Relationships among the Genotypes of *Malassezia Globosa* Colonizing Patients with Atopic Dermatitis, the Clinical Severity of the Disease, and the Level of Specific IgE Antibodies

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**Abstract**

**Background:** Various types of microorganisms colonize the skin surface, and some such as the bacterium *Staphylococcus aureus* and fungus *Malassezia*, exacerbate the symptoms of atopic dermatitis. *Malassezia*-specific IgE antibodies are present in the sera of patients with atopic dermatitis and the level thereof correlates with the severity of the condition. *Malassezia* has many genotypes. In the present study, we explored the relationships among the genotypes of *Malassezia* species colonizing patients with atopic dermatitis, the clinical severity of the disease, and the level of specific IgE antibody.

**Methods:** Scale samples were obtained from head or neck lesions of 74 patients with atopic dermatitis and 40 healthy subjects. The intergenic spacer (IGS) 1 region of the rRNA genes of *M. globosa* (the major flora of patients with atopic dermatitis) were amplified by PCR and directly sequenced. *M. globosa*-specific IgE antibody levels were determined using the AlaSTAT™ microplate system.

**Results:** Eighteen *M. globosa* IGS1 genotypes were detected in scale samples from patients with atopic dermatitis and healthy individuals. Of these, the proportion of the (GT)10:(CT)8 genotype increased with the clinical severity of disease and increasing levels of *M. globosa*-specific IgE antibodies, whereas this genotype was not found on the skin of healthy subjects.

**Conclusion:** A specific genotype of *Malassezia* selectively colonizes the skin of patients with atopic dermatitis, contributing to the clinical severity of disease. Thus, a “bad *Malassezia*” may be present on the skin of patients with atopic dermatitis.

**Keywords:** Atopic dermatitis; *Malassezia globosa*; Genotype; IgE

**Abbreviations:** AD: Atopic Dermatitis; IGS: Intergenic Spacer

**Introduction**

Atopic dermatitis (AD) is a chronic disease exhibiting alternating periods of remission and deterioration. Environmental and genetic factors are involved in AD pathogenesis. AD patients generally exhibit dysfunctions of the skin barrier, allowing environmental substances to penetrate the body. Indeed, environmental allergen specific-IgE antibodies are readily detectable in patient sera [1–4].

The body is covered with various skin microorganisms including viruses, bacteria, and fungi, some of which exacerbate AD symptoms. Specific IgE antibodies against superantigens (enterotoxins) produced by *Staphylococcus aureus* are found in the sera of AD patients [5]. Of the cutaneous fungal microbiome, *Malassezia* species predominate at all sites on the body and comprise approximately 40–90% of all fungi [6,7]. *Malassezia* requires lipids for growth, and thus preferentially colonizes sebum-rich areas such as the head, face, and neck. Many studies have found that *Malassezia* exacerbates AD. Thus, AD symptoms improve after the administration of antifungal agents such as itraconazole and ketoconazole *Malassezia*-specific IgE antibodies are evident in the sera of AD patients but not healthy individuals, and the antibody level is correlated with symptom severity of the 14 *Malassezia* species, both *M. globosa* and *M. restricta* colonize the skin of all AD patients but the remaining species are detected in less than 40% of cases and the extent of *Malassezia* colonization is also correlated with symptom severity [8-11]. Together, these evidences suggest that cutaneous *Malassezia* species exacerbate AD. In our previous analysis of the skin microbiome of AD patients, we found that *M. globosa* (the major cutaneous fungus in AD patients) had many RNA genotypes, and that the genotypes of species obtained from AD patients and healthy subjects differed [12]. The fungal rRNA gene encodes four rRNA subunits (5S, 5.8S, 18S, and 26S), and there are spacer regions between each one (Figure 1). The intergenic spacer (IGS) 1 region located between the 26S and 5S rRNAs has the short sequence repeats (CT)n and (GT)n. The number of (CT)n and (GT)n repeats in the *M. globosa* rRNA gene seems to be strain-specific.

In the present study, we analyzed relationship among the number of (CT)n and (GT)n repeats in the *M. globosa* rRNA gene, the clinical severity of AD, and the level of *M. globosa*-specific IgE antibodies.

**Materials and Methods**

**Patients**

Our sample included 74 Japanese AD patients and 40 healthy
Subjects as controls. Our study protocol was approved by the Institutional Review Board of our institution and informed consent was obtained from each individual (Table 1). The clinical severity of AD was graded using the criteria of Hanifin and Rajka [13].

