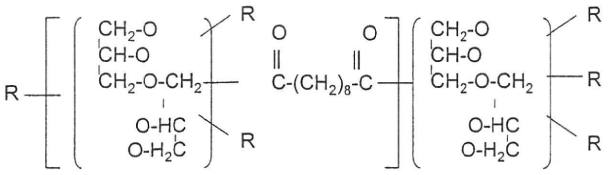

Fig 7. Chemical structure of isopalmityl diglyceryl sebacate (DGS)

陽性反応を呈し，最低惹起濃度を決定することができなかった。過去の報告例における DGS の PT 陽性最低濃度は 0.05% pet.であり[7]，さらに低濃度で貼布すべきであったと考えている。また，化粧品関連アレルゲンの Balsam of Peru と PPD にも陽性であったが，提供された成分には含まれていなかった．患者は5，6年前から毛染めをしているが，ヘアダイ皮膚炎の既往はないため，PPD の陽性反応は硫酸フラジオマイシンの近傍に貼布したために起こった excited skin syndrome の可能性があると考えた。

症例9は，PT で PPD，PAP，*p*-Aminoazobenzene に陽性を呈した。PPD は芳香族アミンの一つで，強い感作能を有し，永久染毛剤による接触皮膚炎の最も多い原因成分である[8]。また，PPD は，ベンゾカイン，プロカイン，アゾ色素，パラアミノサリチル酸，パラアミノ安息香酸，サルファ剤などのパラアミノ化合物と化学構造が類似しており交叉反応を生じる[9]ことが知られている。本症例においても，PPD とパラアミノ化合物に陽性を呈しており，両者は交叉反応であったと考えられる。

接触皮膚炎症候群とは，遅延型接触アレルギーの成立後もその原因アレルゲンが，さらに経皮的に吸収され，全身に皮疹が拡大した場合をいい[10]，Fisher が定義した接触皮膚炎を引き起こす抗原が，経口，吸入，注射など非経皮的に吸収された場合の全身性接触皮膚炎と区別される。症例9はアレルゲンが頭や手から経皮的に吸収され，全身に散布疹を生じたことから，接触皮膚炎症候群と考えた。しかし，伝染性膿痂疹が合併していた可能性も考えられる。

本例は今後も毛染めの希望があり，Maron（シュワルツコフ・ヘンケル製）という，パラアミノ化合物を含まないヘアダイ（鉄剤）のオープンテストを施行したところ，陰性であったため，現在患者は Maron で染毛することができ，皮疹の再燃はない。その他のパラアミノ化合物を含まないヘアダイとして henna（植物成分）があるが，henna と称するヘアダイの中には，PPD が含まれているものがある[9]ため，十分に成分を確かめるか，使用テストを施行してから用いることが望ましい。また，PPD を含まない染毛剤がヘアマニキュアやヘアカラーであるが，これらの中にも PAP や *p*-Aminotoluene などのパラアミノ化合物を含有しているものがあるため，PPD にアレルギーがある場合，注意が必要である。

2007 年は当院において新製品の出現による新たなアレルゲンは認めなかったが，陽性製品数の移り変わりを比較すると 1992 年～94 年，95 年～97 年，2006 年，2007 年と，毎年口紅や日焼け止め製品による接触皮膚炎が発生している（**Fig. 6**）。われわれが医学中央雑誌を調べ得た限りでは，2007 年に新しい化粧品アレルゲンの報告はなかった。そして今後も新しい成分を含有した新種の化粧品だけではなく，これまでに報告されている化粧品による接触皮膚炎が今なお発生していることを忘れてはならず，PT を積極的に行い，原因を追究することが大切であると考えた。

結　論

2007 年に持参香粧品による PT 陽性であった製品を検討し，以下の結論が得られた。

1．1年間に PT で持参香粧品に陽性反応を示したのは 61 例中 10 例（16.4%）であった。

2．陽性製品数の移り変わりは 1992 年～94 年，95 年～97 年，2006 年，2007 年と，毎年，口紅・日焼け止め製品による接触皮膚炎が発生していた。

3．当院において新製品の出現による新たなアレルゲンは認めなかった。

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Patch Test Results of Cosmetic Products and Allergens in 2007

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We investigated current tendencies in cosmetic dermatitis by patch testing of cosmetic products and allergens in 2007.

A total of 61 subjects were suspected of having cosmetic dermatitis and underwent patch testing in 2007. We performed patch testing on the backs of subjects for 48 hours using the Japanese standard series and cosmetic products that each subject had brought in. Patch test reactions were interpreted in accordance with the International Contact Dermatology Research Group (ICDRG) recommendations. We evaluated stronger or equal to (+) as a positive reaction.

Among those tested, 10 subjects exhibited positive reactions to cosmetics. The types of products involved were as follows: hair dyes (2), lipsticks (2), creams (2), UV creams (2), shampoos (2) and makeup foundation (1).

No new allergens were identified as a cause of cosmetic dermatitis in 2007.

(J Environ Dermatol Cutan Allergol, 4 (2): 89-98, 2010)

Key words: contact dermatitis, patch test, cosmetic products, isopalmityl diglyceryl sebacate, hair dye

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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.

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

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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* (nonlipophylic) 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 cm^2 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 targeted 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)/cm^2, and that of the nonculture method was 50 cells/cm^2 [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/cm^2; culture method for *Malassezia* species: 5 CFU/cm^2; culture method for other cutaneous resident microorganisms: 10 CFU/cm^2) was assumed to be 10^0/cm^2. 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-

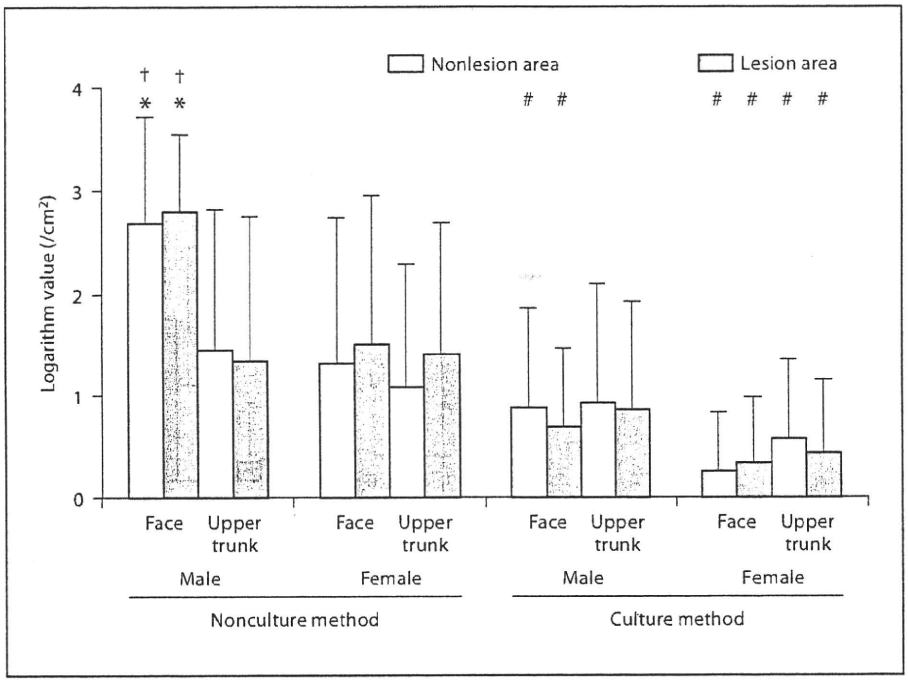

Fig. 1. Numbers of *Malassezia* species on the skin of AD patients. The total numbers of *Malassezia* species by the nonculture method and the culture method in AD patients are shown. The samples from 20 AD males and 47 AD females were examined. Error bars: SD. *Significant difference versus upper trunk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

