



## Paper II

## ORIGINAL ARTICLE

## Epidemiology and Genetics

# *Staphylococcus aureus* enterotoxin sensitization is associated with allergic poly-sensitization and allergic multimorbidity in adolescents

M. Sørensen<sup>1,2</sup>  | C. Klingenberg<sup>1,2</sup> | M. Wickman<sup>3,4</sup> | J. U. E. Sollid<sup>5</sup> |  
A-S. Furberg<sup>6,7</sup> | C. Bachert<sup>8</sup>  | J. Bousquet<sup>9,10,11</sup>

<sup>1</sup>Department of Paediatric and Adolescent medicine, University Hospital of North Norway, Tromsø, Norway

<sup>2</sup>Paediatric Research Group, Department of Clinical Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway

<sup>3</sup>Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden

<sup>4</sup>Sachs Children's Hospital, Södersjukhuset, Stockholm, Sweden

<sup>5</sup>Research Group for Host-Microbe Interactions, Department of Medical Biology, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway

<sup>6</sup>Department of Microbiology and Infection Control, University Hospital of North Norway, Tromsø, Norway

<sup>7</sup>Epidemiology of Chronic Diseases Research Group, Department of Community Medicine, Faculty of Health Sciences, UiT The Arctic University of Norway, Tromsø, Norway

<sup>8</sup>Upper Airways Research Laboratory, Department of Otorhinolaryngology and Head and Neck Surgery, Ghent University, Ghent, Belgium

<sup>9</sup>Hospital Montpellier France, European Innovation Partnership on Active and Healthy Ageing Reference Site MACVIA-LR Contre les MALadies Chroniques pour un Vieillissement Actif en Languedoc-Roussillon, Paris, France

<sup>10</sup>VIMA: Ageing and Chronic Diseases, Epidemiological and Public Health Approaches, U1168 INSERM, Paris, France

<sup>11</sup>Université Versailles St-Quentin-en-Yvelines UVSQ, UMR-S 1168, Versailles, France

## Correspondence

Martin Sørensen, Department of Paediatric and Adolescent medicine, University Hospital of North Norway, Tromsø, Norway.  
Email: martin.sorensen@unn.no

## Funding information

Universitetet i Tromsø; Helse Nord RHF

Edited by: Bodo Niggemann

## Abstract

**Background:** *Staphylococcus aureus* (*S. aureus*) carriage and sensitization to *S. aureus* enterotoxins (SEs) have been associated with allergic diseases. From the Tromsø Study Fit Futures 2, we have previously shown an association between *S. aureus* carriage and severe allergic disease and allergic multimorbidity. However, the role of *S. aureus* carriage and SE sensitization on allergic multimorbidity and allergic sensitization is unclear.

**Objective:** To study associations of both nasal *S. aureus* carriage and SE sensitization to allergic disease and allergic sensitization.

**Methods:** A cross-sectional study of a school-based cohort in late adolescence (aged 18-19 years: The Tromsø Study Fit Futures 2). Self-reported allergic diseases were assessed using the Mechanisms of the Development of ALLergy questionnaire (MeDALL). Participants were tested for nasal *S. aureus* carriage, serum total IgE and specific IgE to SEs, and food and inhalant allergens.

**Results:** A total of 868 participants were studied. Sensitization to at least one food or inhalant allergen was found in 319 of 765 (41.7%), and to at least one SE in 173 of 656 (26.2%) participants. SE sensitization, but not *S. aureus* carriage, was associated with poly-sensitization to food and inhalant allergens. SE-sensitized participants had higher median specific IgE to inhalant allergens (41.4 kU<sub>A</sub>/L, IQR 10.1-118.4)

compared to non-SE-sensitized participants (18.0 kU<sub>A</sub>/L, IQR 5.5-48.6,  $P=0.004$ ), but not to food allergens. SE sensitization was associated with allergic multimorbidity.

**Conclusion:** Sensitization to SEs may play a role in the development of allergen poly-sensitization and allergic multimorbidity.

