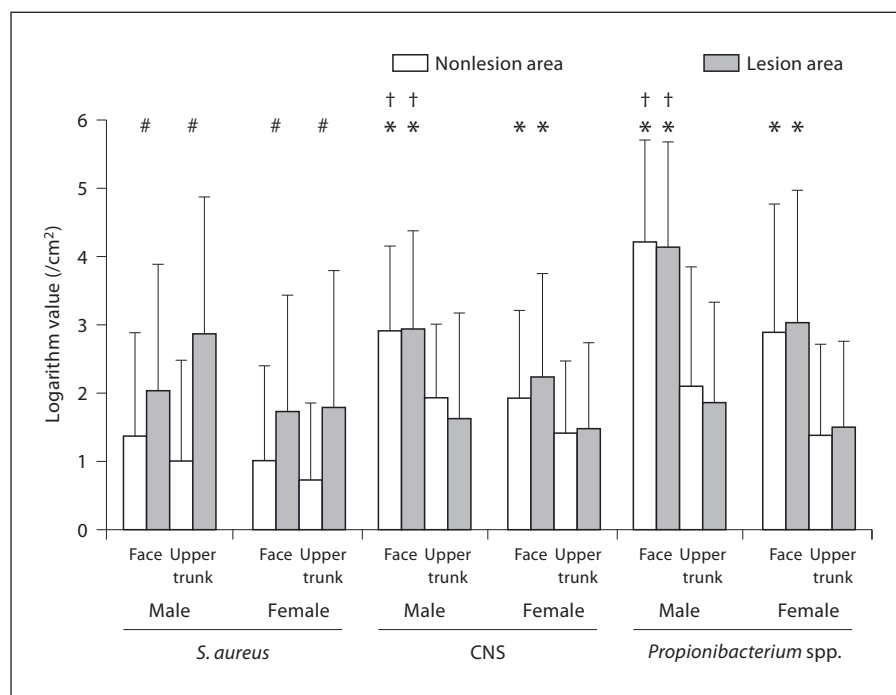


Fig. 2. Numbers of *S. aureus*, CNS and *Propionibacterium* species on the skin of AD patients. The total numbers of *S. aureus*, CNS and *Propionibacterium* species by the culture method in AD patients are shown. The samples from 20 AD males and 47 AD females were examined. Error bars: SD. *Significant difference versus upper trunk ($p < 0.05$); †significant difference versus female ($p < 0.05$); #significant difference between nonlesion and lesion areas ($p < 0.05$).



parison between face and upper trunk in males, the total number of *Malassezia* species on the face was greater than that on the upper trunk found by the nonculture method, although there were no significant differences in numbers found by the culture method. In females, there were no significant differences assessed by either meth-

od. In the comparison between genders, the number of *Malassezia* species on males was greater than that on females as assessed by either method on the face, although there were no significant differences between genders for the upper trunk. The numbers found by the nonculture method were greater than those by the culture method on