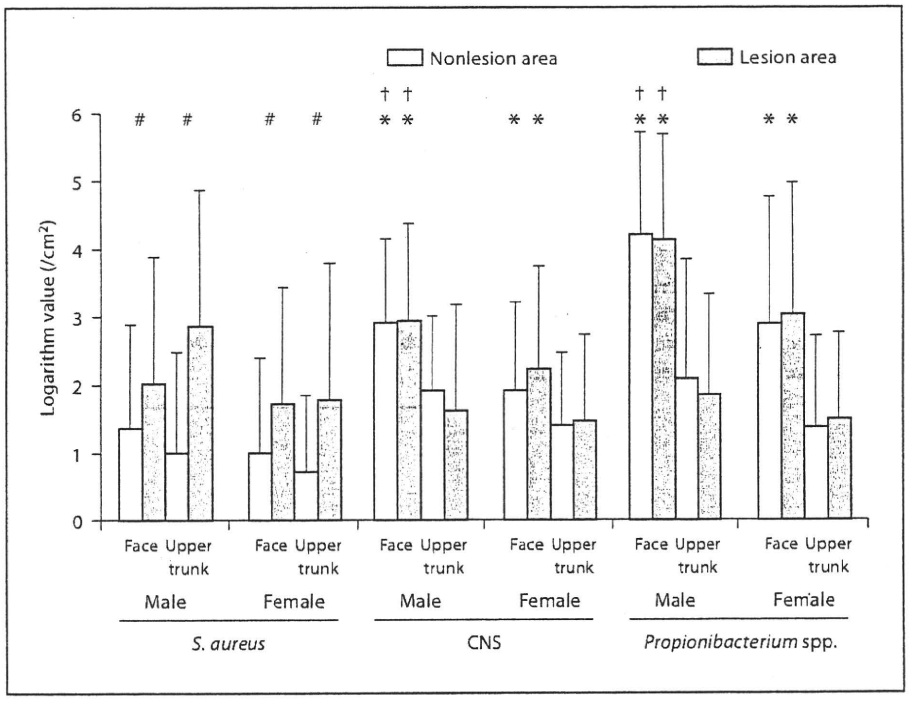

Fig. 2. Numbers of *S. aureus*, CNS and *Propionibacterium* species on the skin of AD patients. The total numbers of *S. aureus*, CNS and *Propionibacterium* species by the culture method in AD patients are shown. The samples from 20 AD males and 47 AD females were examined. Error bars: SD. *Significant 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 method. 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

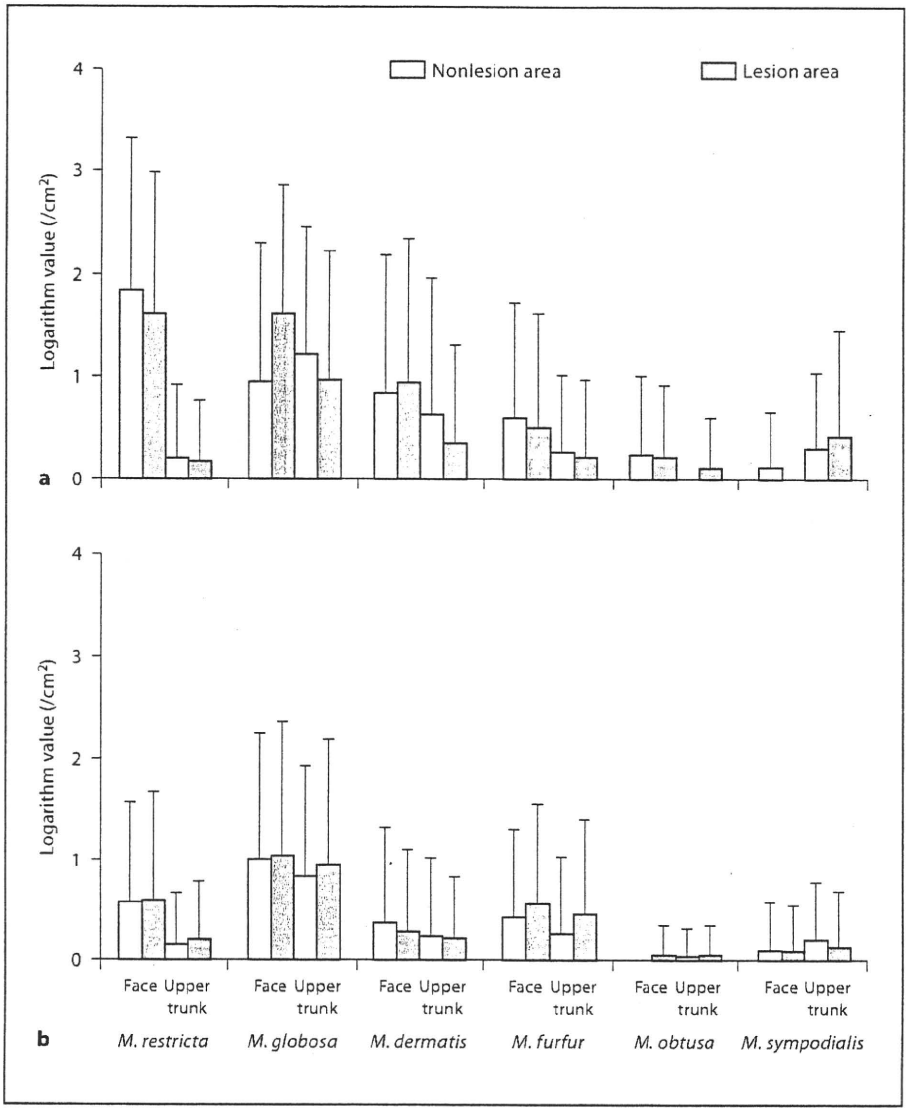

Fig. 3. *Malassezia* species detected from the skin of AD patients by the nonculture method. The samples from 20 AD males (**a**) and 47 AD females (**b**) were examined. *M. slooffiae, M. japonica* and *M. yamatoensis* were not detected in any samples. Error bars: SD.

