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The association between *Staphylococcus aureus* colonization on cheek skin at 2 months and subsequent atopic dermatitis in a prospective birth cohort

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Abstract

Background *Staphylococcus aureus* may worsen already established atopic dermatitis (AD), but its primary role in the aetiopathogenesis and severity of AD is unclear.

Objectives To compare the prevalence of *S. aureus* colonization in early infancy in children who developed AD during the first 2 years of life with children who did not.

Methods In this prospective birth cohort study, which included 450 infants, we analysed bacterial swabs collected from cheek skin at 0 and 2 months of age. The development of AD, and its severity, was diagnosed by a physician and monitored prospectively for 2 years. Information on parental atopy, filaggrin gene mutation status and use of antibiotics and emollients was included in the analyses.

Results At birth, the occurrence of *S. aureus* colonization was similar in infants who developed subsequent AD and those who did not. At 2 months of age, *S. aureus* colonization was more common in children who later developed AD (adjusted hazard ratio 1.97, 95% confidence interval 1.21–3.19; $P=0.006$). No association was found between *S. aureus* colonization and AD severity or age at onset.

Conclusions It remains unknown whether colonization with *S. aureus* may directly increase the risk of AD, or whether it should be considered as secondary to skin barrier impairment or a skewed immune activity, but according to our findings, *S. aureus* colonization is more commonly increased at 2 months of age in children who later developed AD.

What is already known about this topic?

- *Staphylococcus aureus* colonization is common in the skin of patients with atopic dermatitis (AD) and *S. aureus* counts increase with increasing AD severity.
- Only a few studies have examined whether *S. aureus* colonization of infant skin is associated with an increased risk of AD.

What does this study add?

- *S. aureus* colonization on cheek skin at 2 months of age is associated with development of AD in the first 2 years of life.
- *S. aureus* colonization on cheek skin at 2 months of age is not associated with severity of AD.

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Atopic dermatitis (AD) is a common inflammatory skin disease that affects about 20% of young children.[1] AD is characterized by dry and pruritic skin, and the anatomical location of eczematous lesions tends to be age-dependent.[1] A parental history of atopic disease is a strong predictor of AD,[2,3] and mutations in the filaggrin (*FLG*) gene is the strongest genetic risk factor.[4]

The pathogenesis of AD involves a combination of an impaired skin barrier and a dysregulated immune response.[5,6] *Staphylococcus aureus* colonization is very common in skin of patients with AD and *S. aureus* counts increase with increasing AD severity.[7] This is partly explained by reduced levels of free fatty acids and acidic degradation products of filaggrin, in addition to the associated increase in skin pH.[8–10] Accordingly, *S. aureus* colonization is found in 39% of nonlesional skin sites and in 70% of lesional skin sites.[11] Despite the close link between AD and *S. aureus*, only a few studies have examined whether colonization is more prevalent in the skin of infants who later develop AD compared with those who do not.[12–14]

This prospective birth cohort study[15] examined whether colonization with *S. aureus* was more common in children who later developed AD during the first 2 years of life compared with children who did not develop AD.

Materials and methods

Study population

The BABY cohort[15] is a longitudinal birth cohort of 150 preterm and 300 term children recruited from August 2017 to August 2019. Children recruited from the maternity and neonatal ward at Rigshospitalet and Nordsjællands Hospital in Denmark were followed from birth until 2 years of age. The preterm cohort [gestational age (GA) $<37+0$ weeks] enrolled newborns without severe congenital abnormalities or conditions affecting their life expectancy. The term cohort (GA $37+0$–$41+6$ weeks) enrolled singleton newborns without antenatal fetal lung maturation using steroids. Recruitment was independent of their family history of atopic disease. Preterm children had the first study visit during the first month after birth, and another visit took place 2 months after the planned due date. Term children had their first study visit within the first 3 days after birth, and again 2, 6 and 12 months after birth. All parents participated in a structured telephone interview when the child was 18 months old and again at 24 months. All study visits were conducted by trained medical doctors. If children in either cohort developed eczema during the first 2 years of life, they underwent an extra study visit to confirm the diagnosis of AD by a physician and assess the severity using the Eczema Area and Severity Index (EASI).[16] DNA collected at birth using buccal swabs was genotyped for three common *FLG* mutations (R501X, 2282del4 and R2447X).[17]