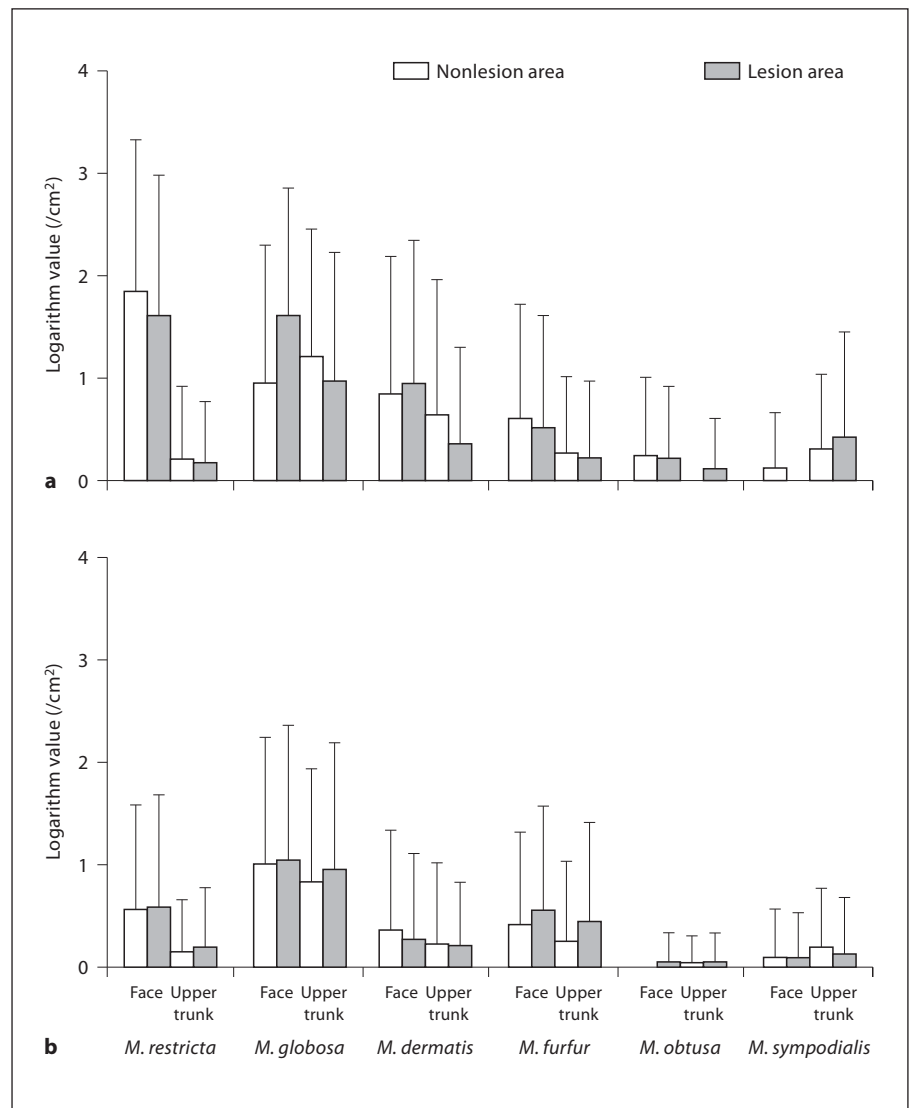


Fig. 3. *Malassezia* species detected from the skin of AD patients by the nonculture method. The samples from 20 AD males (a) and 47 AD females (b) were examined. *M. slooffiae*, *M. japonica* and *M. yamatoensis* were not detected in any samples. Error bars: SD.

the face of males and females and on the upper trunk of females, although there was no significant difference in numbers on the upper trunk in males.

The total numbers of *S. aureus*, CNS and *Propionibacterium* species by the culture method in AD patients are shown in figure 2. The numbers of *S. aureus* in the lesion areas were greater than those in the nonlesion areas. The presence of the *mecA* gene was confirmed in 261 strains of *S. aureus* isolated from AD patients; MRSA comprised 6 strains (table 1). There were no significant differences between the lesion and nonlesion areas for CNS and *Propionibacterium* species. For CNS and *Propionibacterium* species, the numbers on the face were greater than those on the upper trunk. The numbers of CNS and *Propioni-*

bacterium species on males were greater than those on the face of females, although there were no significant differences by gender regarding their numbers on the upper trunk. For non-*Malassezia* fungi, the numbers detected were 0.22–0.35 (logarithm value)/cm², and there were no significant differences by gender or body part (data not shown).

For the numbers of total *Malassezia* species (assessed by both the nonculture method and the culture method), *S. aureus*, CNS, *Propionibacterium* species and non-*Malassezia* fungi, there were no significant differences between the forehead and cheek or between the upper chest and upper trunk (data not shown).

Fig. 4. Number of *S. aureus* for each clinical severity of AD. 10 severe AD males, 6 moderate AD males, 4 mild AD males, 19 severe AD females, 16 moderate AD females and 10 mild AD females were examined. Error bars: SD. I = Mild AD patients; II = moderate AD patients; III = severe AD patients. *Significant difference between each clinical degree ($p < 0.05$); †significant difference versus nonlesion area ($p < 0.05$).

