



Original Article

Transmission of the major skin microbiota, *Malassezia*, from mother to neonate

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Abstract **Background:** Skin surface colonization starts after birth. It is thought that early microbial colonization affects the development of skin immune functions. Although *Malassezia* is the predominant fungus in the skin microbiota in healthy individuals, the microorganism is associated with atopic dermatitis and seborrheic dermatitis. In the present study, transmission of skin microbiota from mothers to their neonates was elucidated using the *Malassezia* microbiota as an indicator.

Methods: Temporal changes in the level of *Malassezia* colonization of the skin from 27 neonates and mothers were investigated by real-time polymerase chain reaction assay. The genotypes of *Malassezia* colonizing the neonate and mother were also determined.

Results: *Malassezia* was detected from 89% and 100% of neonate samples on days 0 and 1 after birth, respectively. Subsequently, the level of *Malassezia* colonization of the neonates increased with time, whereas that of the mothers did not change. The *Malassezia* diversity of neonates shifted to the adult type by day 30. The genotype of *Malassezia* colonizing the skin of neonates agreed well with that of *Malassezia* colonizing the skin of the mothers.

Conclusion: Fungal microbiota colonization of neonates began on day 0, and the fungal microbiota of neonates had changed to the adult type by day 30. To our knowledge, this is the first report of a molecular analysis of the fungal microbiota of neonates.

Key words neonate, *Malassezia*, skin microbiota, transmission.

A fetus develops for approximately 10 months in the uterus, a microorganism-free environment, and is subsequently delivered into the external environment. Exposure of the neonate to a wide variety of microbes begins immediately at birth. The skin microbiota of a newborn baby is probably greatly influenced by that of the mother. Recent 16S rRNA gene pyrosequencing revealed that babies acquired the bacterial community of their mothers' microbiota; *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* species predominated in the babies' skin.¹

The bacterial skin microbiota shows remarkable species diversity among individuals;^{2,3} however, of the fungal skin microbiota, the lipophilic fungi *Malassezia* are predominant in all body sites (>50–80% of overall fungi).^{4,5} These microorganisms require lipid for growth and thus colonize sebum-rich areas such as the head, face, and neck, as opposed to the trunk or limbs.^{4,6} Although *Malassezia* species colonize healthy skin, they are associated

with several skin diseases, including atopic dermatitis, seborrheic dermatitis, and folliculitis.^{7,8} *Malassezia* is also considered to be a causative agent of seborrheic dermatitis in infants, producing so-called infantile seborrheic dermatitis (ISD).^{9,10} Some patients with ISD subsequently develop atopic dermatitis.

In the present study, to investigate the possible transmission of cutaneous *Malassezia* from a mother to her neonate, we examined the *Malassezia* genotypes colonized on the skin of babies and mothers and the temporal change in the cutaneous *Malassezia* microbiota in the neonates/infants compared with the mothers, using a highly accurate, molecular-based method.

Methods

Subjects

Sampling from 27 neonates (eight men and 19 women) and their mothers was performed at the Department of Obstetrics and Gynecology, Juntendo University Nerima Hospital. The protocol was approved by the institutional review board of the university, and informed consent was obtained from each subject. Subjects who had received any antimicrobial agents were excluded from the study.

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Table 1 Primer and probe sequences for real-time polymerase chain reaction detection of *Malassezia* DNA

Primer and probe	Sequence (5' to 3')
<i>All Malassezia</i> species	
Reverse	CTAAATATCGGGGAGAGACCGA
Forward	GTACTTTTAACTCTCTTTCCAAAGTGCTT
TaqMan probe	FAM-TTCATCTTTCCCTCACGGTAC-MGB
<i>Malassezia globosa</i>	
Reverse	GGCCAAGCGCGCTCT
Forward	CCACAACCAAATGCTCTCCTACAG
TaqMan probe	FAM-ATCATCAGGCATAGCATG-MGB
<i>Malassezia restricta</i>	
Reverse	GGCGGCCAAGCAGTGTTT
Forward	AACCAACATTCTCTCTTTAGGTGA
TaqMan probe	FAM-TTCTCCTGGCATGGCAT-MGB