#### KEYWORDS

Allergic disease, Allergic multimorbidity, Poly-sensitization, *Staphylococcus aureus*, *Staphylococcus aureus* Enterotoxins

## 1 | INTRODUCTION

*Staphylococcus aureus* carriage on eczematous skin is directly correlated to eczema severity,<sup>1</sup> but there are conflicting results regarding the associations between *S. aureus* carriage and allergic rhinitis or asthma.<sup>2-5</sup> In our first paper from the Tromsø Study Fit Futures 2 (TFF2), we found that nasal *S. aureus* carriage was associated with eczema, severe eczema, severe asthma, severe allergic rhinitis, and allergic multimorbidity in adolescents.<sup>6</sup>

Sensitization to *S. aureus* enterotoxins (SEs) is also associated with allergic disease. Local and/or systemic specific IgE to SEs may play a role in the development and/or disease severity of allergic disease.<sup>7-11</sup> SE sensitization is associated with asthma in adults and in the elderly, but an association has not yet been clearly demonstrated among children and adolescents.<sup>7,8,12,13</sup> Some studies indicate an association between SE sensitization and polyclonal allergen sensitization, reflected by higher total IgE levels among SE-sensitized individuals.<sup>7,8</sup> However, the patterns and the magnitude of allergen poly-sensitization related to SE sensitization have not been studied.

Recently, it was hypothesized that allergic multimorbidity and IgE poly-sensitization are associated and related to the persistence or re-occurrence of fetal type 2 signaling.<sup>14</sup> It has been suggested that reoccurrence of type 2 signaling may be due to epigenetic changes induced by viral infections or *S. aureus* infection/carriage and subsequent SE sensitization.

The aim of this study was to study associations between both nasal *S. aureus* carriage and SE sensitization to allergic disease and allergic sensitization in the cross-sectional Tromsø Study Fit Futures 2 (TFF2).

## 2 | MATERIAL AND METHODS

### 2.1 | Setting

The TFF cohort in Northern Norway included students from all eight high schools in the municipalities of Tromsø and Balsfjord.<sup>6</sup> In this region, more than 90% of the population in the age group of 16-19 years attend high school.

### 2.2 | Participants

The TFF cohort was initiated in 2010-11. Eligibility criteria and the sources and methods of study recruitment are previously published.<sup>6</sup>

Briefly, all first-year high school students in both academic and vocational educational programs were invited to participate (TFF1) and 92.8% attended.<sup>15</sup> The current study is part of the second wave of the study (2012-13), where all third-year high school students, including all participants from TFF1, were invited for follow-up (TFF2). Among 1208 invited students, 868 (71.9%) participated in TFF2. Each participant completed a web-based general health and lifestyle questionnaire (<http://www.questback.com>) and underwent clinical examination during a one-day session at the Clinical Research Unit, University Hospital of North Norway, Tromsø, between November 2012 and June 2013.

### 2.3 | Definition of allergy-related disease outcomes

The Mechanisms of the Development of ALLergy (MeDALL) core questionnaire for adolescents was incorporated in the web-based questionnaire.<sup>16</sup> The MeDALL questionnaire was translated from Swedish to Norwegian and back-translated in good agreement with the original Swedish and English versions. The classification of allergic diseases was based on standardized self-reported questions (MeDALL) used by European population-based birth cohort studies on asthma and allergy, and validated in the International Study of Asthma and Allergies in Childhood.<sup>16</sup> Outcome definitions of allergic diseases have previously been published<sup>6</sup> (Table S1, Online Repository) and are based on reported symptoms from the MeDALL questionnaire.