Parents completed a questionnaire about lifestyle factors, pregnancy and parental atopic diseases before the 2-month study visit. A parental history of atopy was defined as either parent having current or previous AD, asthma, rhinitis or aeroallergen sensitization measured via skin prick testing or specific IgE. At the clinical 2-month visit, information on use of any antibiotics or emollients within the last 2 months was recorded.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the scientific Ethical Committee of the Capital of Region H (H-16042289 and H-16042294) and the local data protection agency (ID no. HGH-3017-040, I-suite no. 05578).

Skin swab collection from the cheeks

At 0 (baseline) and 2 months follow-up, children had one bacterial swab collected from cheek skin (Eswab Collection and Transport System, Copan Italia, Brescia, Italy). The samples were automatically inoculated, spread on agar plates and incubated using BD-Kiestra (Becton Dickinson, Drachten, the Netherlands). The plates were cultured for 16–18 h at 35 °C. Columbia agar plates with 5% horse blood (Difco, Thermo Fisher Scientific, Waltham, MA, USA), CHROMagar *Staph aureus* (CHROMagar, Paris, France) were used to culture and identify *S. aureus*. Colonies resembling either *S. aureus* were finally identified by MALDI-TOF MS (Biotyper, Bruker Daltonics, Bremen, Germany). In the first years of the study, Staphaurex (Thermo Fisher Scientific) was used for the identification of *S. aureus* rather than CHROMagar *Staph aureus*. Antimicrobial susceptibility testing was interpreted according to European Committee on Antimicrobial Susceptibility Testing breakpoints and guidelines. Isolates were frozen at −80 °C in bouillon with 10% glycerol until further investigation.

Confirmed *S. aureus* isolates were submitted to the Danish National Reference Laboratory for Antimicrobial Resistance for typing. Polymerase chain reaction (PCR) detection of the *spa* gene confirmed the submitted isolates to be *S. aureus*. PCR simultaneously detected the *spa*, *mecA*, *mecC* and *lukF/S-pv* genes.[18] The isolates were typed by sequencing of the *spa* gene and *spa* types were annotated using Bionumerics 7.6 (Applied Maths, Sint-Martens-Latem, Belgium) and RidomStaphType 1.4 (Ridom GmbH, Würzburg, Germany). The *spa* types were approximated to multilocus sequence typing clonal complexes (CCs). Distribution of *spa* types found in this study was compared with data from the national *S. aureus* bacteraemia (SAB) database.[19]

Corneocyte sampling from the hands and analysis

At 0 and 2 months of age the children born to term had eight skin tape strips collected from the dorsal side of one hand. No preference was given to the left or right side, but selection depended on the positioning of the child. Similarly, around the date of birth and 2 months after the due date, the children born preterm had eight skin tape strips collected from the upper back. Tape strip no. 5 was examined for natural moisturizing factor (NMF).[20] Tape strip no. 6 was analysed for thymus and activation-regulated chemokine (TARC/CCL17) and other cytokines.[21] Cytokine concentrations in the extracts were measured on multiplex panels using MESO QuickPlex SQ 120 (MSD, Rockville, MA, USA).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk,

NY, USA) and R statistical software version 4.1.0. All distributions were presented as medians with interquartile range (IQR).

Hazard ratios (HR) with 95% confidence intervals (CIs) were calculated using multivariate Cox proportional hazard regression analysis with 'development of AD in the first 2 years of life' as the outcome, 'colonization with *S. aureus* on cheek skin' as the exposure and 'age in months' as the underlying time variable. The analyses were adjusted for history of parental atopy, sex, *FLG* mutation status, maturity at birth and use of antibiotics since last visit. In additional regressions, we further adjusted for use of emollients, birth method, birth season and levels of TARC/CCL17 and NMF. The risk was visualized using cumulative risk curves, and the log-rank test was used to compare the event-free time of the *S. aureus* colonized group with the noncolonized group. All models were investigated for confounding and proportional hazard assumptions were tested using Schoenfeld residuals. The proportional hazard assumptions were met for all analyses. Tests for interaction between 'colonization with *S. aureus*' and 'maturity' and '*FLG* mutations' were performed in the final Cox regression model. All twin B and triplets B and C were excluded from our analyses. Only children without onset of AD at the time of examination were included in the analyses. Missing data were omitted from the analyses. No correction for multiple analyses was performed, as this was an exploratory study. A significance level of $P<0.05$ was used.

