

ORIGINAL ARTICLE

Sensitization to *Malassezia* in children with atopic dermatitis combined with food allergy

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Keywords

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Abstract

Background: The yeast *Malassezia* belongs to our normal cutaneous flora, but is capable of sensitizing individuals with atopic dermatitis (AD). Our objective was to investigate the prevalence of sensitization to *Malassezia* with a 10-yr follow-up among children suffering from AD combined with food allergy (FA) in relation to the extent of AD in infancy.

Methods: One hundred and eighty seven infants diagnosed with AD and milk/wheat allergy before 1 yr of age were included in the study. The area of AD was estimated from patient records of the first visit and measured with SCORAD at the 10-yr follow-up. Specific IgE against *Malassezia* was determined with ImmunoCAP™ at 11 yr of age.

Results: In infancy, 24 children (13%) were allergic to milk, 71 (38%) to wheat, and 92 (49%) to both milk and wheat, and 94 (50%) children had mild, 57 (30%) moderate and 36 (19%) severe AD. At the 10-yr follow-up visit, 19 (10%) of the children had ongoing milk and/or wheat allergy; 147 children (79%) had mild AD and 30 (16%) had SCORAD index of 0. Specific IgE against *Malassezia* mix was positive (≥ 0.35 kU/l) in 27% and specific IgE against *M. sympodialis* in 20% of the 187 children. The area of AD in infancy was associated with a greater risk of having allergen-specific IgE to *Malassezia* at the 10-yr follow-up. The risk ratio for FA was 3.11 (95% CI: 2.05–4.72; $p < 0.001$) if specific IgE to *Malassezia* was positive.

Conclusions: Infants with severe AD and FA seem to have a greater risk of becoming sensitized to *Malassezia* during a 10-yr follow-up.

Atopic dermatitis (AD) is a chronic itchy skin disease, 50%–60% of which begins in infancy (1, 2). A combination of genetic background, inappropriate immune responses, defective skin barrier, and environmental factors contributes to the development of AD (2). The role of food allergies (FA) in causing AD has not been exhaustively settled. The importance of FA is, however, regarded as being greatest in infancy (3–5). Sampson has reported that children with AD and multiple FAs following an elimination diet experienced a marked improvement in their eczematous rash during follow-up (6–8).

Mothes et al. (9) stated in 2005 that IgE autoreactivity to a variety of endogenous proteins develops in the first years of life. Food allergens may act as the first triggers of IgE autoimmunity. This emphasizes the importance of preventive measures against allergies, such as an elimination diet in patients with FA in early infancy. Effective treatment of AD restores the skin

barrier function and reduces the load of environmental antigens on the skin, which, in turn, decreases the skewing toward T helper (Th) 2-type inflammation and increases Th 1-type reactivity toward the normal (10). Therefore, it is beneficial to suppress skin inflammation as early as possible (11).

The yeast *Malassezia* belongs to our normal cutaneous flora, but it can also cause skin disorders and even trigger allergic symptoms by acting as an allergen (12). *Malassezia* colonization takes place immediately after birth, and IgE-mediated sensitization can occur as early as in infancy (13). Repeated episodes of oozing lesions have been found to predict sensitization in this age group (13), supporting with the assumption that the sensitization most probably occurs through the impaired skin barrier observed in patients with AD. IgE sensitization to *Malassezia* seems to be connected to AD, as allergen-specific IgE has only been found occasionally in

patients with atopic respiratory diseases without AD or in patients with seborrheic dermatitis (14–17).

Sensitization to *Malassezia* in children with AD and FA has not been addressed previously. We hereby investigated the prevalence of *Malassezia* sensitization in children at 11 yr of age who had suffered from FA and AD of varying severity grades in infancy.

Methods

Study population

The patients were selected from among infants under 1 yr of age with AD who entered the Dermatologic Allergy Unit of Tampere University Hospital (TAUH) during January 1996–June 1997 and were diagnosed as being allergic to milk and/or wheat. A letter inviting participants to the study was sent to the parents of 283 children a few months prior to the beginning of the study. Eighty-two families declined to take part in the study. A total of 187 children with a mean age of 10.7 yr, including 69 girls and 118 boys, fulfilled the inclusion criteria (AD from under the age of 1 yr and milk and/or wheat allergy) and attended the 10-yr follow-up study.