Sampling and fungal DNA extraction

Cutaneous microorganisms were collected by stripping a 5 × 7-cm transparent OpSite dressing (Smith & Nephew Medical, Hull, UK) from the right and left cheeks in non-lesional sites of each subject. Neonates were sampled immediately after birth and at 1 day, 3 days, and 1 month after birth. Mothers were sampled immediately before giving birth and at 1 day, 3 days, and 1 month after giving birth. The collected OpSite transparent dressings were cut into pieces measuring 3 × 3 cm, and *Malassezia* DNA was extracted directly from the OpSite by using the method of Sugita *et al.*¹¹ Briefly, the collected OpSite dressing was placed in a 1.5-mL Eppendorf tube with 1000 µL of lysing solution (100 mM Tris-HCl [pH 8.0], 30 mM ethylenediaminetetraacetic acid [EDTA; pH 8.0], and 0.5% sodium dodecyl sulfate). The tube was freeze-thawed three times using liquid nitrogen and subsequently incubated for 15 min at 100°C. The OpSite dressing was then removed from the tube, and the suspension was extracted with phenol-chloroform-isoamyl alcohol (25:24:1, v/v/v). Subsequently, the samples were extracted with chloroform-isoamyl alcohol (24:1, v/v) and the DNA was precipitated with ethanol, using Ethatimate (Nippon Gene, Toyama, Japan) as a precipitation activator. The DNA pellet was resuspended in 50 µL of TE (10 mM Tris-HCl [pH 8.0], 1 mM EDTA [pH 8.0]). An unused OpSite dressing was used as a negative control.

Quantitative analysis of the *Malassezia microbiota* by real-time polymerase chain reaction assay

Total *Malassezia* DNA and that of the two major species, *Malassezia globosa* and *Malassezia restricta*, were quantified using universal or species-specific primers and TaqMan probes, shown in Table 1, according to the method of Sugita *et al.*¹² Briefly, *Malassezia* universal and TaqMan probes were designed from the conserved region of the *Malassezia* 26S rDNA sequence, and species-specific primers for *M. globosa* and *M. restricta* were designed from the internal transcribed spacer (ITS) 2 region of the respective rRNA genes (Fig. 1). The polymerase chain reaction (PCR) products of the targeted 26S rDNA or ITS 2 region were inserted into the plasmid vector pCR 2.1 (Invitrogen, Carlsbad, CA, USA), and the plasmid was extracted from an overnight culture of *Escherichia coli* using QIAprep (QIAGEN, Santa

Clarita, CA, USA) according to the manufacturer's instructions. Ten-fold serial dilutions were made in triplicate, ranging from 10¹ to 10⁸ copies, and these serial dilutions were used to derive a standard curve for quantification. For real-time PCR analysis, 50 µL of a PCR mixture (Applied Biosystems, Foster, CA, USA), 200 nM of each primer, and 250 nM TaqMan probe were placed in each well of a 96-well plate. Amplification and detection were performed with the ABI PRISM 7500 sequence detection system (Applied Biosystems) with the following cycle parameters: 50°C for 2 min, 95°C for 10 min, and 40 cycles of 95°C for 15 s and 60°C for 1 min. Standard curves were plotted for each primer-probe set using the cycle threshold (Ct) (the cycle number at which the threshold fluorescence was reached) values obtained by amplifying successive tenfold dilutions of a known concentration of plasmid DNA. The assay was repeated in triplicate with the samples from each cheek.

Genotyping of *M. globosa*

To identify the strain-specific genotype of *M. globosa*, the DNA sequence of the intergenic spacer region (IGS), which is located between the large subunit (LSU) and 5S in the rRNA gene, was determined.¹³ The DNA (10 µL) extracted from each sample of both mother and her neonate obtained at day 30 was added to 40 µL of PCR master mixture, which consisted of 5 µL of 10× PCR buffer (Takara, Shiga, Japan), 4 µL of 200 µM deoxynucleoside triphosphates, 10 pmol of each primer, and 0.5 U of Takara Ex Taq DNA polymerase (Takara). PCR was performed with an initial denaturation at 94°C for 1 min, followed by 30 cycles of 30 s at 94°C, 1 min at 54°C, and 30 s at 72°C, with a final extension at 72°C for 10 min using primers gb-F (GCTTTCGAGTGCAT ACCACAC) and gb-R (GGAAATAGGATGAGAGAAAC). The PCR products were cloned with a TA cloning kit (Invitrogen Corp., Carlsbad, CA, USA), and 20 positive clones were sequenced with an ABI PRISM 3100 Genetic Analyzer (Applied Biosystems, CA, USA) in accordance with the manufacturers' instructions.

Results

Quantitative analysis of the *Malassezia microbiota*

The levels of *Malassezia* colonization of all 27 neonates and their mothers were sequentially quantified using a real-time PCR

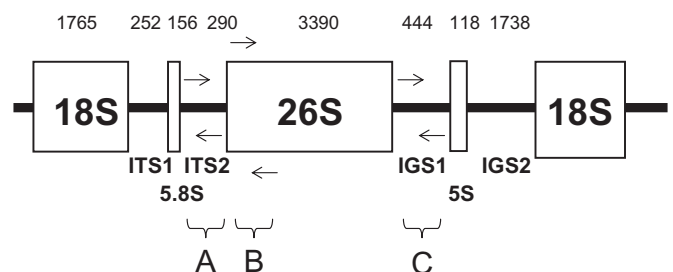


Fig. 1 Schematic representation of the rRNA locus in *Malassezia*. Numerals indicate base-pair length of the subunits or regions of the *Malassezia globosa* rRNA gene. A, target site for detection of *M. globosa* or *Malassezia restricta*. B, target site for detection of all *Malassezia* species. C, target site for genotyping of *M. globosa*. ITS, internal transcribed spacer; IGS, intergenic spacer region.