### 2.4 | Specific IgE in serum

Serum specific IgE and total IgE were measured consecutively in fresh serum samples with the Phadia ImmunoCAP<sup>®</sup> allergen-specific IgE test panel system (Phadia-Thermo Fisher, Uppsala, Sweden). We analyzed two screening panels of inhalant allergens (birch, timothy, mugwort pollen, *Alternaria alternata*, and *Cladosporium herbarum*; and cat, horse, dog, house dust mite, and rabbit), one screening panel for food allergens (egg white, cow's milk, cod, wheat, peanut, and soy) and total IgE. In addition, specific IgE to SE-A, SE-B, SE-C, and TSST (toxic shock syndrome toxin) were analyzed in spring 2016 in serum samples stored at  $-70^{\circ}\text{C}$ . Specific IgE values  $<0.35$  kU<sub>A</sub>/L for a screening panel were interpreted as negative for all included allergens. If screening panels had IgE  $\geq 0.35$  kU<sub>A</sub>/L, then specific IgE was measured to all allergens included in the screening panel. In line with

previous publications, a lower cutoff level for SE sensitization, specific IgE >0.1 kU<sub>A</sub>/L, was chosen.<sup>7,8,12,13</sup> All serum analyses were performed at the Department of Laboratory Medicine, University Hospital of North Norway. To assess number of allergen sensitizations, we divided participants, separately for inhalant and food allergens, in nonsensitized, mono-sensitized (one allergen), and poly-sensitized groups (>1 allergen).<sup>17</sup> To assess levels of specific IgE values, all values from either food or inhalant allergens were added and reported as median sum of inhalant or food allergen values with 95% confidence intervals.

## 2.5 | Nasal *Staphylococcus aureus* carriage

Using a NaCl-moistened sterile rayon-tipped swab, we swabbed both anterior nares (taking care to avoid skin contact) in 819 participants. *S. aureus* was identified using methods previously described<sup>18</sup> with a few modifications; all swabs were submerged in Bacto® *Staphylococcus* medium broth (Difco laboratories, Sparks, MD, USA) for enrichment and incubated for 18-24 hours at 37°C before plating. The liquid culture was then plated on blood agar (Oxoid, Cambridge, UK) and *S. aureus* ID agar (SAID, bioMérieux, Marcy l'Etoile, France) and incubated for 18-24 hours at 37°C. One colony representative for the most dominating morphology was selected and confirmed as *S. aureus* by the Staphaurex Plus (Remel, Lenexa, KS, USA) agglutination test. Only observations of bacterial growth on blood agar (Oxoid, Cambridge, UK) and/or SAID agar (BioMérieux, Marcy l'Etoile, France) plates were included. We defined a positive *S. aureus* culture result as *S. aureus* carriage. We did not perform quantitative analysis of bacterial load.

## 2.6 | Statistical methods

We performed statistical analyses with IBM SPSS® statistics, version 23. The characteristics of the study participants and the prevalence of symptoms of allergic diseases were described with summary statistics. Pearson's chi-square test was used in comparisons of categorical variables, and Student's *t* test (normally distributed data) and Mann-Whitney U test (non-normally distributed data) were used for comparisons of continuous variables. Multivariate logistic regression models were used to analyze associations between *S. aureus* carriage and SE sensitization with allergic disease. Separate analyses were performed with each allergic entity as dependent variable and with *S. aureus* carriage or SE sensitization as independent variables, adjusting for gender, body mass index (BMI), smoking, use of snuff tobacco, and physical activity. Comparison between *S. aureus* carriage and SE sensitization with food and inhalant allergen sensitization was made with multinomial logistic regression models, comparing poly-sensitized or mono-sensitized groups with the nonsensitized group as reference category. A two-way between-groups analysis of variance was conducted to explore effect modification of SE sensitization on *S. aureus* carriage. Statistical significance was assumed at a 5% level.

## 2.7 | Ethics

The study was approved by The Regional Committee for Medical and Health Research Ethics - North, Norway. Each participant in TFF2 signed informed consent.