Results

A total of 300 children born to term were enrolled, and 97% ($n=290$) were eligible for analyses. *FLG* mutations were detected in 9% ($n=25$), and 64% ($n=153$) had a parental history of atopy. Of 150 enrolled children born prematurely, 79% ($n=118$) had complete data and were eligible for analysis. A total of 4% ($n=5$) had *FLG* mutations, and 76% ($n=89$) had a history of parental atopy. Baseline demographics stratified by *S. aureus* colonization at 2 months of age are provided in (Table 1).

Among term-born children, 34% ($n=99$) developed AD in the first 2 years of life. Term-born children developed AD at a median age of 6 months (IQR 3.0–11.3) and had a median EASI score of 4.8 (IQR 2.20–7.9). A total of 21% ($n=25$) preterm children developed AD in the first 2 years of life. The preterm children had a median age at AD onset of 9 months (IQR 6.3–11.8) and had a median EASI score of 1.6 (IQR 1.1–3.3) at onset. Information on onset and severity of AD stratified by *S. aureus* colonization is provided in Table S1 (see Supporting Information).

The crude risk of developing AD was increased in children (term and preterm together) with a history of parental atopy (HR 2.17, 95% CI 1.31–3.60; $P<0.01$) and *FLG* mutations (HR 3.74, 95% CI 2.22–6.30; $P<0.01$), but was lower in children born prematurely (HR 0.49, 95% CI 0.26–0.81; $P<0.01$) when compared with children born at term (Table 2).

Staphylococcus aureus colonization on cheek skin at birth and 2 months and development of atopic dermatitis

In analysis including both mature and premature-born children, a total of 116 children exhibited *S. aureus* colonization at the 0-month visit and/or the 2-month visit. A total of 19.4% of children with AD onset in the first 2 years of life were colonized with *S. aureus* at birth compared with 17.3% in the group of children who did not develop AD (Table 3). There was no association between *S. aureus* colonization at birth and development of AD (crude HR 1.06, 95% CI 0.67–1.69; $P=0.88$). At 2 months of age, 20.7% of preterm and term children who later developed AD exhibited *S. aureus* colonization compared with 12.8% of the children who did not develop AD (crude HR 1.63, 95% CI 1.03–2.59; $P=0.04$) (Tables 2–3).

The prevalence of AD was 29.7% in children with no colonization at any point, 31.6% in children with *S. aureus* colonization at birth, 45% in children with *S. aureus* colonization at 2 months of age and 42.9% in children with *S. aureus* colonization both at birth and at 2 months of age. We found no association between *S. aureus* colonization and early

Table 1 Baseline demographics stratified by *Staphylococcus aureus* colonization at 2 months of age[a]

	Mature		Premature		Overall	
	***S. aureus* colonization (*n*=36)**	**No *S. aureus* colonization (*n*=230)**	***S. aureus* colonization (*n*=21)**	**No *S. aureus* colonization (*n*=75)**	***S. aureus* colonization (*n*=57)**	**No *S. aureus* colonization (*n*=305)**
AD onset	16 (44)	80 (34.8)	8 (38)	10 (13)	24 (42)	90 (29.5)
Male sex	21 (58)	113 (57.8)	15 (71)	43 (57)	36 (63)	176 (57.7)
Birth method						
Vaginal	18 (50)	144 (62.6)	5 (24)	33 (44)	23 (40)	177 (58.0)
Caesarean section	18 (50)	86 (37.4)	16 (76)	42 (56)	34 (60)	128 (42.0)
Birth season						
Winter	14 (39)	55 (23.9)	1 (5)	8 (11)	15 (26)	63 (20.7)
Spring	7 (19)	51 (22.2)	1 (5)	20 (27)	8 (14)	71 (23.3)
Summer	3 (8)	55 (23.9)	18 (86)	34 (45)	21 (37)	89 (29.2)
Autumn	12 (33)	69 (30.0)	1 (5)	13 (17)	13 (23)	82 (26.9)
FLG mutation status						
Mutation carrier	1 (3)	21 (9.2)	1 (5)	2 (3)	2 (4)	23 (7.6)
Parental history of atopy[b]						
Yes	27 (79)	119 (61.0)	16 (76)	55 (73)	43 (78)	174 (64.4)