The study protocol was approved by the ethical committee of TAUH. Written informed consent was obtained from the parents and from the children at the 10-yr follow-up visit.

Severity of AD

The severity of AD in infancy was determined by two independent dermatologists (KT, OMK) based on the doctors' notes in the medical record from the initial visit. Atopic dermatitis severity was divided into three levels, where 1 stood for mild (affecting <25% of the total skin area), 2 for moderate (25%–50%), and 3 for severe AD (>50%). In the case of diverging scores, the investigators negotiated a mutual agreement. At the 10-yr follow-up, the severity of AD was determined using the SCORAD index (Scoring Atopic Dermatitis index) (18). In addition to a food elimination diet, AD was treated with mild local corticosteroids periodically, and emollients were used for dry skin. The aim was total clearance of AD due to the elimination diet and local treatment. The time of the first remission of AD was documented in patient records. The children visited the outpatient clinic regularly until milk/wheat was tolerated and AD was under control.

Diagnosis of food allergy

The diagnosis of milk/wheat allergy was based either on a positive oral challenge test at the hospital or on a positive skin prick test (SPT), and/or specific IgE against milk and/or wheat combined with a favorable outcome of eliminating the respective food at home (19–21). All milk challenges were performed at the outpatient clinic.

Challenge test

The challenged food was first eliminated from the diet for at least 2 wk then openly challenged at the outpatient clinic under

the supervision of a pediatrician/dermatologist. Milk substitute (containing milk protein 15 mg/ml) or wheat (flour diluted in water, containing wheat protein 13 mg/ml) was given orally in increasing doses at 30-min intervals until adverse symptoms (in the skin, gastrointestinal tract, or respiratory system) appeared or a total dosage of 186 ml of milk (2.8 g of milk protein) or 155 ml of wheat porridge (2.0 g of wheat protein) was achieved (22).

For the infants with positive SPT/specific IgE against wheat, the food was eliminated from the diet, and the outcome of elimination was verified at a control visit at the outpatient clinic after a few weeks. When wheat allergy was suspected, rye and barley were also avoided because of the close similarity of the allergens in all three (23). Only total clearance or a clear decrease in AD was accepted for the diagnosis of an allergy. All milk and/or wheat allergic infants were put on egg elimination because egg is one of the most common food allergens in small children. No challenge tests with egg were performed.

Skin prick tests

Skin prick tests were performed at baseline and at the 10-yr follow-up for all the children as described earlier (16). In brief, a commercially available milk allergen (ALK-Abelló A/S Hørsholm, Denmark) and wheat flour (Vaasan Mylly, Espoo, Finland) diluted in physiologic saline (1 g/ml NaCl) were used at baseline. At the 10-yr follow-up, the SPT was performed with skimmed milk (Valio, Helsinki, Finland) and wheat flour as at baseline. Histamine dihydrochloride 10 mg/ml (ALK-Abelló) was used as the positive and physiologic saline as the negative control. A wheal diameter of ≥ 3 mm at 15 min was considered positive provided that the negative control was zero.

IgE serology

In infancy, specific IgE antibodies in serum against milk and wheat were analyzed using ImmunoCAPTM (Pharmacia CAP System Specific IgE RIA, Uppsala, Sweden). Values ≥ 0.4 kU/l were considered positive.

IgE serology to *Malassezia*

Blood was drawn for the determination of total serum IgE as well as specific IgE against *M. sympodialis* (m70) and *Malassezia* mix (m227), a mixture of three *Malassezia* species (*M. sympodialis*, *M. globosa* and *M. restricta*), only at follow-up. All samples were analyzed simultaneously by Phadia with ImmunoCAPTM (Phadia AB, Uppsala, Sweden). Allergen-specific values of ≥ 0.35 kU/l were regarded as positive.

Statistical analysis

The data are presented as means with standard deviations, medians with interquartile ranges (IQR) or number, and percentage with 95% confidence intervals. Statistical comparisons between the groups were performed using the *t*-test, the Fisher-Pitman permutation test, the chi-square test, or Fisher's exact test, when appropriate. The statistical significance for hypotheses of linearity was evaluated using the Cuzick's test and logistic regression models. The time-to-event (cumulative remission) analysis was based on the product-limit estimate

(bootstrap estimation was used to derive a 95% CI) of the cumulative survival function, and the log-rank test with exact p values was used to identify the cumulative proportion of the statistical difference. Correlations were estimated with Spearman's correlation coefficient method. All statistical analyses were performed using STATA, version 11.1 (StataCorp, College Station, TX, USA).