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 *Propionibacterium* 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)/cm^2, 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).

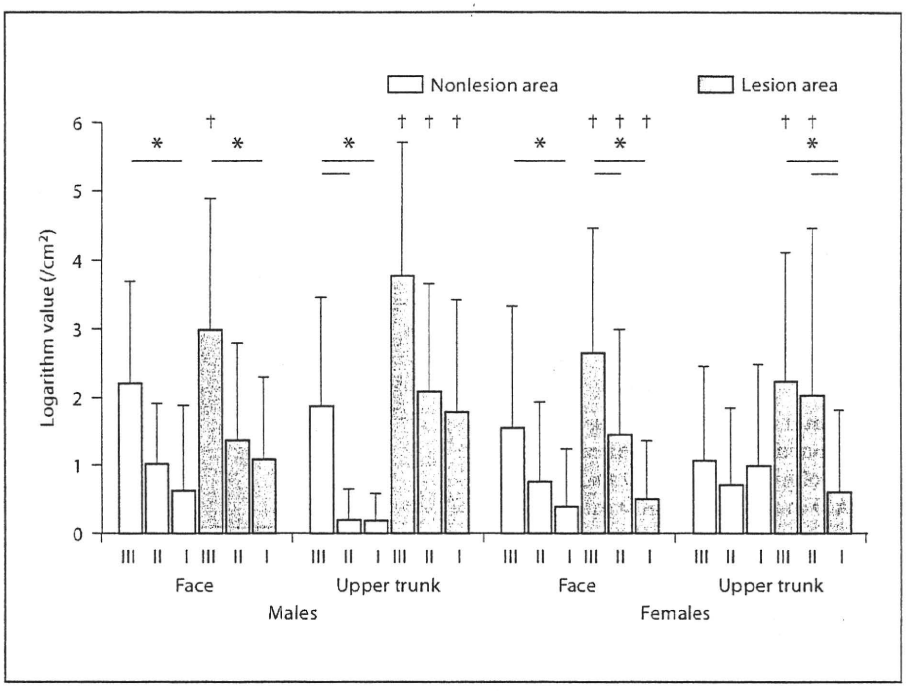

Fig. 4. Number of *S. aureus* for each clinical severity of A D. 10 severe A D males, 6 moderate AD males, 4 mild AD males, 19 severe A D females, 16 m oderate A D females and 10 mild AD females were examined. Error bars: SD. I = Mild AD patients; II = moderate AD patients; III = severe AD patients. *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. restricta* 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 t he n umber o f *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.

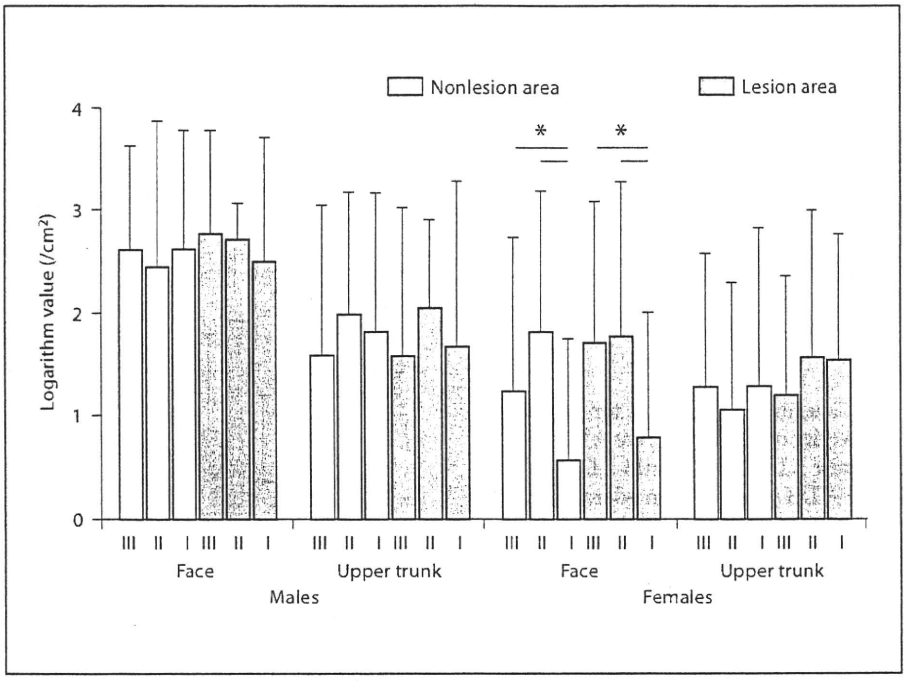

Fig. 5. Total number of *Malassezia* species by the nonculture method for each clinical severity of AD. 10 severe AD males, 6 moderate AD males, 4 mild AD males, 19 severe AD females, 16 moderate AD females and 10 mild AD females were examined. Error bars: SD. I = Mild AD patients; II = moderate AD patients; 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.

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ORIGINAL ARTICLE

Cutaneous *Malassezia* microbiota of healthy subjects differ by sex, body part and season

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ABSTRACT

Malassezia is a component of normal cutaneous resident microbiota. The aim of this study was to quantitatively clarify the differences in cutaneous *Malassezia* microbiota in healthy subjects by sex, body part and season. Samples were collected from the forehead, cheek, upper chest and upper back of 20 healthy men and 20 healthy women (average age 32 years) in summer and winter by the swab method. *Malassezia* DNA was analyzed using a real-time PCR system. As a result, in sex, body parts and season, men, the upper trunk and summer showed the highest total numbers of cutaneous *Malassezia* species on average. There were also differences depending on the analytical method. The predominant species were *M. restricta* on the face of men, *M. globosa* and *M. dermatis* on the upper trunk of men, and *M. globosa* and *M. sympodialis* on the upper trunk of women. This study clarified that the cutaneous *Malassezia* microbiota of healthy subjects differed by sex, body part and season.

Key words: cutaneous resident microbiota, healthy subject, *Malassezia*, *Propionibacterium*, real-time polymerase chain reaction, *Staphylococcus*.