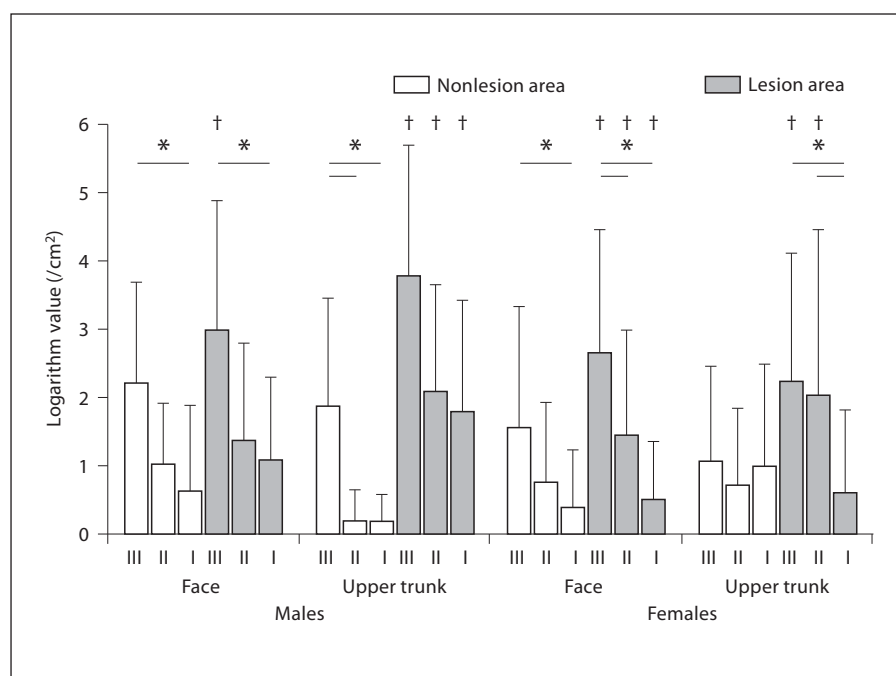


Table 1. Recovery rate of MRSA from the skin of AD patients

	Area	Males	Females	Total
Face	nonlesion	0/17	3/47	3/64
	lesion	0/20	3/63	3/83
Upper trunk	nonlesion	0/15	0/20	0/35
	lesion	0/26	0/53	0/79
Total		0/78	6/183	6/261

The number of samples positive for the *mecA* gene to the number of strains analyzed is shown. MRSA was detected from the lesion and nonlesion areas of 1 severe and 2 mild AD female subjects.

Malassezia Species Detected from Skin of AD Patients

The numbers of *Malassezia* species detected from the skin of AD patients by the nonculture method are shown in figure 3. The *Malassezia* species detected were *M. restricta*, *M. globosa*, *M. dermatis*, *M. furfur*, *M. obtusa* and *M. sympodialis*. *M. slooffiae*, *M. japonica* and *M. yamatoensis* were not detected in any samples. There were no significant differences in the numbers of each *Malassezia* species between lesion and nonlesion areas. The predominant *Malassezia* species on the face of males were *M. re-*

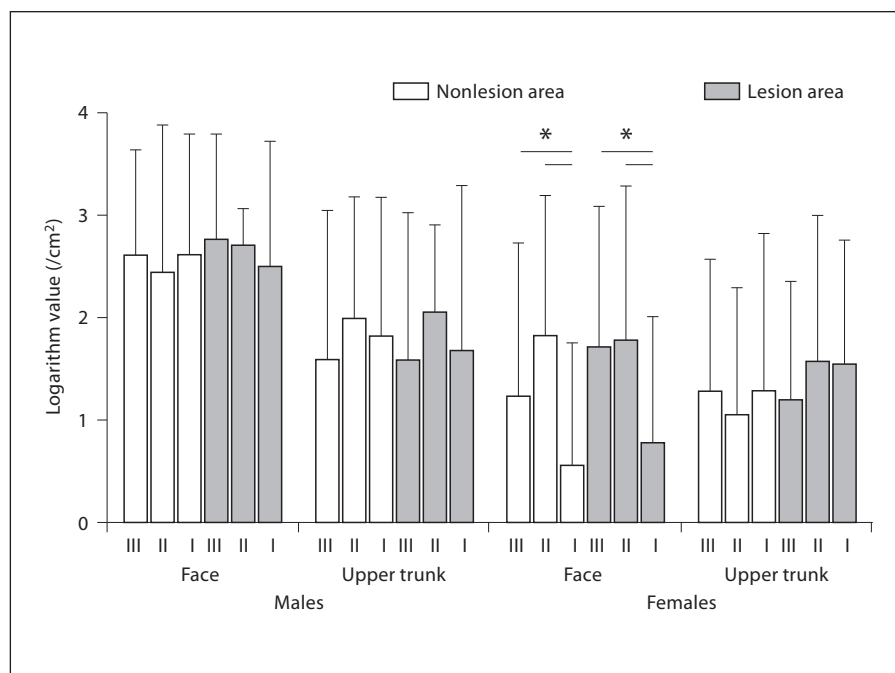
stricta and *M. globosa*. On the other hand, the number of *M. restricta* on the upper trunk of males was small, and the predominant species was *M. globosa*. In females, *M. globosa* was the predominant species on both the face and upper trunk.