AD, atopic dermatitis. [a]Children without information on *S. aureus* colonization at 2 months of age were excluded. [b]Parental history of AD, asthma, rhinitis, or aeroallergen sensitization measured via skin prick testing or specific IgE allergies in at least one parent. Data are presented as *n* (%).

Table 2 The association between study participant characteristics and atopic dermatitis (AD) in the first 2 years of life

	Atopic dermatitis, *n*/*N* (%)	Crude HR (95% CI)	*P*-values	Adjusted HR[a] (95% CI)	*P*-values	Adjusted HR[b] (95% CI)	*P*-values
Staphylococcus aureus colonization at 2 months							
Positive	24/57 (42)	**1.63 (1.03–2.59)**	**0.04**	**1.97 (1.21–3.19)**	**< 0.01**	**1.99 (1.22–3.25)**	**< 0.01**
Negative	90/305 (29.5)	1		1		1	
Sex							
Male	72/240 (30.0)	0.99 (0.68–1.46)	1.0	0.98 (0.64–1.48)	0.9	1.01 (0.66–1.54)	1.0
Female	52/171 (30.4)	1		1		1	
Parental atopy							
Yes	87/242 (36.0)	**2.17 (1.31–3.60)**	**< 0.01**	**2.35 (1.41–3.94)**	**< 0.01**	**2.36 (1.40–3.95)**	**< 0.01**
No	22/114 (19.3)	1		1		1	
FLG mutations							
Yes	19/30 (63)	**3.74 (2.22–6.30)**	**< 0.01**	**3.80 (2.17–6.67)**	**< 0.01**	**3.56 (2.02–6.29)**	**< 0.01**
No	104/372 (28.0)	1		1		1	
Maturity							
Term	99/293 (33.8)	1	**< 0.01**	1	**< 0.01**	1	**0.04**
Preterm	25/118 (21.2)	**0.49 (0.30–0.81)**		**0.45 (0.27–0.77)**		**0.53 (0.28–0.97)**	
Use of any antibiotics[c]							
Yes	10/36 (28)	0.86 (0.43–1.70)	0.7	0.80 (0.36–1.74)	0.6	0.75 (0.33–1.67)	0.47
No	105/330 (31.8)	1		1		1	
Birth season							
Winter	29/89 (33)	1				1	
Spring	23/86 (27)	0.68 (0.38–1.24)	0.2			0.82 (0.42–1.57)	0.5
Summer	38/130 (29.2)	0.86 (0.51–1.44)	0.6			1.15 (0.62–2.13)	0.7
Autumn	34/106 (32.1)	0.87 (0.51–1.48)	0.6			1.06 (0.57–1.95)	0.9
Use of emollients[c]							
Yes	39/110 (35.5)	1.14 (0.76–1.73)	0.6			1.02 (0.65–1.59)	0.9
No	76/256 (29.7)	1				1	
Birth method							
Vaginal	72/221 (32.6)	1				1	
Caesarean section							
Planned	27/84 (32)	1.17 (0.73–1.85)	0.5			0.98 (0.57–1.68)	0.9
Acute	25/106 (23.6)	0.58 (0.34–0.97)	**0.04**			0.59 (0.32–1.08)	0.09

CI, confidence interval; HR, hazard ratio. [a]Cox regression adjusted for swab result, sex, history of parental atopy, *FLG* mutation status, maturity and use of antibiotics. [b]Cox regression adjusted for swab result, sex, history of parental atopy, *FLG* mutation status, maturity, use of antibiotics, birth season, use of emollient and birth method. [c]Use of emollient and antibiotics during the first 2 months. Bold values are significant.

(2–5 months) vs. late (6–24 months) onset of AD compared with children who did not develop AD ($P=0.15$).