INTRODUCTION

The genus *Malassezia* consists of basidiomycetous yeasts associated with *Malassezia* folliculitis, pityriasis versicolor, atopic dermatitis, seborrheic dermatitis, dandruff and psoriasis, although they are also members of the normal resident flora on human skin.[1–3] This genus consists of 13 species.[4–11] The taxonomy of genus *Malassezia* was revised in 1996 to include the following seven species: *M. furfur*, *M. globosa*, *M. obtusa*, *M. restricta*, *M. pachydermatis*, *M. slooffiae* and *M. sympodialis*.[4] Moreover, six new species, *M. dermatis*, *M. japonica*, *M. yamatoensis*, *M. nana*, *M. caprae* and *M. equina*, were proposed in 2000.[7–11]

Clarification of the normal cutaneous resident *Malassezia* microbiota is indispensable for investigations into the cause of *Malassezia*-related disorders. There are several previous reports on the *Malassezia* microbiota of healthy subjects,[12–18] although differences by sex, body part and season of *Malassezia* species isolation from healthy subjects have not been investigated thoroughly. Moreover, these previous studies used either non-culture or culture methods, although there is a report that there were differences between the results from non-culture methods and those from culture methods in an analysis of *Malassezia* species in *Malassezia* folliculitis.[19] The aim of this study was to quantitatively clarify the differences in cutaneous *Malassezia* microbiota in healthy subjects by sex, body part, season, and analytical method. At the same time, the predominant microorganisms in the cutaneous resident microbiota, namely *Propionibacterium* species and *Staphylococcus* species, and non-*Malassezia* (non-lipophilic) fungi were

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also analyzed in order to compare with cutaneous *Malassezia* microbiota.

METHODS

Subjects

The study design was reviewed and approved by the Institutional Review Board of Fujita Health University. The authors took the informed consent from all the subjects. Forty healthy Japanese subjects who did not have a skin disease (20 men aged 33 ± 8 years [24–47] and 20 women aged 32 ± 6 [24–43]) were analyzed from June to September (summer). In addition, 40 healthy Japanese subjects (20 men aged 33 ± 7 years [25–46] and 20 women aged 31 ± 6 [24–43]) were analyzed from January to February (winter). All subjects were indoor workers. We took samples from 26 subjects (15 men and 11 women) in both summer and winter, from 24 subjects in only summer and from 24 subjects in only winter.

Sample collection

Samples were collected from 10 cm^2 of skin surface on the forehead, cheek, upper chest and upper back of healthy subjects by a swab method using 5 mL of phosphate buffer containing 2% polysorbate 80 (pH 7.0) in the morning between 09.00 and 12.00 hours. We collected the samples from the skin without make-up in the female subjects.

Quantitative analysis of *Malassezia* species

Malassezia species were identified using both non-culture and culture methods. For analysis by the non-culture method, *Malassezia* DNA was extracted directly from 3 mL of the collected samples. *Malassezia* strains picked from the culture medium and the collected samples were placed in lysing solution (100 mmol/L Tris-HCl, 10 mmol/L ethylene diamine tetra acetate (EDTA), 0.5% sodium dodecyl sulfate, pH 8.0) and incubated for 15 min at 100°C.[14] The suspension was extracted with phenol-chloroform-isoamyl alcohol (25:24:1). Subsequently, the samples were extracted with chloroform-isoamyl alcohol (24:1) and DNA was precipitated using 2-propanol, using Dr genTLE precipitation carrier (Takara, Shiga, Japan). The DNA pellet was resuspended in TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0). For analysis by the culture method, 0.1 mL of the collected samples were cultivated at 32°C on Leeming and Notman agar medium (LNA) for 14 days.[20] The colonies that appeared were sub-cultured on LNA for 2–7 days for purification and extraction of *Malassezia* DNA.

Table 1. Oligonucleotides used in the detection of *Malassezia* DNA

Species		Sequence (5′→3′)
Malassezia globosa	Forward	GGCCAAGCGCGCTCT
	Reverse	CCACCACCAAATGCTCTCCTACAG
Malassezia sympoctalis	Forward	GACCCTCGCTACCGCTCTCT
	Reverse	GCCCACACACAGCAAATGAC
Malassezia restricta	Forward	TGCCATGAAATCTCCCAC
	Reverse	AGGCACCCATCCAGACCCCAT
Malassezia dermatitis	Forward	GGCTGGATGCCTGGTGTATT
	Reverse	CCTTCTCCGGCGACTCAA
Malassezia furfur	Forward	CCCAAGCGGTTGCGATT
	Reverse	CCTCCCTTTCAGAGCGGTTT
Malassezia obtusa	Forward	GGTTCCCACCCGTTAGCCA
	Reverse	GCACACAGCAAATGAC
Malassezia slooffiae	Forward	ATCCACTATTTATCCACAAA
	Reverse	CCGGACGCCATTAAGCAA
Malassezia japonica	Forward	GACTGCTGATAATGCTCCAGT
	Reverse	GTCTGCTGATAAGTCTCACTG
Malassezia yamatoensis	Forward	TGAATTCTCTCCCCCCTTTG
	Reverse	GGCATGGCCCATCCAA

Malassezia DNA was analyzed by the same method as in the previous study, using a real-time PCR system (7300 Real Time PCR system; Applied Biosystems, Foster City, CA, USA) and a quantitative PCR reagent (Platinum SYBR Green qPCR SuperMix-UDG with ROX; Invitrogen, Carlsbad, CA, USA).[19] The primer sets used are shown in Table 1. Nine *Malassezia* species isolated from human skin were targeted in this study. The standard curves of each *Malassezia* species using quantitative analysis of the cutaneous *Malassezia* microbiota were generated with *M. globosa* CBS 7966, *M. restricta* CBS 7877, *M. sympodialis* CBS 7222, *M. furfur* NBRC 0656, *M. obtusa* CBS 7876, *M. slooffiae* CBS 7956, *M. dermatis* JCM 11348, *M. japonica* JCM 11963 and *M. yamatoensis* CBS 9725 counted beforehand using a bacteria counting chamber.

Quantitative analysis of other cutaneous resident microorganisms

For the analysis of *Propionibacterium* species, 0.05 mL collected samples or the buffer diluted appropriately were cultivated on modified GAM 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 as *Propionibacterium* species. For the analysis of *Staphylococcus* species, the samples were cultivated on mannitol salt agar medium (Eiken Chemical, Tokyo, Japan) at 35°C for 3 days. *Staphylococcus* species were distinguished into *S. aureus* and coagulase-negative staphylococci (CNS) by the resolvability of mannitol and coagulase test (Eiken Chemical). The samples were cultivated on potato dextrose agar medium (Nihon Pharmaceutical, Tokyo, Japan) at 32°C for 7 days for the isolation of non-*Malassezia* fungi.

Statistical analysis

In the quantitative analysis, the number of cells less than the detection limit (non-culture method for *Malassezia* species, 50 cells/cm^2; culture method for *Malassezia* species, 5 colony forming units [CFU]/cm^2; culture method for other cutaneous resident microorganism, 10 CFU/cm^2) was assumed to be $10^0/cm^2$. The Student's *t*-test and paired *t*-test were used for statistical analysis in this study.

RESULTS

Cutaneous resident microbiota in healthy subjects

The total numbers of *Malassezia* species (assessed by both the non-culture method and the culture method), *Propionibacterium* species, CNS and non-*Malassezia* fungi on the skin of healthy subjects are shown in Figure 1. For the total number of *Malassezia* species by the non-culture method in men, there were no differences by body part or season. On the other hand, in women, the total number of *Malassezia* species on the forehead and cheek were less than that on the upper chest and upper back. In the comparison of men and women, the total numbers of *Malassezia* species on men were greater than that on women.