The *Malassezia* species detected by the culture method were *M. restricta*, *M. globosa*, *M. furfur*, *M. obtusa* and *M. sympodialis*. *M. dermatis*, *M. slooffiae*, *M. japonica* and *M. yamatoensis* were not detected in any samples. There were no significant differences in the numbers of each *Malassezia* species between the lesion and nonlesion areas. The predominant *Malassezia* species was *M. globosa* (data not shown).

Relationship between Number of *S. aureus* and *Malassezia* Species and Clinical Severity of AD

The numbers of *S. aureus* for each clinical severity of AD are shown in figure 4. There were positive correlations between the number of *S. aureus* and clinical severity. The total numbers of *Malassezia* species for each clinical severity of AD are shown in figure 5. On the face of females, the numbers of *Malassezia* species in severe and moderate AD patients were greater than the number in mild AD patients. This result was due to the difference in the number of *M. globosa* (data not shown). There were no significant differences by clinical severity on the face of males or on the upper trunk of either gender.

Fig. 5. Total number of *Malassezia* species by the nonculture method for each clinical severity of AD. 10 severe AD males, 6 moderate AD males, 4 mild AD males, 19 severe AD females, 16 moderate AD females and 10 mild AD females were examined. Error bars: SD. I = Mild AD patients; II = moderate AD patients; III = severe AD patients. *Significant difference between each clinical degree ($p < 0.05$).



Effect of Medical Agents on Cutaneous Resident Microbiota

No statistical difference was found between AD patients using tacrolimus and those using steroids with regard to the total numbers of *Malassezia* species (assessed by both the nonculture method and the culture method), *S. aureus*, CNS, *Propionibacterium* species or non-*Malassezia* fungi (data not shown).

Discussion

The result of this study indicates that there was a difference in the cutaneous *Malassezia* microbiota of AD patients according to gender or body part, as also found in healthy subjects [27]. Moreover, there were differences between the results of the nonculture method and those of the culture method. Some previous studies have demonstrated the prevalence and species composition of *Malassezia* species in AD patients as well [20–25]. However, in these reports, the influences on the data of gender, body part and analytical method were hardly considered. Batra et al. [11] discussed the differences in sampling and identification methods used in various studies that may have contributed to the differences observed in the prevalence and species composition of *Malassezia* species. The results of the present study provide a basis for these

discrepancies. It was considered that it is necessary to consider analytical factors such as gender, body part and the experimental method when previous reports are quoted.

In the analysis of AD females by the nonculture method, the numbers of *M. globosa* on lesion areas showed positive correlations with clinical severity. Moreover, the number of *M. globosa* on the face of AD females was clearly greater than that on healthy females analyzed by same method [27]. *M. globosa* is a typical antigen in AD [7]. On the other hand, the numbers of *Malassezia* species including *M. globosa* in AD males, and on the upper trunk of AD females, were not greater than those on healthy subjects [27]. The present results suggest that the influence on inflammation by *Malassezia* antigens on the face of AD females may be greater than that on the upper trunk or on the face of AD males. There was a previous report that the efficacy of ketoconazole in the treatment of AD was most significant in female patients with positive *Malassezia* culture [30].