Using a cumulative risk curve, *S. aureus* colonization at 2 months was found to be significantly associated with development of AD in the first 2 years of life (log-rank test $P=0.04$, Figure 1). In an analysis adjusted for sex, parental atopy, *FLG* mutations, maturity and use of antibiotics during the first 2 months, *S. aureus* colonization at 2 months remained significantly associated with subsequent AD (aHR 1.97, 95% CI 1.21–3.19; $P<0.01$) (Table 2). In additional regression analysis, birth method, birth season and parental report of emollient use during the first 2 months were included as possible confounders, but the association remained significant (aHR 1.99, 95% CI 1.22–3.25; $P<0.01$) (Table 2). No interaction between *S. aureus* colonization and *FLG* mutation status ($P=0.17$) or maturity ($P=0.12$) was observed. However, when stratifying children based on preterm vs. term status, the association between *S. aureus* colonization at 2 months of age and development of AD was stronger among preterm children than among term children

Table 3 Distribution of skin swab test results for *Staphylococcus aureus* taken at birth and 2 months of age in children born to term or preterm by atopic dermatitis (AD)

	Mature		Premature		Overall	
	Children with AD	Children without AD	Children with AD	Children without AD	Children with AD	Children without AD
At birth	*n*=99	*n*=191	*n*=25	*n*=93	*n*=124	*n*=284
Positive	17 (17)	30 (15.7)	7 (28)	19 (20)	24 (19.4)	49 (17.3)
Negative	76 (77)	143 (74.9)	15 (60)	62 (67)	91 (73.4)	205 (72.2)
Lost/not taken	6	18	3	12	9	30
At 2 months of age[a]	*n*=97	*n*=177	*n*=19	*n*=81	*n*=116	*n*=258
Positive	16 (17)	20 (11.3)	8 (42)	13 (16)	24 (20.7)	33 (12.8)
Negative	80 (83)	150 (84.7)	10 (53)	65 (80)	90 (77.6)	215 (83.3)
Lost/not taken	1	7	1	3	2	10

[a]Preterm age is 2 months after due date. Mature: removed dropouts/lost to follow-up ($n=10$). Premature: removed twins/triplets ($n=22$) and dropouts/lost to follow-up ($n=10$). Data are presented as *n* (%).

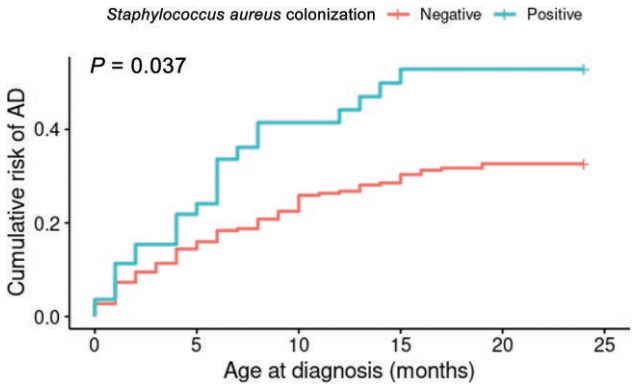

Figure 1 Cumulative risk of atopic dermatitis (AD) during the first 2 years of life in children with *Staphylococcus aureus* colonization on the skin vs. no colonization on the cheek at 2 months of age. *P*-values were obtained using the log-rank test.

(preterm children aHR 4.21, 95% CI 1.32–13.4; $P=0.02$; term children aHR 1.80, 95% CI 0.99–3.28; $P=0.05$) (Tables S2–S3; see Supporting Information).

While current levels of TARC/CCL17 and NMF in tissue collected from the dorsal aspects of the hands were measured, adjustment for these variables did not change the association between AD and *S. aureus* colonization (data not shown).

The median EASI scores were 4.2 (IQR 2.0–7.9) in children with no colonization at any time ($n=55$), 2.5 (IQR 1.6–5.8) for children with *S. aureus* colonization at birth ($n=13$), 2.0 (IQR 1.2–5.4) for children with *S. aureus* colonization at 2 months of age ($n=16$), and 10.4 (IQR 2.5–19.1) in children with *S. aureus* colonization at both birth and 2 months of age ($n=6$). There was no association between colonization at 2 months of age and onset of moderate-to-severe AD in the fully adjusted analysis (aHR 1.94, 95% CI 0.71–5.34; $P=0.20$).