**Determination of M. globosa genotypes**

Scale samples were obtained from head or neck lesions by stripping using OpSite transparent dressings (Smith & Nephew, Hull, UK) [14]. *M. globosa* genotypes were determined by DNA sequencing of the IGS 1 regions of rRNA genes [12]. *Malassezia* DNA was extracted directly from dressings and the IGS 1 regions were amplified by PCR using specific primers. PCR consisted of initial denaturation at 94°C for 1 min; 30 cycles of 30 s at 94°C, 30 s at 54°C, and 30 s at 72°C; then a final extension at 72°C for 10 min. The primers used were gb-F2 (TGCATACCACACTCGAGCGCTT) and gb-R1 (GGAAATAGGATGAGAGAAACA). For nested PCR, 1 µL of the first amplification product was added to a new reaction tube and PCR was performed with an initial denaturation at 94°C for 1 min; 30 cycles of 30 s at 94°C, 30 s at 54°C, and 30 s at 72°C; then a final extension at 72°C for 10 min. The primers used were gb-F2 (TGCTTCCAGTGGATACGACACT) and gb-R1 (GGAAATAGGATGAGAGAAACA). For nested PCR, 1 µL of the first amplification product was added to a new reaction tube and PCR was performed with an initial denaturation at 94°C for 1 min; 30 cycles of 30 s at 94°C, 30 s at 54°C, and 30 s at 72°C; then a final extension at 72°C for 10 min. The primers used were gb-F2 (TGCTTCCAGTGGATACGACACT) and gb-R2 (ATGTTGATGATCAGACATAGA). PCR products were directly sequenced.

**Determination of M. globosa-specific IgE antibody levels**

*M. globosa*-specific IgE levels were determined using the AlaSTAT™ microplate system (Diagnostic Products Corporation, Los Angeles, CA) according to the method of Kato et al. [15]. Briefly, the wells of a microtiter plate were coated with 100 μL amounts of *Malassezia* antigen in phosphate-buffered saline (PBS). Then serum (50 μL) was added, followed by peroxidase-labeled anti-IgE antibodies. After addition of a TMB (3,3′,5,5′-tetramethylbenzidine dissolved in hydrogen peroxide) substrate solution, the absorbance at 650 nm was measured at 1 s intervals for 5 min. IgE levels (U/mL) were calculated with the aid of a standard curve, and an IgE level greater than 0.35 U/mL was defined as a positive result.

**Results and Discussion**

The PCR products amplified were about 300 bp in length and included the GT and CT repeats (Figure 2). A total of 9-19 GT repeats and 3-9 CT repeats detected in DNAs of the patients and healthy individuals. A total of 18 combinations were noted. Of these, 16 were found in samples from patients with mild disease and the genotype (GT)_{19}(CT)_{5} was present in 24.3% (9/37) of cases. In patients with moderate disease, (GT)_{15}(CT)_{9} was present in 41.3% (12/29). In patients with severe disease, 87.5% (7/8) of the patients had the (GT)_{9}(CT)_{5} genotype although a limited number of patients were examined. The genotypes (GT)_{19}(CT)_{5} and (GT)_{9}(CT)_{5} predominated in healthy subjects, and (GT)_{15}(CT)_{9} was not found (Figure 3). These findings suggest that *M. globosa* of a specific genotype becomes more predominant as disease severity increases. The levels of *M. globosa*-specific IgE antibodies in the sera of AD patients also increased as disease severity increased (Figure 4). Of the 9 and 12 patients with mild and moderate disease, respectively, who were colonized with the genotype (GT)_{9}(CT)_{5} strain, 5 (56%) and 11 (92%) had IgE-specific antibody levels over 30 IU/mL. When these patients were compared to those with less than 30 IU/mL, 92.3% (24/26) of the former patients had the genotype (GT)_{9}(CT)_{5} compared to only 10.4% (5/48) of the latter. Generally, both total IgE and *Malassezia*-specific IgE antibody levels are correlated with the clinical severity of AD [9]. Even in patients with mild or moderate disease, the specific genotype predominated in patients with high *M. globosa*-specific IgE antibody levels.
Why does a specific genotypic strain of *M. globosa* seem to affect (or at least appear to be associated with) the clinical severity of AD? We have no clear explanation as yet. However, physiological conditions including the lipid composition, water content, and/or pH of the skin surface may influence *Malassezia* colonization. Therapeutic agents given to AD patients may also affect selective skin microbial colonization. However, no patients in the present study received any antibacterial agent.

In conclusion, we found that *M. globosa* of a specific genotype was closely associated with the clinical severity of AD and increasing levels of specific IgE antibodies in sera. Further genotypic work is required.

References