The numbers of CNS and *Propionibacterium* species on the face of AD patients were greater than those on the upper trunk, and the number of non-*Malassezia* fungi was small, as found on healthy subjects [27]. On the other hand, *Malassezia* microbiota on AD patients differed from those on healthy subjects. For example, the total number of *Malassezia* species on the face was greater

than that on the upper trunk in AD males, while there was no significant difference between the face and upper trunk in healthy subjects. Moreover, *Malassezia* species were detected on the face at the same level as on the upper trunk in AD females, while *Malassezia* species were hardly detected on the face of healthy subjects. In analyzing each *Malassezia* species, it was characteristic that the number of *M. sympodialis*, which was the predominant species on the upper trunk of healthy subjects, was small. This study clarified that the cutaneous *Malassezia* microbiota on AD patients differed from that on healthy subjects. It was considered that the elucidation of this clarifies the relationship of AD to *Malassezia* species.

The numbers of *S. aureus* related closely to the lesions in AD patients and were greater than those in nonlesion areas of AD patients, which has previously been noted [31]. However, for *Malassezia* species, both the total numbers and the species composition did not show significant differences between them. There is also a report that the detection rate of *Malassezia* species in lesion areas was lower than that in nonlesion areas [24]. These results may indicate that *Malassezia* species do not induce skin inflammation immediately but via the action of antigens on the lesional skin, which has had its barrier function damaged due to the primary inflammation.

The total numbers of *Malassezia* species in AD patients assessed by the nonculture method were greater than those found by the culture method in many cases. One reason may be that *M. restricta* constituted the detection limit for the culture method but is a predominant species [26]. However, the numbers of cultivatable *Malassezia* species, i.e. *M. globosa*, *M. dermatis*, *M. furfur* and *M. obtusa*, were also smaller than those assessed by the nonculture method. These results differed from those of healthy subjects [27]. It was indicated that the proportion of dead cells out of the total number of *Malassezia* species in AD patients was large compared with healthy subjects. It may be considered that dead *Malassezia* cells are not adequately excreted from the skin due to a reduction in skin renewal in AD patients because the impaired epidermal proliferation and disturbed differentiation show on the skin of AD patients [32]. Moreover, there is a possibility that the use of medical agents for AD may increase the amount of dead *Malassezia* cells.

In AD treatment, various topical agents are used, such as tacrolimus, a therapeutic agent, and steroids. In this study, most AD subjects used either of these medicines. It was reported that tacrolimus has an antifungal effect on some *Malassezia* strains [33]. Moreover, it is generally known that steroids affect sebum secretion. However, in

the present study, no statistical difference was found between AD patients using tacrolimus and those using steroids. In *Malassezia* microbiota on the skin of AD patients, there may not be much influence of topical agents. It was also considered that studies with subjects in whom the use of agents was completely controlled are necessary to clarify the influence of medicines on cutaneous *Malassezia* species.

In this study, the existence of a *mecA* gene in MRSA was also analyzed. A previous report suggested that the detection rate of MRSA from the skin of AD patients was 43.8% in AD inpatients and 29.4% in AD outpatients [34]. Moreover, there has been a report as well that the detection rate of MRSA from pediatric AD outpatients was 18.3% [35]. In this study of AD outpatients, the rate of detection of MRSA was 2.3% (6/261). These results may suggest that the infection rate of MRSA on the skin of AD patients is strongly influenced by the medical facilities and environment of these different studies.

In conclusion, this study clarified that cutaneous *Malassezia* species on AD patients differ by gender, body part and analytical method. This result indicates the possibility that the influence of *Malassezia* antigens is different according to gender or body part. It is also necessary to consider the suitability of the examination method when cutaneous *Malassezia* microbiota in AD patients are examined. Moreover, it was clarified that *Malassezia* microbiota on AD patients differ from those on healthy subjects in that the influence on inflammation by *Malassezia* antigens on the face of AD females may be greater, but there was no difference in *Malassezia* microbiota between lesion and nonlesion areas in AD, and that the proportion of dead cells of *Malassezia* species in AD patients was larger.

Disclosure Statement

The authors report no conflicts of interest.

References

- 1 Gupta AK, Batra R, Bluhm R, Boekhout T, Dawson TL: Skin disease associated with *Malassezia* species. *J Am Acad Dermatol* 2004;51:785–798.
- 2 Jensen-Jarolim E, Poulsen LK, With H, Kieffer M, Ottevanger V, Stahl SP: Atopic dermatitis of the face, scalp, and neck: type 1 reaction to the yeast *Pityrosporum ovale*? *J Allergy Clin Immunol* 1992;89:44–51.