## 3 | RESULTS

### 3.1 | General characteristics of study participants

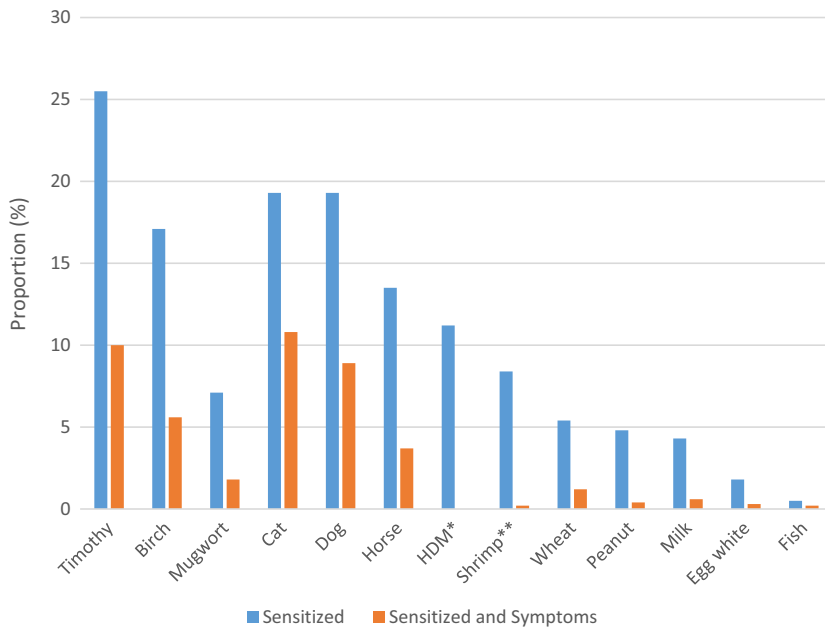
Among the 868 participants, 844 (97.2%) answered the questionnaire and 825 (95.1%) underwent clinical examinations. We obtained blood samples for specific IgE measurements from 765 (88.1%) participants, of which 656 were available for analysis of specific IgE to SEs. Another 819 (94.4%) participants had a nasal swab for analysis of *S. aureus* carriage (Fig. S1, Online Repository). Background characteristics of the participants are previously published<sup>6</sup> (Table S2, Online Repository). Analysis of background characteristics related to SE sensitization showed that BMI  $\geq 25$  kg/m<sup>2</sup> ( $P=.045$ ) and not being physical active outside school time ( $P=.001$ ) was associated with SE sensitization, whereas no difference was seen for age, gender, smoking, use of snuff tobacco, and screen time. Among the 480 (55.3%) females and 388 males, the mean age was 18.6 years and 26% were overweight (BMI  $\geq 25$  kg/m<sup>2</sup>). Male participants were more often overweight and used more snuff tobacco compared to female participants. Nasal *S. aureus* carriage was found in 420 of 819 (51.3%) participants, with no gender difference, and was twice as prevalent as sensitization to at least one SE in 173 of 656 participants (26.4%). Of 173 SE-sensitized participants, 102 (59.0%) were sensitized to one SE, 36 (20.8%) to two SEs, 19 (11.0%) to three SEs, and 16 (9.2%) to all four SEs. There was no significant association between *S. aureus* carriage and SE sensitization ( $P=.062$ ), and only 56% of SE-sensitized participants were current nasal *S. aureus* carriers.

### 3.2 | Specific IgE reactivity and relation to reported allergic symptoms and allergic disease

Figure 1 shows the prevalence of sensitization to food and inhalant allergens compared with prevalence of self-reported allergic symptoms to these allergens. We found specific IgE reactivity to at least one food or inhalant allergen in 319 of 765 (41.7%) participants, more frequently in men (47.7%) than in women (36.7%,  $P=.003$ ). Specific IgE reactivity to at least one allergen was found in 54 of 85 (63.5%) participants with asthma, 48 of 79 (60.8%) participants with eczema, 149 of 189 (78.8%) participants with allergic rhinitis, and 66 of 75 (88%) participants with allergic multimorbidity.