For the total numbers of *Malassezia* species by the culture method in men, there were less on the forehead and cheek than on the upper chest and upper trunk. The numbers in summer were greater than those in winter on the forehead and upper back. In women, as with men, the numbers on the forehead and cheek were less than those on the upper chest and upper back. The numbers on the forehead in summer were greater than those in winter. In the comparison of men and women, the numbers of total *Malassezia* species on men were greater than those on females.

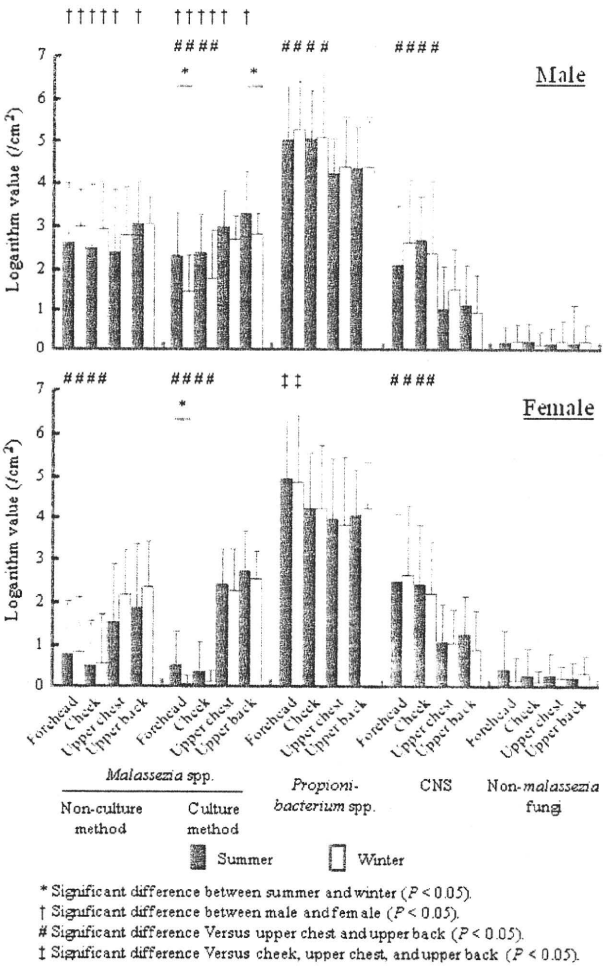

Figure 1. Cutaneous resident microbiota of healthy subjects. The total numbers of *Malassezia* species by the non-culture method and the culture method, *Propionibacterium* species, coagulase-negative staphylococci (CNS) and non-*Malassezia* fungi by culture methods on the skin of healthy subjects are shown. Twenty samples from healthy men and 20 samples from healthy women were examined. Error bars show standard deviation. The Student's *t*-test was used in the statistical analysis.

The number of *Propionibacterium* species on the forehead and cheek were greater than those on the upper chest and upper back in men. In women, the number of *Propionibacterium* species was the greatest on the forehead. All *Staphylococcus* spp. detected from the skin of healthy subjects were CNS. The numbers of CNS on the forehead and cheek

were greater than those on the upper chest and upper back in each sex. There are no significant differences by sex or season for *Propionibacterium* species and CNS. For the number of non-*Malassezia* species, there were no significant differences by body part, sex or season.

Changes in cutaneous resident microbiota of healthy subjects during summer and winter

The changes in the total number of *Malassezia* species (assessed by both the non-culture method and the culture method) on the skin of each healthy subject during summer and winter are shown in Figure 2.

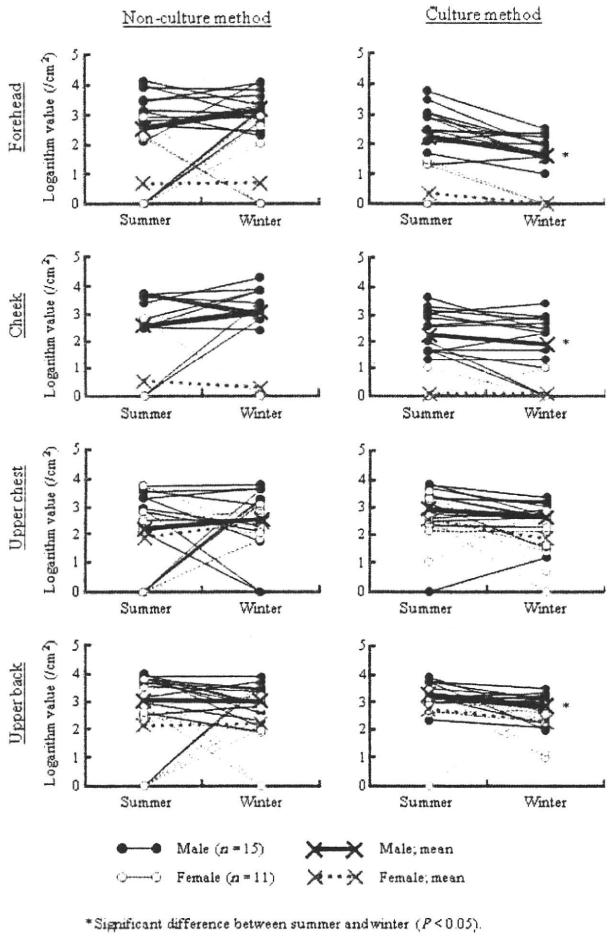

Figure 2. Changes in cutaneous resident microbiota of healthy subjects in summer and winter. The total numbers of *Malassezia* species by the non-culture method and the culture method, and coagulase-negative staphylococci by a culture method on the skin of each healthy subject are shown. Fifteen samples from healthy men and 11 from healthy women were examined. The Student's paired *t*-test was used in the statistical analysis.

For the total number of *Malassezia* species by the non-culture method, there were no differences according to season on any body part in either men or women. On the other hand, using the culture method, the numbers on the forehead, cheek and upper back in summer were greater than those in winter on men.