Spa types distribution in the baby cohort

From a total of 130 skin swabs that were positive for *S. aureus*, collected at 0 and 2 months of age in both premature and mature children, 87 were eligible for *spa* typing. No *spa* types were more prevalent in the AD group compared with the non-AD group, or among groups of premature and mature children. The five most common *spa* types were t084/CC15 (12.6%), t008/CC8 (6.9%), t012/CC30 (5.7%), t005/CC22 (4.6%) and t065/CC45 (4.6%), whereas singletons made up 44.8% of the studied isolates. The distribution of *spa* types among the isolates was similar to the *spa* type distribution among all Danish SAB cases and among SAB cases in children who were < 1 year of age from the period 2017–2021 ($n=217$).

Discussion

We showed that colonization with *S. aureus* on cheek skin at 2 months of age, but not at birth, was significantly more prevalent in infants who developed AD within the first 2 years of life. *S. aureus* colonization was not predictive of AD severity.

Our results support findings from a Swiss study in which *S. aureus* colonization of the axillary skin at 3 months was predictive of AD at 2 years.[14] However, other studies[12,13] have failed to show an association between *S. aureus* colonization in infancy and subsequent AD, and the pathogenetic role of *S. aureus* in AD remains unclear. *S. aureus* is known to bind to corneodesmosin displayed on the tip of corneocyte protrusions using fibronectin-binding protein B clumping factor B.[22] These corneocyte protrusions are higher in number in children with AD and *FLG* mutations, and are associated with dry skin and any deficiency in the filaggrin axis.[23] The expression of fibronectin is increased by interleukin-4, a key cytokine in AD pathogenesis.[24] After binding, *S. aureus* then penetrates the epidermis by proteolytic mechanisms, and then triggers the release of proinflammatory cytokines,[25] which in turn downregulate skin barrier proteins. The decreased capacity of the epidermis of patients with AD to produce antimicrobial peptides, including cathelicidin and β-defensins, may further promote the growth of *S. aureus*.[26] We attempted to adjust for skin tape strip biomarker data collected from the dorsal hands in our fully adjusted analysis as we previously showed that increased levels of TARC/CCL17, in addition to reduced levels of phytosphingosine and urocanic acid, measured at 2 months of age was predictive of AD in the first year.[27] However, adjustment did not change the observed association between *S. aureus* colonization in cheek skin and AD. Moreover, analyses adjusted for emollient and antibiotic use did not alter the associations. We did not find an association between having *S. aureus* colonization at birth and 2 months of age and more severe AD. A previous Danish study found significantly higher AD scores in children with neonatal *S. aureus* colonization [mean SCORing AD (SCORAD) score 31.0, SD 12.7] compared with children without neonatal *S. aureus* colonization (mean SCORAD score 22.8, SD 11.1).[13] As we included both preterm and term children, adjustment was also made for maturity, but the association remained significant and there was no interaction between *S. aureus* colonization and maturity. However, when stratifying children according to maturity, we observed that the association appeared to be stronger among preterm children when compared with term children.

One particular strength of our study is the collection site of *S. aureus*. AD most often begins in facial skin areas, on the cheeks in particular,[28] and facial AD is more common in children with *FLG* mutations.[29] In this context, we previously showed that > 50% of the children in our birth cohort developed AD on cheek skin.[30] An Irish study showed how the skin barrier matures slowly in cheek skin of infants compared with other skin sites, reinforcing the idea that cheek skin would be a sensitive skin area to study the role of *S. aureus* colonization.[31] This may explain why a Danish birth cohort, where bacteria were collected from the vestibulum nasi and/or perineum at week 2 and week 11 (median age 4 weeks), did not show an association between colonization of *S. aureus* and AD.[13]

Based on our recent data,[27] along with findings from the recent STOP-AD trial,[32] there appears to be a window starting more than 3 days after birth and lasting until at least 8 weeks into life where skin biomarkers that can be predictive of AD can be collected and where intervention with specific lipid-rich emollients may reduce the risk of AD. Our

present findings corroborate this notion, as we identified alterations in cheek skin at 2 months of age, indicated by increased colonization with *S. aureus*, in those who developed AD.