### 3.3 | Nasal *Staphylococcus aureus* carriage and association with allergic sensitization

*Staphylococcus aureus* carriage was more frequent in participants poly-sensitized to inhalant allergens (OR 1.65, 95% CI; 1.19-2.31) compared to nonsensitized participants (Table 1), but the difference



**FIGURE 1** Prevalence of sensitization to specific food and inhalant allergens compared to sensitized participants with self-reported symptoms. Sensitized: >0.35 kU<sub>A</sub>/L. \*No questions about symptoms to house dust mite (HDM) was included in the questionnaire. \*\*The questionnaire had only questions regarding symptoms to shellfish, not specified to shrimp. Only one participant reported symptoms to shellfish

was no longer statistically significant when SE-sensitized participants were excluded from the analysis. There was no difference between *S. aureus* carriers and noncarriers in sensitization to food allergens (Table 2).

### 3.4 | SE sensitization and association with allergic sensitization

The number and proportion of participants sensitized to SE and the corresponding groups of participants that were non-, mono-, and poly-sensitized to food and inhalant allergens are shown in Tables 3 and 4. Sensitization to all four SEs was more frequent in those who were poly-sensitized to food and inhalant allergens, compared to nonsensitized participants. The largest difference was seen in participants sensitized to all four SEs with OR 50.5 (6.6-389.3) comparing poly-sensitization to inhalant allergens with nonsensitization (not shown in the tables).

Among participants with inhalant allergen sensitization, participants sensitized to at least one SE had significantly higher median

sum of specific IgE values to inhalant allergens (41.4 kU<sub>A</sub>/L, IQR 10.1-118.4) compared to non-SE-sensitized participants (18.0 kU<sub>A</sub>/L, IQR 5.5-48.6, *P*=.004). In contrast, no significant difference was seen in median sum of IgE values to inhalant allergens between *S. aureus* carriers (22.1 kU<sub>A</sub>/L, IQR 5.7-53.3) and noncarriers (16.4 kU<sub>A</sub>/L, IQR 5.1-48.3, *P*=.104). When SE-sensitized participants were excluded from the analysis, the median sum of IgE values to inhalant allergens was similar between *S. aureus* carriers and noncarriers (14.8 kU<sub>A</sub>/L vs 14.3 kU<sub>A</sub>/L, *P*=.893). Moreover, there were no differences in median sum of IgE values to food allergens between SE-sensitized and non-SE-sensitized participants (2.33 kU<sub>A</sub>/L vs 1.30 kU<sub>A</sub>/L, *P*=.166), or between *S. aureus* carriers and noncarriers (1.5 kU<sub>A</sub>/L vs 1.7 kU<sub>A</sub>/L, *P*=.529).

Overall, SE-sensitized participants had higher median total IgE levels (113.0 kU<sub>A</sub>/L, IQR 42.0-116.3) compared to nonsensitized participants (24.1 kU<sub>A</sub>/L, IQR 9.2-60.0, *P*<.001), whereas no differences were seen between *S. aureus* carriers (34.5 kU<sub>A</sub>/L, IQR 13.0-116.3) and noncarriers (34.0 kU<sub>A</sub>/L, IQR 13.2-91.5, *P*=.744).

**TABLE 1** Association between *S. aureus* carriage and inhalant allergen sensitization

Inhalant Allergens	SE-sensitized Included				SE-sensitized Excluded			
	<i>S. aureus</i> carriage n=382	<i>S. aureus</i> Noncarriage n=379	OR	95% CI	<i>S. aureus</i> carriage n=225	<i>S. aureus</i> Noncarriage n=253	OR	95% CI
Nonsensitized	228 (59.9%)	252 (66.5%)			151 (67.1%)	178 (70.4%)		
Mono-sensitized	31 (8.1%)	45 (11.9%)	0.71	0.47-1.24	23 (10.2%)	34 (13.4%)	0.80	0.45-1.41
Poly-sensitized	123 (32.2%)	82 (21.6%)	1.65	1.19-2.31	51 (22.7%)	41 (16.2%)	1.47	0.92-2.33

SE, *S. aureus* Enterotoxin; OR, odds ratio; CI, confidence interval.