Malassezia species detected from the skin of healthy subjects by the non-culture method

Malassezia species detected by the non-culture method are shown Figure 3. The predominant species on the forehead and cheek of men was *M. restricta*. On the other hand, the predominant species on the upper chest and upper back of men were *M. globosa* and *M. dermatis*. Moreover, the

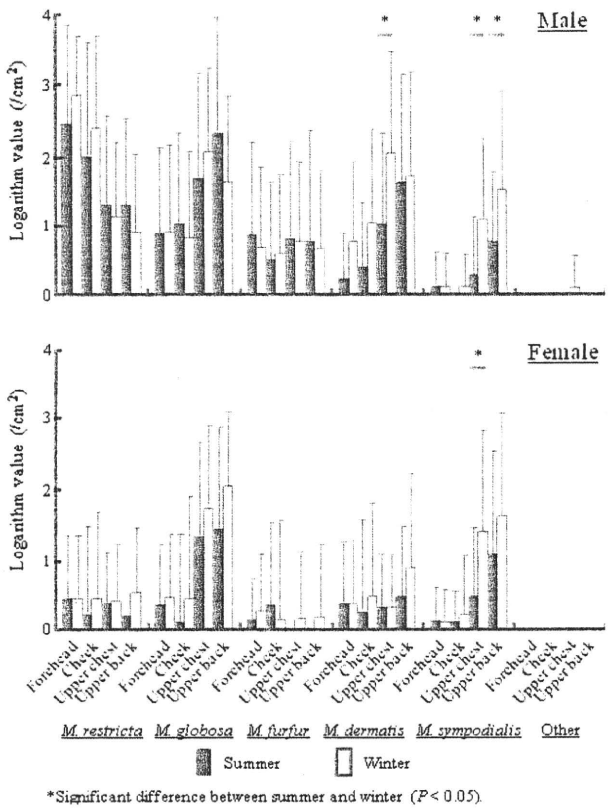

Figure 3. *Malassezia* species detected on the skin of healthy subjects by the non-culture method. Twenty samples from healthy men and 20 samples from healthy women were examined. Error bars show standard deviation. *M. restricta*, *M. globosa*, *M. furfur*, *M. dermatis*, *M. sympodialis* and *M. yamatoensis* were detected. *M. obtusa*, *M. slooffiae* and *M. japonica* were not detected in any samples. The Student's *t*-test was used in the statistical analysis.

predominant species on the upper chest and upper back of women were *M. globosa* and *M. sympodialis*. In the comparison of summer and winter, the number of *M. dermatis* on the upper chest, and *M. sympodialis* on the upper chest and upper back of men in winter were greater than that in summer. Moreover, the number of *M. sympodialis* on the upper chest of women in winter was greater than that in summer.

Malassezia species detected from the skin of healthy subjects by the culture method

Malassezia species detected by the culture method are shown Figure 4. The predominant *Malassezia* species were the same as the results by the non-culture method. Moreover, *M. sympodialis* was also readily detected from the upper chest and upper back of men. In the comparison of summer and winter, the numbers of *M. restricta* on the upper back, and *M. globosa* on the forehead and upper back in summer of men were greater than those in winter. Moreover, the number of *M. globosa* on the forehead of women in summer was greater than that in winter.

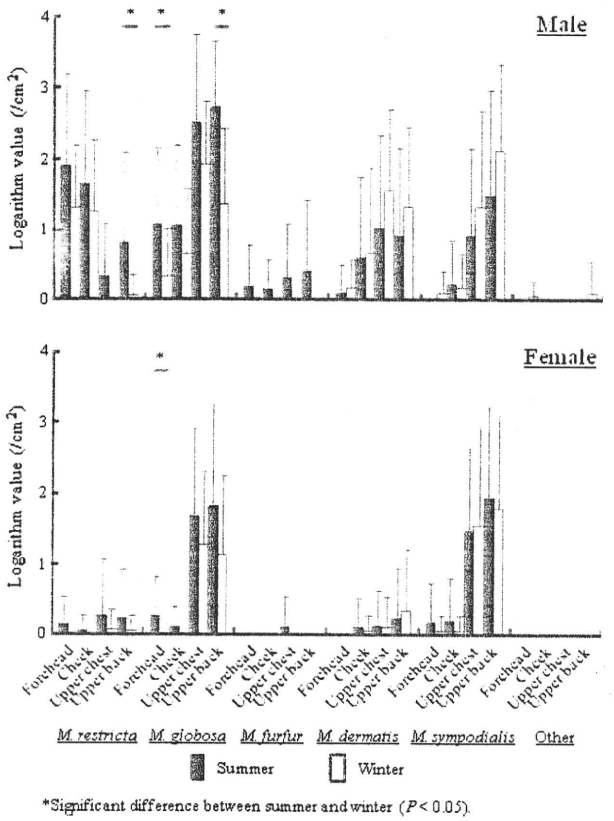

Figure 4. *Malassezia* species detected on the skin of healthy subjects by the culture method. Twenty samples from healthy men and 20 samples from healthy women were examined. Error bars show standard deviation. *M. restricta*, *M. globosa*, *M. furfur*, *M. dermatis*, *M. sympodialis*, *M. obtusa* and *M. slooffiae* were detected. *M. yamatoensis* and *M. japonica* were not detected in any samples. The Student's *t*-test was used in the statistical analysis.

DISCUSSION

This study clarified the differences in the total number of cutaneous *Malassezia* species on healthy subjects by sex, body part, season and analytical method. In the comparison of sex, the number on men was greater than that on women for each body part. It was considered that this difference by sex depends on the difference in the amounts of sebum by sex.[21]

In the comparison of body parts, on men, although the results by the culture method were the same as the result of women, there were no significant differences between the number on the face and on the upper trunk in the analytical results from the non-culture method. The face of men had a high proportion of *M. restricta*, and it was considered that a lot of non-cultivatable types were included.[19] It may be considered that there are no clear differences between the face and upper trunk in the total number of *Malassezia* species in men. On women, the number of *Malassezia* species on the upper trunk was greater than that on the face by both the non-culture and culture methods. On the other hand, the numbers of *Propionibacterium* species and CNS on the face were greater than those on the upper trunk. These results suggested the possibility that *Malassezia* species are rivals to *Propionibacterium* species and CNS. Moreover, it was also considered that the difference of the composition of sebum in each body area influences the geographical distribution of *Malassezia* microbiota. However, there was no detailed report about the composition of sebum in each body area. We believe this to be an interesting topic for further research.

In the comparison of seasons, there were no differences in the total number of *Malassezia* species by the non-culture method. On the other hand, using the culture method, there were some body parts where the number in summer was greater than that in winter. To be certain, *M. globosa* and *M. restricta*, which are the predominant species, increased in summer on

some body parts. These results suggested the possibility that the number of living *Malassezia* cells in summer is greater in comparison with winter, although the total number of *Malassezia* cells (living and dead cells) did not differ by season. The skin diseases caused by *Malassezia* yeasts, namely pityriasis versicolor and *Malassezia* folliculitis, tend to be more prevalent in the summer months and in tropical locations than in temperate regions.[2] The development of these diseases may relate to the increases in the number of living *Malassezia* cells in summer. There is a report that there was no significant difference in the amounts of sebum by season in Japanese subjects.[22] It was considered that the number of living *Malassezia* cells in summer increased by the effect of temperature, humidity or sweat. On the other hand, *M. dermatis* and *M. sympodialis* numbers increased in winter in comparison with summer in some cases by the non-culture method. This result indicates the possibility that there are some interrelations between *Malassezia* species. This was also considered as an interesting topic for further research.