Results

Extent of AD

At the first visit, 94 (50%) of the infants were classified as having mild AD, while 57 (30%) had moderate and 36 (19%) had severe AD (Table 1). There was no difference between boys and girls.

At the 10-yr follow-up visit, the majority of the children (n = 147 [79%]) had mild AD, that is, SCORAD index of <25, and 30(16%) had no AD-related symptoms, that is, SCORAD index of 0.

Food allergy

Twenty-four (13%) of the children were allergic to milk, 71 (38%) to wheat, and 92 (49%) to both milk and wheat in infancy (Table 1). At the 10-yr follow-up visit, 19 (10%) of the children had ongoing milk and/or wheat allergy based on challenge tests.

IgE serology against *Malassezia* at the follow-up

At the follow-up visit, specific IgE against the *Malassezia* mix (≥ 0.35 kU/l) was detected in 50 children (27% [95% CI:

21–34]), and specific IgE against *M. sympodialis* in 38 (20% [95% CI: 15–27]) of the 187 children (Table 1).

The area of AD at the first visit was associated with specific IgE to the *Malassezia* mix at the 10-yr follow-up. In the groups with moderate or severe AD, the risk (OR) of having specific IgE to the *Malassezia* mix was greater when compared with those with mild AD: in the group with moderate AD (n = 57), the risk (OR) was 1.41 (95% CI: 0.65–3.06) and in the severe group 3.16 (1.38–7.23; p for linearity 0.011). In the group with severe AD (n = 36) at baseline, 44% of the patients tested positive for specific IgE to the *Malassezia* mix and 31% for specific IgE to *M. sympodialis* at the 10-yr follow-up. In the group with mild AD (n = 94), specific IgE against the *Malassezia* mix was positive in 20% of the cases. The statistically significant linear relationship between the area of AD in infancy and the amount of specific IgE against the *Malassezia* mix but not against *M. sympodialis* at the 10-yr follow-up study is shown in Fig. 1.

The mean (s.d.) SCORAD index at follow-up was 8(8) for children without specific IgE against the *Malassezia* and 12(11) for those with specific IgE against the *Malassezia* mix (p = 0.011; Fig. 2).

The children with specific IgE against *Malassezia* mix at follow-up had suffered from more long-standing AD symptoms. For them, the cumulative remission rates of AD were 36% (95% CI: 24–51) at 1 yr and 88% (77–95) at 10 yr after the FA diagnosis, while for their counterparts testing negative for specific IgE to *Malassezia* mix, the corresponding rates were 58% (95% CI: 50–66) and 93% (88–97), respectively (p = 0.034) (Fig. 3).

At the follow-up visit, specific IgE to *Malassezia* was found in 13 (68%) of the 19 children with ongoing milk and/or wheat

Table 1 Characteristics of 187 infants under the age of 1 yr according to their status of specific IgE against the *Malassezia* mix at the 10-yr follow-up.

Variables	All children	Specific IgE to <i>Malassezia</i> mix at 10-yr follow-up*		p value ^{††}
	n = 187	Negative (<0.35 kU/l) n = 137	Positive (≥ 0.35 kU/l) n = 50	
Number of boys, %	118 (63)	86 (63)	32 (64)	0.88
Age at first visit, months mean (range)	6.1 (2–11)	6.0 (2–11)	6.2 (3–11)	0.65
Total breast-feeding duration, months, mean (range)	6.0 (0–17)	5.9 (0–13)	6.3 (0–17)	0.47
Percentage of skin affected by AD, n (%)				
Mild (0%–24%)	94 (50)	75 (55)	19 (38)	0.02 [‡]
Moderate (25%–49%)	57 (30)	42 (31)	15 (30)	
Severe ($\geq 50\%$)	36 (19)	20 (15)	16 (32)	
Milk and/or wheat allergy, n (%) [†]				
Milk only	24 (13)	22 (16)	2 (4)	0.073
Wheat only	71 (38)	52 (38)	19 (38)	
Milk and wheat	92 (49)	63 (46)	29 (58)	
Total serum IgE, median (IQR) [‡]	22 (5, 70)	12 (5, 61)	51 (14, 154)	0.028

*ImmunoCap™ m227 (Phadia AB).