Comparison between *S. aureus* carrier and noncarrier groups of inhalant allergen sensitization is made with multinomial logistic regression with comparison of poly-sensitized or mono-sensitized with nonsensitized as reference category. SE-sensitized: SE sIgE >0.1 kU/L to at least one of SE-A, SE-B, SE-C, or SE-TSST. Sensitized to inhalant allergens: sIgE ≥0.35 kU/L. Poly-sensitized:>1 allergen sensitization.

**TABLE 2** Association between *S. aureus* carriage and food allergen sensitization

Food Allergen Sensitizations	<i>S. aureus</i> Carriage n=382	<i>S. aureus</i> Noncarriage n=379	OR	95% CI
Nonsensitized	313 (81.9%)	327 (86.3%)		
Mono-sensitized	45 (11.8%)	31 (8.2%)	0.70	0.43-1.15
Poly-sensitized	24 (6.3%)	21 (5.5%)	2.23	0.57-8.71

OR, odds ratio; CI, confidence interval.

Comparison between *S. aureus* carrier and noncarrier groups of food allergen sensitization is made with multinomial logistic regression with comparison of poly-sensitized or mono-sensitized with nonsensitized as reference category. SE-sensitized: SE sIgE >0.1 kU/L to at least one of SE-A, SE-B, SE-C or SE-TSST. Sensitized to food allergens: sIgE ≥0.35 kU/L. Poly-sensitized: >1 allergen sensitization.

### 3.5 | Allergic disease and association with *Staphylococcus aureus* carriage and SE sensitization

Associations between each allergic disease entity and *S. aureus* carriage or SE sensitization were analyzed in separate multivariate logistic regression models (Table 5). After stratification, SE sensitization was only associated with allergic multimorbidity. A two-way between-groups analysis of variance was conducted to explore effect modification of SE sensitization on the previously published associations between *S. aureus* carriage and multimorbidity, severe asthma, eczema, and allergic rhinitis.<sup>6</sup> Effect modification was found only for severe eczema,  $P=0.021$ .

## 4 | DISCUSSION

In this large cross-sectional study of late adolescents, our aims were to study associations between allergic diseases and allergic sensitization with *S. aureus* carriage and SE sensitization. From the same study, we have previously shown that *S. aureus* carriage is associated with allergic multimorbidity, severe asthma, eczema, and allergic rhinitis.<sup>6</sup> In the present study, we found that SE sensitization was

significantly associated with allergic multimorbidity. Moreover, SE sensitization was significantly associated with poly-sensitization to both food and inhalant allergens, and specific IgE values to inhalant allergens were higher in the SE-sensitized group. In contrast, *S. aureus* carriage was not associated with food or inhalant poly-sensitization and no differences were found in specific IgE levels between *S. aureus* carriers and noncarriers. Our results suggest that the proposed MeDALL hypothesis on multimorbidity and poly-sensitization<sup>14</sup> is, at least partly, associated with SE sensitization.<sup>19</sup>

### 4.1 | Strengths and limitations

The main strengths of our study are the school-based approach recruiting late adolescents from the general population in the two municipalities of Northern Norway, the large sample size with a high participation rate and the combination of both self-reported and objective health measurements. We believe our data represent good estimates of prevalence rates of allergic sensitization, allergic disease, and *S. aureus* carriage in this population.

The cross-sectional design is a limitation as it only permits us to discuss observed associations and no true causality. Even after adjusting for relevant confounding factors, we cannot rule out that the observed association between SE sensitization and allergic poly-sensitization is due to other unknown confounding factors. However, a dose-response effect with increasing prevalence of SE sensitization in participants poly-sensitized to inhalant and food allergens, and significantly higher total sum of specific IgE levels in SE-sensitized participants strengthens our observations. We only obtained one nasal swab and we can therefore not distinguish between intermittent and persistent *S. aureus* carriage. Persistent carriage of enterotoxin-producing *S. aureus* strains is more likely to be associated with SE sensitization due to longer exposure time. However, we cannot rule out that intermittent carriers are exposed to a greater diversity of *S. aureus* strains, and thereby have greater probability of SE exposure compared to persistent carriers with less diverse exposure. Another limitation is that we did not assess skin carriage in order to obtain a broader understanding of the overall importance of nasal and/or skin