Not only the total number but also the *Malassezia* species detected from the skin of healthy subjects differed according to sex and body part. It was also considered that these differences in *Malassezia* microbiota in healthy subjects depend on the difference in the properties of sebum and sweat by sex or body part. There is a report of an *in vitro* experiment that some ingredients of sebum and sweat influence the increase in *Malassezia* species, although this influence differs according to the species.[23] However, this previous study did not support the current *Malassezia* classification. Moreover, it is necessary to study the relationship of sebum or sweat and *Malassezia* microbiota using human skin *in vivo*. The influence of the quantity and properties of sebum and sweat on *Malassezia* species will be studied in the future.

There are several reports about the normal resident *Malassezia* microbiota on human skin.[12–18] Lee *et al.*[17] reported that the total numbers of *Malassezia* species were different according to age and body part by a culture method. Sugita *et al.*[18] reported that the total numbers of *Malassezia* species on the cheek were different according to age and sex by a non-culture method. The result where *Malassezia* microbiota was different by sex and body part corresponds with the present findings. With regard to *Malassezia* species detected on the skin of healthy subjects, there are no reports concerning the quantitative analysis but there are some reports concerning the recovery rates of each *Malassezia* species on the skin in healthy subjects. Using the culture method, Lee *et al.*[17] reported that the predominant *Malassezia* species were *M. restricta* on the forehead and *M. globosa* on the chest. Nakabayashi *et al.*[12] reported that *M. globosa* was predominant on the trunk. The difference by sex was not considered, although their results related to the present findings. On the other hand, Gupta *et al.*[13] reported *M. sympodialis* was predominant on both the face and trunk. It is considered that this result may have been because there were many female subjects in their experiment in comparison with the above two reports. It may also be the case that the white subjects are different from Asian subjects. Using the non-culture method, there are reports that the predominant species were *M. restricta* and *M. globosa* on the face and neck.[15,18] Moreover, there is also a report that the recovery rate of *M. sympodialis* was also high.[14] These results correlate with the present findings. It was considered that cutaneous *Malassezia* microbiota in healthy subjects was clarified by the present quantitative analysis using the non-culture and culture methods as well as the previous qualitative analysis.

In conclusion, this study clarified that the cutaneous *Malassezia* microbiota of healthy subjects differed by sex, body part and season. Moreover, there were also differences between the results by the non-culture method and those by the culture method. It is considered that it is necessary to take account of the sex, body part, season and analytical method when cutaneous *Malassezia* microbiota is studied.

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SPECIAL REPORT

Prevalence of dermatological disorders in Japan: A nationwide, cross-sectional, seasonal, multicenter, hospital-based study

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ABSTRACT

To clarify the prevalence of skin disorders among dermatology patients in Japan, a nationwide, cross-sectional, seasonal, multicenter study was conducted in 69 university hospitals, 45 district-based pivotal hospitals, and 56 private clinics (170 clinics in total). In each clinic, information was collected on the diagnosis, age, and gender of all outpatients and inpatients who visited the clinic on any one day of the second week in each of May, August, and November 2007 and February 2008. Among 67 448 cases, the top twenty skin disorders were, in descending order of incidence, miscellaneous eczema, atopic dermatitis, tinea pedis, urticaria/angioedema, tinea unguium, viral warts, psoriasis, contact dermatitis, acne, seborrheic dermatitis, hand eczema, miscellaneous benign skin tumors, alopecia areata, herpes zoster/postherpetic neuralgia, skin ulcers (nondiabetic), prurigo, epidermal cysts, vitiligo vulgaris, seborrheic keratosis, and drug eruption/toxicoderma. Atopic dermatitis, impetigo, molluscum, warts, acne, and miscellaneous eczema shared their top-ranking position in the pediatric population, whereas the most common disorders among the geriatric population were tinea pedis, tinea unguium, psoriasis, seborrheic dermatitis, and miscellaneous eczema. For some disorders, such as atopic dermatitis, contact dermatitis, urticaria/angioedema, prurigo, insect bites, and tinea pedis, the number of patients correlated with the average high and low monthly temperatures. Males showed a greater susceptibility to some diseases (psoriasis, erythroderma, diabetic dermatoses, *inter alia*), whereas females were more susceptible to others (erythema nodosum, collagen diseases, livedo reticularis/racemosa, hand eczema, *inter alia*). In conclusion, this hospital-based study highlights the present situation regarding dermatological patients in the early 21st century in Japan.

Key words: age, Japan, prevalence, sex, skin diseases.

INTRODUCTION

Skin forms the outermost part of the human body and it acts as a vital barrier to external and internal damage. Various external and internal stimuli, which can be either short- or long-term, can affect the homeostasis of the skin, leading to a variety of disorders. The development and perpetuation of skin disorders are multifactorial in nature, and can result from genetic, environmental, mechanical, meteorological and even cultural effects. Skin disorders therefore include a vast range of diseases.

Although it is difficult to know the exact prevalence or incidence of skin diseases, several hospital-based

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studies have shown that skin diseases are very common. Of a total of 11 191 patients seen by a general practitioner in the UK, 2386 (21%) presented dermatological complaints. Among these there was a preponderance of females (1604, 67%), and the most common skin diseases seen were viral warts, eczema and benign tumors.[1] In the Netherlands, 235–460/1000 person-years of children aged 0–17 years contacted general practitioners in 1987 and 2001,[2] and these contacts frequently involved bacterial, viral, fungal, eczematous or traumatic skin diseases.[2] Tamer *et al.* reported on 6300 pediatric cases aged 0–16 years who visited dermatological clinics in Turkey; this group showed a preponderance of bacterial, viral and eczematous skin diseases.[3] In the case of Japan, there is no authentic report in the published work on any investigation of the prevalence of skin diseases; therefore, the Japanese Dermatological Association conducted a nationwide, cross-sectional, seasonal, multicenter, hospital-based study.