[†]Number examined. [‡]Mean $\pm$ standard deviation. [§]Comparison of male and female neonates. [¶]Comparison of neonate and mother. The levels of *Malassezia* colonization of neonates and their mothers were sequentially quantified using a real-time polymerase chain reaction assay and *Malassezia* universal primers and TaqMan probes and expressed as plasmid copy numbers.

The *Malassezia* DNA was slightly greater in the male neonates ($n = 8$) than in the female neonates ($n = 19$), although there was no statistically significant difference (Table 2). The level of the mothers' *Malassezia* DNA remained almost unchanged from immediately before giving birth to 30 days afterwards (15944 ± 19739 to 24720 ± 19638 copies).

The levels of colonization of the predominant *Malassezia* species, *M. globosa* and *M. restricta*, were determined using species-specific primers and TaqMan probes. These two species accounted for more than 90% of all *Malassezia* DNA colonization in both the mother and her neonate (Table 3). In the mothers' microbiota, *M. restricta* predominated over *M. globosa*. The former was 75.3–77.6%, while the latter was 17.2–18.2%. From just before giving birth to 30 days after giving birth, the ratio of these two major species remained almost unchanged. In contrast, the species distribution in the neonate differed greatly from that in the mother. Immediately following birth to 30 days after birth, the rates of *M. globosa* and *M. restricta* were similar; however, the rates changed at day 30 (34.2% for *M. globosa* and 63.3% for *M. restricta*).

IGS 1 of *M. globosa* had four short sequence repeats (SSR) of (CT)_n (CT)_n (CT)_n, and (GT)_n at positions 21–45, 226–242, 320–335, and 180–215, respectively, in the IGS sequence of strain CBS 7996 (type strain of *M. globosa*) (Fig. 2). Because *M. globosa* had three (CT)_ns in its IGS 1 region, these are referred to as (CT1)_n (CT2)_n, and (CT3)_n in this article. In all cases, the IGS 1 genotype of the neonate sample agreed with that of the mother (Table 4). For example, of the 20 IGS 1

Table 3 Temporal change in the ratio (%) of the two predominant *Malassezia* species in neonates and their mothers

[†]Mean $\pm$ standard deviation. The ratio (%) shows the level of *Malassezia globosa* or *Malassezia restricta* colonization compared to that of all colonizing *Malassezia* species. [‡]Comparison of neonate and mother.

Table 4 Analyses of short sequence repeats, CT and GT, of the intergenic spacer region 1 region in *Malassezia globosa* rRNA gene

Subject	Mother					Neonate				
	Number of clone analyzed	CT1	CT2	CT3	GT	Number of clone analyzed	CT1	CT2	CT3	GT
1	20	8	4	3	15	20	8	4	3	15
2	17	10	11	8	19	20	10	11	8	19
	3	8	6	2	15					
3	18	10	7	7	19	20	10	7	7	19
	2	11	11	9	15					
4	19	11	9	3	15	20	11	9	3	15
	1	10	6	9	19					
5	18	11	5	7	19	20	11	5	7	19
	2	10	7	9	19					
6	15	8	3	3	10	19	8	3	3	10
	4	10	7	9	19	1	10	7	9	19
	1	8	6	3	9					
7	20	8	7	9	19	20	8	7	9	19
8	16	10	11	11	15	20	10	11	11	15
	4	11	5	9	19					
9	20	13	8	9	15	20	13	8	9	15
10	20	4	5	3	15	16	4	5	3	15
						4	11	7	5	15
11	11	11	7	7	15	17	11	7	7	15
	9	10	7	9	15	3	10	7	9	15
12	18	11	9	3	19	19	11	9	3	19
	2	10	11	3	15	1	10	9	9	19
13	20	8	11	7	19	20	8	11	7	19
14	20	6	11	8	15	20	6	11	8	15
15	20	7	11	3	19	18	7	11	3	19
						2	8	6	11	15
16	19	7	8	3	19	15	7	8	3	19
	1	10	11	7	15	4	11	6	4	19
17	17	9	9	9	15	17	9	9	9	15
	3	11	9	9	19	3	9	8	5	19
18	15	11	9	5	15	19	11	9	5	15
	4	10	11	9	19					
	1	9	7	3	19	1	9	7	3	19
19	17	10	7	9	15	20	10	7	9	15
	3	11	9	9	19					
20	15	7	8	11	11	18	7	8	11	11
	5	11	5	7	15	2	11	5	7	15
21	17	11	9	11	19	20	11	9	11	19
	3	10	7	9	19					
22	20	6	7	8	15	20	6	7	8	15
23	18	10	9	9	19	20	10	9	9	19
	2	8	5	3	10					
24	20	11	7	8	19	16	11	7	8	19
						4	10	8	5	19
25	17	8	7	7	19	17	8	7	7	19
	3	9	7	5	19	9	7	5	8	19
26	20	11	6	7	15	18	11	6	7	15
						2	11	7	6	19
27	15	8	7	3	19	18	8	7	3	19
	5	8	11	7	15	2	7	11	5	19