- 3 Kieffer M, Bergbrant IM, Faergemann J, Jemec GB, Ottevanger V, Stahl SP, Svejgaard E: Immune reactions to *Pityrosporum ovale* in adult patients with atopic and seborrheic dermatitis. *J Am Acad Dermatol* 1990;22:739–742.
- 4 Tengvall LM, Johansson C, Scheynius A, Wahlgren C: Positive atopy patch test reactions to *Pityrosporum orbiculare* in atopic dermatitis patients. *Clin Exp Allergy* 2000;30:122–131.
- 5 Kim TY, Jang IG, Park YM, Kim HO, Kim CW: Head and neck dermatitis: the role of *Malassezia furfur*, topical steroid use and environmental factors in its causation. *Clin Exp Dermatol* 1999;24:226–231.
- 6 Savolainen J, Lintu P, Kosonen J, Kortekangas-Savolainen O, Viander M, Pène J, Kalimo K, Terho EO, Bousquet J: *Pityrosporum* and *Candida* specific and non-specific humoral, cellular and cytokine responses in atopic dermatitis patients. *Clin Exp Allergy* 2001;31:125–134.
- 7 Koyama T, Kanbe T, Ishiguro A, Kikuchi A, Tomita Y: Isolation and characterization of a major antigenic component of *Malassezia globosa* to IgE antibodies in sera of patients with atopic dermatitis. *Microbiol Immunol* 2000;44:373–379.
- 8 Koyama T, Kanbe T, Ishiguro A, Kikuchi A, Tomita Y: Antigenic components of *Malassezia* species for immunoglobulin E antibodies in sera of patients with atopic dermatitis. *J Dermatol Sci* 2001;26:201–208.
- 9 Bayrou O, Pecquet C, Flahault A, Artigou C, Abuaf N, Leynadier F: Head and neck atopic dermatitis and *Malassezia-furfur*-specific IgE antibodies. *Dermatology* 2005;211:107–113.
- 10 Kato H, Sugita T, Ishibashi Y, Nishikawa A: Detection and quantification of specific IgE antibodies against eight *Malassezia* species in sera of patients with atopic dermatitis by using an enzyme-linked immunosorbent assay. *Microbiol Immunol* 2006;50:851–856.
- 11 Batra R, Boekhout T, Guého E, Cabañes FJ, Dawson TL Jr, Gupta AK: *Malassezia* Bailon, emerging clinical yeasts. *FEMS Yeast Res* 2005;5:1101–1113.
- 12 Guého E, Midgley G, Guillot J: The genus *Malassezia* with description of four new species. *Antonie Van Leeuwenhoek* 1996;69:337–355.
- 13 Guillot J, Guého E: The diversity of *Malassezia* yeasts confirmed by rRNA sequence and nuclear DNA comparison. *Antonie Van Leeuwenhoek* 1995;67:297–314.
- 14 Guillot J, Guého E, Chermette R: Confirmation of the nomenclatural status of *Malassezia pachydermatis*. *Antonie Van Leeuwenhoek* 1995;67:173–176.
- 15 Sugita T, Takashima M, Shinoda T, Suto H, Unno T, Tsuboi R, Ogawa H, Nishikawa A: New species, *Malassezia dermatis*, isolated from patients with atopic dermatitis. *J Clin Microbiol* 2002;40:1363–1367.
- 16 Sugita T, Takashima M, Kodama M, Tsuboi R, Nishikawa A: Description of a new yeast species, *Malassezia japonica*, and its detection in patients with atopic dermatitis and healthy subjects. *J Clin Microbiol* 2003;41:4695–4699.
- 17 Sugita T, Tajima M, Takashima M, Amaya M, Saito M, Tsuboi R, Nishikawa A: A new yeast, *Malassezia yamatoensis*, isolated from a patient with seborrheic dermatitis, and its distribution in patients and healthy subjects. *Microbiol Immunol* 2004;48:579–583.
- 18 Hirai A, Kano R, Makimura K, Duarte ER, Hamdan JS, Lachance MA, Yamaguchi H, Hasegawa A: *Malassezia nana* sp. nov., a novel lipid-dependent yeast species isolated from animals. *Int J Syst Evol Microbiol* 2004;54:623–627.
- 19 Cabañes FJ, Theelen B, Castellá G, Boekhout T: Two new lipid-dependent *Malassezia* species from domestic animals. *FEMS Yeast Res* 2007;7:1064–1076.