METHODS

A total of 190 dermatology clinics at 76 university hospitals, 55 district-based pivotal hospitals and 59 private clinics participated in this study. At each clinic,

Table 1. Numbers of patients recruited in each season

	Number of patients				
	May 2007	August 2007	November 2007	February 2008	Total
University Hospitals n = 69	8558	7944	7782	7778	32 062 (47.54%)
District-based Hospitals n = 45	3505	3450	2890	2864	12 709 (18.84%)
Private clinics n = 56	5779	6709	5364	4825	22 677 (33.62%)
Total	17 842	18 103	16 036	15 467	67 448 (100%)

Table 2. Age distribution and sex difference of patients

Age distribution (years old)	Number of patients	Male patients	Female patients	Sex undescribed
0–5	4192 (6.22%)	2200 (7.12%)	1983 (5.49%)	9
6–10	2099 (3.11%)	1047 (3.39%)	1047 (2.9%)	5
11–15	1711 (2.54%)	815 (2.64%)	893 (2.47%)	3
16–20	2270 (3.37%)	995 (3.22%)	1266 (3.5%)	9
21–25	3219 (4.77%)	1245 (4.03%)	1960 (5.43%)	14
26–30	3516 (5.21%)	1378 (4.46%)	2126 (5.89%)	12
31–35	4050 (6%)	1546 (5%)	2483 (6.87%)	21
36–40	3807 (5.64%)	1604 (5.19%)	2180 (6.03%)	23
41–45	3298 (4.89%)	1387 (4.49%)	1879 (5.2%)	32
46–50	3201 (4.75%)	1326 (4.29%)	1848 (5.12%)	27
51–55	4062 (6.02%)	1763 (5.71%)	2279 (6.31%)	20
56–60	5543 (8.22%)	2503 (8.1%)	3012 (8.34%)	28
61–65	5413 (8.03%)	2533 (8.2%)	2846 (7.88%)	34
66–70	5629 (8.35%)	2775 (8.98%)	2824 (7.82%)	30
71–75	6157 (9.13%)	3195 (10.34%)	2923 (8.09%)	39
76–80	4777 (7.08%)	2487 (8.05%)	2259 (6.25%)	31
81–85	2636 (3.91%)	1297 (4.2%)	1318 (3.65%)	21
86–90	1098 (1.63%)	508 (1.64%)	583 (1.61%)	7
91–100	427 (0.63%)	166 (0.54%)	259 (0.72%)	2
≥101	16 (0.02%)	3 (0.01%)	2 (0.01%)	11
Age undescribed	327 (0.48%)	126 (0.41%)	155 (0.43%)	46
Total	67 448 (100%)	30 899 (100%)	36 125 (100%)	424

information on diagnosis, age and sex was collected from all outpatients and inpatients who visited the clinics or who were hospitalized on any single day of the second week in each of May, August and November 2007 and February 2008. Reports on the monthly average values of the high and low temperatures and humidities were collected from the Meteorological Agency. The information on 67 448 cases from 170 clinics (69 university hospitals, 45 district-based pivotal hospitals and 56 private clinics) that participated in all of the four seasonal examinations was analyzed. Statistical analyses were performed by using Spearman's rank correlation coefficient. A P-value of <0.05 was considered to be statistically significant. This study was approved by the internal ethical review boards of the Japanese Dermatological Association.

Table 3. Prevalence of skin diseases in 67 448 patients

Burn	899 (1.33%)	Syphilis	24 (0.04%)
Trauma	409 (0.61%)	Miscellaneous sexually transmitted diseases	41 (0.06%)
Skin ulcer (nondiabetic)	1334 (1.98%)		
Pressure ulcer	608 (0.9%)	Bullous pemphigoid	510 (0.76%)
Miscellaneous physico-chemical skin damage	681 (1.01%)	Pemphigus	424 (0.63%)
		Miscellaneous bullous diseases	141 (0.21%)
Diabetic dermatoses	436 (0.65%)	Systemic sclerosis	619 (0.92%)
Atopic dermatitis	6733 (9.98%)	Systemic lupus erythematosus	525 (0.78%)
Hand eczema	2024 (3%)	Dermatomyositis	304 (0.45%)
Contact dermatitis	2643 (3.92%)	Miscellaneous collagen diseases	915 (1.36%)
Seborrheic dermatitis	2213 (3.28%)	Anaphylactoid purpura	171 (0.25%)
Miscellaneous eczema	12590 (18.67%)	Reticular/racemous livedo	81 (0.12%)
Urticaria/angioedema	3369 (4.99%)	Miscellaneous vasculitis/purpura/ circulatory disturbance	632 (0.94%)
Prurigo	1229 (1.82%)		
Drug eruption/toxicoderma	1018 (1.51%)	Mycosis fungoides	427 (0.63%)
Psoriasis	2985 (4.43%)	Miscellaneous lymphomas	285 (0.42%)
Palmoplantar pustulosis	832 (1.23%)	Pigmented nevus	709 (1.05%)
Miscellaneous pustulosis	172 (0.26%)	Seborrheic keratosis	1095 (1.62%)
Lichen planus	200 (0.3%)	Soft fibroma/acrochordon	231 (0.34%)
Miscellaneous inflammatory keratotic disorders	241 (0.36%)	Epidermal cyst	1194 (1.77%)
		Lipoma	173 (0.26%)
Tylosis/clavus	917 (1.36%)	Dermatofibroma	111 (0.16%)
Ichthyosis	61 (0.09%)	Miscellaneous benign skin tumors	1666 (2.47%)
Miscellaneous keratinization disorders	502 (0.74%)	Actinic keratosis	261 (0.39%)
Ingrown nail	597 (0.89%)	Basal cell carcinoma	324 (0.48%)
Miscellaneous nail disorder	397 (0.59%)	Squamous cell carcinoma/Bowen's disease	455 (0.67%)
Alopecia areata	1653 (2.45%)		
Androgenic alopecia	210 (0.31%)	Paget's disease	224 (0.33%)
Miscellaneous skin appendage disorders	266 (0.39%)	Malignant melanoma	808 (1.2%)
Scabies	98 (0.15%)	Miscellaneous malignant skin tumors	534 (0.79%)
Insect bite	762 (1.13%)	Vitiligo vulgaris	1134 (1.68%)
Tinea pedis	4379 (6.49%)	Chloasma/senile freckle	336 (0.5%)
Tinea unguium	3231 (4.79%)	Miscellaneous pigmented disorders	154 (0.23%)
Miscellaneous tinea	610 (0.9%)	Erythema multiforme	197 (0.29%)
Candidiasis	408 (0.6%)	Erythema nodosum	111 (0.16%)
Miscellaneous mycosis	211 (0.31%)	Miscellaneous disorders with erythematous plaques	130 (0.19%)
Acne	2430 (3.6%)		
Impetigo contagiosum	507 (0.75%)	Nevus/phacomatosis (other than pigmented nevus)	267 (0.4%)
Folliculitis	755 (1.12%)		
Erysipelas	81 (0.12%)	Rosacea/rosacea-like dermatitis	150 (0.22%)
Cellulitis	594 (0.88%)	Granulomatous diseases	192 (0.28%)
Miscellaneous bacterial infection	914 (1.36%)	Keloid/hypertrophic scar	186 (0.28%)
Molluscum contagiosum	604 (0.9%)	Cheilitis/angular cheilitis/mucous membrane diseases	95 (0.14%)
Herpes simplex	691 (1.02%)		
Herpes zoster/zoster-associated pain	1609 (2.39%)	Erythroderma	63 (0.09%)
Viral wart	3028 (4.49%)	Other diseases	666 (0.99%)
Miscellaneous viral disorders	353 (0.52%)	Total	67 448 (100%)