[‡]p for linearity.

[†]Diagnosed by allergen-specific IgE (ImmunoCap™ ≥ 0.4 kU/l) or SPT and oral challenge.

[‡]ImmunoCap™, kU/l.

^{††}p value between *Malassezia*-negative and *Malassezia*-positive groups.

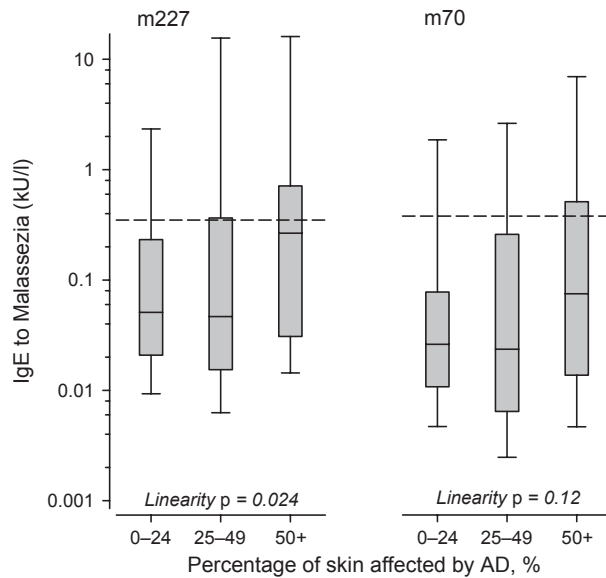


Figure 1 IgE against the *Malassezia* mix (m227) and *M. sympodialis* (m70) at the 10-yr follow-up in relation to the area of atopic dermatitis (0%–24% $n = 94$, 25%–49% $n = 57$, >50% $n = 36$) at the first visit when the patients were under the age of 1 yr. The dotted line represents specific IgE (≥ 0.35 kU/l). The box shows the median with interquartile range, and whiskers represent the 5th and 95th percentiles.

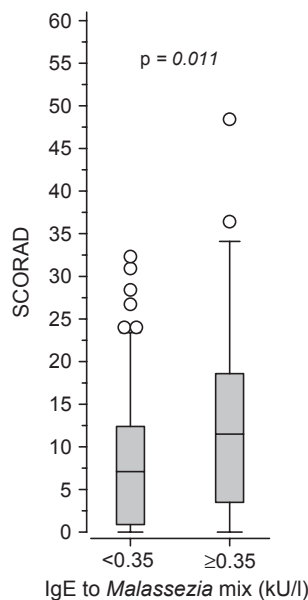


Figure 2 Box and plots of the SCORAD values of 187 children ($n = 137 < 0.35$ kU/l and $n = 50 \geq 0.35$ kU/l) at the 10-yr follow-up in relation to their status of IgE against the *Malassezia* mix (m227) (ImmunoCap™).

allergy and in 37 (22%) of the 168 children who had outgrown their allergy to milk and/or wheat (RR = 3.11 [95% CI: 2.05–4.72]; $p < 0.001$).

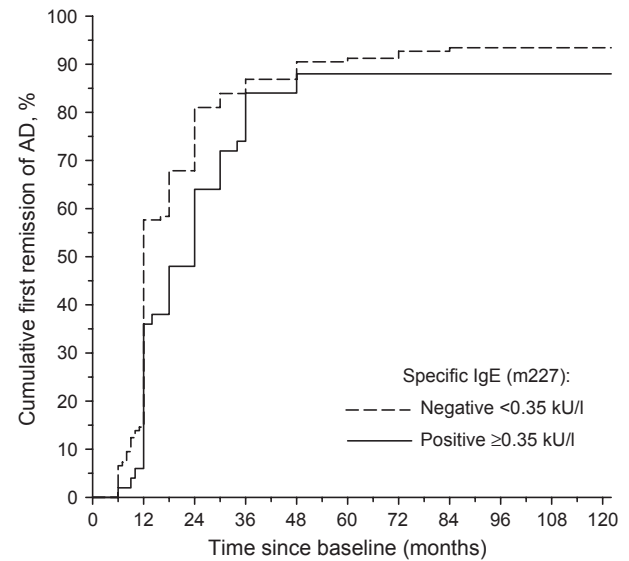


Figure 3 Cumulative first remission of atopic dermatitis according to the status of IgE against the *Malassezia* mix (m227).