- 20 Nakabayashi A, Sei Y, Guillot J: Identification of *Malassezia* species isolated from patients with seborrheic dermatitis, atopic dermatitis, pityriasis versicolor and normal subjects. *Med Mycol* 2000;38:337–341.
- 21 Gupta AK, Kohli Y, Summerbell RC, Faergemann J: Quantitative culture of *Malassezia* species from different body sites of individuals with or without dermatoses. *Med Mycol* 2001;39:243–251.
- 22 Sugita T, Suto H, Unno T, Tsuboi R, Ogawa H, Shinoda T, Nishikawa A: Molecular analysis of *Malassezia* microflora on the skin of atopic dermatitis patients and healthy subjects. *J Clin Microbiol* 2001;39:3486–3490.
- 23 Sugita T, Tajima M, Tsuboku H, Tsuboi R, Nishikawa A: Quantitative analysis of cutaneous *Malassezia* in atopic dermatitis patients using real-time PCR. *Microbiol Immunol* 2006;50:549–552.
- 24 Sandström Falk MH, Tengvall Linder M, Johansson C, Bartosik J, Bäck O, Särnhult T, Wahlgren CF, Scheynius A, Faergemann J: The prevalence of *Malassezia* yeasts in patients with atopic dermatitis, seborrheic dermatitis and healthy controls. *Acta Derm Venerol* 2005;85:17–23.
- 25 Tajima M, Sugita T, Nishikawa A, Tsuboi R: Molecular analysis of *Malassezia* microflora in seborrheic dermatitis patients: comparison with other diseases and healthy subjects. *J Invest Dermatol* 2008;128:345–351.
- 26 Akaza N, Akamatsu H, Sasaki Y, Kishi M, Mizutani H, Sano A, Hirokawa K, Nakata S, Nishijima S, Matsunaga K: *Malassezia* folliculitis is caused by cutaneous resident *Malassezia* species. *Med Mycol* 2009;47:618–624.
- 27 Akaza N, Akamatsu H, Sasaki Y, Takeoka S, Kishi M, Mizutani H, Sano A, Hirokawa K, Nakata S, Matsunaga K: Cutaneous *Malassezia* microbiota of healthy subjects differ by sex, body part, season, and analytical method. *J Dermatol*, in press.
- 28 Furue M, Saeki H, Furukawa F, Hide M, Ohtsuki M, Nakamura T, Sasaki R, Suto H, Takehara K: Guideline for management of atopic dermatitis (in Japanese). *Jpn J Dermatol* 2008;118:325–342.
- 29 Leeming JP, Notman FH: Improved methods for isolation and enumeration of *Malassezia furfur* from human skin. *J Clin Microbiol* 1987;25:2017–2019.
- 30 Lintu P, Savolainen J, Kortekangas-Savolainen O, Kalimo K: Systemic ketoconazole is an effective treatment of atopic dermatitis with IgE-mediated hypersensitivity to yeasts. *Allergy* 2001;56:512–517.
- 31 Baker BS: The role of microorganisms in atopic dermatitis. *Clin Exp Immunol* 2006;144:1–9.
- 32 Jensen JM, Fölster-Holst R, Baranowsky A, Schunck M, Winoto-Morbach S, Neumann C, Schütze S, Proksch E: Impaired sphingomyelinase activity and epidermal differentiation in atopic dermatitis. *J Invest Dermatol* 2004;122:1423–1431.
- 33 Sugita T, Tajima, Ito T, Saito M, Tsuboi R, Nishikawa A: Antifungal activities of tacrolimus and azole agents against the eleven currently accepted *Malassezia* species. *J Clin Microbiol* 2005;43:2824–2829.
- 34 Akiyama H, Yamasaki O, Tada J, Arata J: Adherence characteristics and susceptibility to antimicrobial agents of *Staphylococcus aureus* strains isolated from skin infections and atopic dermatitis. *J Dermatol Sci* 2000;23:155–160.
- 35 Chung H, Jeon H, Sung H, Kim M, Hong S: Epidemiological characteristics of methicillin-resistant *Staphylococcus aureus* isolates from children with eczematous atopic dermatitis lesions. *J Clin Microbiol* 2008;46:991–995.