genotype clone samples from the mother of Subject 5, 18 clones from the mother's sample were completely identical to 20 clones from the neonate. In Subject 6, three and two genotypes were obtained from the mother's and neonate's samples, respectively. Fifteen clones from the mother's sample agreed with 19 clones from the neonate's sample. The remaining four clones from the mother's sample also agreed with one clone from the neonate's sample.

Discussion

The human skin microbiota comprises a wide variety of bacteria and fungi.^{2,3,5} The majority of fungi belong to a lipophilic yeast genus, *Malassezia*.^{4,5} Therefore, we chose the genus *Malassezia* as an indicator for elucidating, with high accuracy, transmission of the skin microbiota from mother to neonate, using quantitative PCR and genotyping analyses. Although *Malassezia* is a part of

the major cutaneous microbiota in healthy individuals, these microorganisms are also involved in atopic dermatitis and seborrheic dermatitis.^{7,8} Molecular-based analysis has revealed that *M. restricta* and *M. globosa* are predominant among the 14 *Malassezia* species in patients with skin disease as well as in healthy individuals.^{11,12,14–17} In addition, the level of *Malassezia* colonization depends on age and sex. In Japan, a large epidemiological study of 770 healthy individuals showed that the level of *Malassezia* colonization increased with aging (up to 16–18 years in male subjects and 10–12 years in female subjects) and subsequently decreased gradually in both sexes until senescence.¹⁸

In the present study, our molecular-based assay succeeded in detecting *Malassezia* DNA from day 0 neonate samples (88%, 24 of 27). *Malassezia* detected in a day-0 neonate sample might have originated in the mother's vulva. Indeed, *Malassezia* has been detected in the vulva (unpublished data).

Regardless of sex, both *M. restricta* and *M. globosa* are the major microbiota present in individuals from infancy to the age of 90 years, although their ratio of colonization changes, with *M. restricta* greatly predominating over *M. globosa* in the adult population.¹⁸ In the neonate population, the ratio of the two microorganisms showed near uniformity, and the proportion of *M. restricta* was increased at day 30. The microbiota ratio of the mothers' skin (*M. restricta* to *M. globosa*) was approximately 8:2. This is a general ratio in the female adult population. The microbiota of the neonate may shift to that of the adult microbiota after 30 days of life.

We also investigated the transmission of cutaneous microbiota from a mother to her baby. The constitution of the microbiota of the neonate is greatly influenced by the mother's microbiota because the baby spends long periods of time with its mother. As *M. globosa* has a remarkable DNA sequence variation in the IGS region of the rRNA gene, the IGS analysis enables strain-typing.¹³ The present study revealed that the *Malassezia* that colonized in the neonate was transmitted from the mother. Another IGS analysis study demonstrated that the IGS genotype detected from patients with atopic dermatitis or seborrheic dermatitis and that of healthy individuals differed significantly.^{13,15,19} There are atopic-dermatitis- and seborrheic-dermatitis-specific genotypes of *M. globosa*. Atopic dermatitis develops in approximately 10–20% of individuals who are 4 months old. Infantile seborrheic dermatitis develops approximately 1–2 months after birth.²⁰ Anti-*Malassezia*-specific IgE antibody is produced in the serum of patients with atopic dermatitis, while the antibody is not produced in the serum of healthy individuals. In the present study, we did not examine anti-*Malassezia*-specific IgE antibody in neonates. Neonates develop erythema, papules, desquamation, and pustules in the skin, however, no neonate developed neonatal cephalic pustulosis, neonatal acne, or neonatal dermatosis. It would be interesting to determine the correlation between the *Malassezia* microbiota and the development of *Malassezia*-associated skin diseases, and this should be investigated in long-term follow-up studies.

In recent years, the analysis of cutaneous microbiota has been actively conducted. One study demonstrated that vaginally delivered infants acquired bacterial communities resembling their

mothers' vaginal microbiota, and that infants delivered via cesarean section harbored bacterial communities similar to those found on the skin surface.¹ These data suggest that the cutaneous microbiota of an infant are significantly influenced by that of the mother and may be associated with the infant's health.

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