Discussion

In the present study, the severity of AD in infancy was associated with specific IgE against the *Malassezia* mix at the 10-yr follow-up. Of the 11-yr-old children with both AD and FA in infancy, 27% had the allergen-specific IgE against the *Malassezia* mix, and 20% of them tested positive for the allergen-specific IgE to *M. sympodialis*. The distribution of allergen-specific IgE against *Malassezia* mix and *M. sympodialis* verifies that the use of a *Malassezia* mix representing three different species (m227) allows the detection of more *Malassezia*-sensitized individuals than does the analysis of specific IgE to *M. sympodialis* only (m70), as suggested earlier (24). Unfortunately, a control group of children without AD was not available in the present study. However, it has been reported previously that specific IgE against *Malassezia* has not been found in healthy controls or in patients with seborrheic dermatitis (14–17).

The retrospective aspect of our study forced us to estimate the severity of AD in infancy according to the notes available in patient files. Fortunately, the status had been carefully described by dermatologists, and therefore, the authors were able to form a clear picture of the AD in infancy. Of the infants studied, 81% showed only mild to moderate AD in spite of diagnosed allergies to milk and/or wheat. This differs from, for example, the study by Wolkerstorfer et al. (25) where small children sensitized to egg and milk but not on the respective elimination diets showed more severe AD.

In addition, the inclusion of children without a uniform diagnosis of FA is the result of the retrospective nature of our study. However, the clinical examination of the children and the repeated yearly challenge tests strengthened the initial FA diagnosis.

Due to different inclusion criteria between our study and previous ones (13, 26, 27), the results are not directly

comparable. In the study by Broberg et al. (26), only 5%–15% of patients with AD aged 0–10 yr had *Malassezia*-specific IgE, whereas the figure was 65%–90% for patients aged 11–21 yr, all suffering from generalized AD. In the present study, the number of children sensitized to *Malassezia* was relatively high, even though the severity of AD in infancy was not remarkable; only 19% of the children had suffered from severe AD. Furthermore, at the follow-up, the mean SCORAD value was low, 12, in the group having specific IgE against the *Malassezia* mix, in contrast to the mean SCORAD index of eight for those without IgE against *Malassezia* mix. The children in our study who tested positive for specific IgE to *Malassezia* suffered from more long-standing AD: 1 yr after the FA diagnosis, the number of children in remission of AD was higher in the group not sensitized to *Malassezia*.

There are two further reports emphasizing the importance of age in the development of IgE sensitization to *Malassezia*. Ong et al. (27) stated that the development of IgE sensitization to microbial allergens (including *Malassezia species*) is associated with older age (≥ 3 yr) in children with mild or moderate AD. A previous study on children with AD found no difference in the prevalence of IgE sensitization to *Malassezia species* between infants aged ≤ 12 months (15.2%) and >12 months (18.1%), but repeated episodes of oozing lesions in infancy were predictive of sensitization, as were high total serum IgE values (13).

In agreement with the results of Lange et al. (13), *Malassezia*-positive children in the present study had significantly higher total serum IgE than those with no IgE antibodies

against *Malassezia*. The mean SCORAD index (36) was markedly higher in the study by Lange et al. than in our patient population. Lange et al. concluded that patients with a severe clinical course of AD and high serum IgE values are at a higher risk of becoming sensitized to *Malassezia*. In our study, mild AD with FA was also associated with a higher risk of sensitization to *Malassezia*.

Until recently, local treatment has been the main practice for alleviating the symptoms of AD. However, a number of studies have reported the beneficial effect of removing foods suspected of provoking symptoms in children with AD (6, 28–30). In our experience, a meticulous diagnosis of food allergies and controlled elimination diets provide an important addition to local treatment.

In conclusion, the severity of AD in infancy and the rate of AD clearance were associated with sensitization to *Malassezia* at the 10-yr follow-up. Rapid and effective treatment aiming at total clearance of AD in infancy may reduce the risk of sensitization to *Malassezia* later in childhood. Furthermore, children with AD and specific IgE to *Malassezia* should be investigated for the possibility of FA.

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