The *spa* and CC types of children in our study were comparable with those of the general Danish population,[19] indicating that the infants in our cohort were unselected in terms of microbial composition. We did not observe any association between *spa* groups or CC groups and the onset of AD during the first 2 years of life. This provides confirmation of earlier findings, which indicated that no specific types of *S. aureus* are associated with AD.[13]

Other strengths of our study included the close prospective follow-up and the requirement of physician-diagnosed AD. While participants were randomly selected at enrolment, we observed a high proportion of children with parental history of atopy. For this reason, and because of our predominantly urban study population, our results may not be generalizable. However, as investigators were unaware of family risk factors during examination, bias was minimized. The physicians and parents were not aware of the colonization status during the follow-up period. Parents to especially preterm-born children were less inclined to attend the hospital owing to fear of illness, particularly during the COVID-19 pandemic. Term and preterm children were examined at different timepoints; term children were examined at 0–3 days after birth and 2 months after birth vs. preterm children examined at 0–28 days after birth and 2 months after their due date. Hence, comparisons of *S. aureus* colonization between the two groups should be interpreted with caution. This paper is a part of a larger study, nested in the BABY cohort, implicating possible type I errors from multiple testing. Owing to the exploratory nature of the study, no multiple testing was performed; hence, our findings should be interpreted with caution, and future studies are needed to confirm the findings of this prospective birth cohort.

In conclusion, in a prospective birth cohort of premature and mature-born children, colonization with *S. aureus* at 2 months of age in cheek skin was associated with subsequent AD in the first 2 years of life but not with severity of AD. Our findings imply that early skin alterations are present and detectable before the onset of AD.

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Conflicts of interest

J.P.T. is an advisor for AbbVie, Almirall, Arena Pharmaceuticals, OM Pharma, Coloplast, Aslan Pharmaceuticals, Union Therapeutics, Eli Lilly & Co, LEO Pharma, Pfizer, Regeneron, and Sanofi-Genzyme, a speaker for AbbVie, Almirall, Eli Lilly & Co, LEO Pharma, Pfizer, Regeneron and Sanofi-Genzyme, and has received research grants from Pfizer, Regeneron and Sanofi-Genzyme. J.T. has been a speaker or has served on advisory boards for Sanofi, AbbVie, LEO Pharma, Pfizer, Eli Lilly and Company, Novartis, UCB Pharma, Union Therapeutics, Almirall and Janssen Pharmaceuticals, and has received research support from Sanofi, AbbVie, LEO Pharma, Novartis, UCB Pharma and Janssen Pharmaceuticals outside the submitted work. L.S. has been an advisor or speaker for AbbVie, Eli Lilly, Novartis, Sanofi, Celegen, LEO Pharma, Janssen-Cilag, UCB, BMS, Boehringer Ingelheim and Almirall, and has received research support from Sanofi, LEO Pharma, UCB, BMS and Janssen outside the submitted work. A.E. has received research funding from Pfizer, Eli Lilly, Novartis, Bristol-Myers Squibb, AbbVie, Janssen Pharmaceuticals, Boehringer Ingelheim, the Danish National Psoriasis Foundation, the Simon Spies Foundation and the Kgl Hofbundtmager Aage Bang Foundation and has also received honoraria as a consultant and/or speaker from AbbVie, Almirall, LEO Pharma, Zuellig Pharma Ltd., Galápagos NV, Sun Pharmaceuticals, Samsung Bioepis Co., Ltd., Pfizer, Eli Lilly and Company, Novartis, Union Therapeutics, Galderma, Dermavant, UCB, Mylan, Bristol-Myers Squibb, McNeil Consumer Healthcare, Horizon Therapeutics, Boehringer Ingelheim and Janssen Pharmaceuticals. A.-S.H. has been a consultant for Coloplast A/S and speaker for LEO Pharma.

Data availability

The data underlying this article cannot be shared publicly owing to the privacy of individuals who participated in the study.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and was approved by the scientific Ethical Committee of the Capital of Region H (H-16042289 and H-16042294) and the local data protection agency (ID-no: HGH-3017-040, I-suite no.:05578).

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website.

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