**TABLE 3** Association between *S. aureus* Enterotoxins (SEs) and inhalant allergen sensitization

	Inhalant Allergen Sensitization				OR Mono	95% CI	OR Poly	95% CI
	Non n=410 (%)	Mono n=67 (%)	Poly n=177 (%)	OR				
Non-SE-sensitized	332 (81.0)	57 (85.1)	92 (52.0)					
SE-A, n=48	12 (2.9)	2 (3.0)	34 (19.2)	1.02	0.22-2.63	7.89	3.97-15.65	
SE-B, n=58	20 (4.9)	2 (3.0)	36 (20.3)	0.60	0.14-2.63	5.00	2.79-8.90	
SE-C, n=77	32 (7.8)	4 (6.0)	41 (23.2)	0.75	0.26-2.20	3.56	2.16-5.88	
TSST, n=112	49 (12.0)	7 (10.4)	56 (31.6)	0.86	0.37-1.99	3.41	2.21-5.27	

SE, *S. aureus* Enterotoxin; TSST, toxic shock syndrome toxin; Non, Nonsensitized; Mono, mono-sensitized; Poly, poly-sensitized: >1 allergen sensitization; OR, odds ratio; CI, confidence interval.

Comparison between each SE-sensitized and non-SE-sensitized groups of inhalant allergen sensitization is made with multinomial logistic regression with comparison of poly-sensitized or mono-sensitized with nonsensitized as reference category.

**TABLE 4** Association between *S. aureus* Enterotoxins (SEs) and food allergen sensitization

	Food Allergen Sensitization				OR Mono	95% CI	OR Poly	95% CI
	Non n=552 (%)	Mono n=65 (%)	Poly n=37 (%)					
Non-SE-sensitized	431 (78.1)	40 (61.5)	10 (27.0)					
SE-A, n=48	24 (4.3)	8 (12.3)	16 (43.2)	3.10	1.33-7.20	16.76	7.77-36.14	
SE-B, n=58	33 (6.0)	8 (12.3)	17 (45.9)	2.21	0.97-5.01	13.37	6.40-27.9	
SE-C, n=77	51 (9.3)	8 (12.3)	18 (48.6)	1.38	0.62-3.05	9.31	4.59-18.86	
TSST, n=112	73 (13.2)	18 (27.7)	21 (56.8)	2.51	1.39-4.56	8.61	4.30-17.26	

SE, *S. aureus* Enterotoxin; TSST, toxic shock syndrome toxin; Non, nonsensitized; Mono, mono-sensitized; Poly, poly-sensitized: >1 allergen sensitization; OR, odds ratio; CI, confidence interval.

Comparison between each SE-sensitized and non-SE-sensitized groups of food allergen sensitization is made with multinomial logistic regression with comparison of poly-sensitized or mono-sensitized with nonsensitized as reference category.

**TABLE 5** Allergic disease and association with *S. aureus* carriage and SE sensitization

	<i>S. aureus</i> carriage <sup>a</sup>		SE Sensitization	
	OR	95% CI	OR	95% CI
Asthma	1.18	0.76-1.82	1.68	0.99-2.83
Asthma, stratified	0.96	0.49-1.88	0.93	0.36-2.41
Severe asthma	3.37	1.34-8.51	2.05	0.83-5.03
Severe asthma, stratified	6.32	0.74-53.7	0.97	0.10-9.17
Eczema	1.79	1.11-2.89	2.27	1.33-3.87
Eczema, stratified	1.63	0.79-3.35	1.67	0.73-3.27
Severe eczema	2.40	1.25-4.58	2.82	1.42-5.55
Severe eczema, stratified	7.12	1.59-31.85	2.67	0.94-7.65
Allergic rhinitis	1.25	0.90-1.72	1.45	0.98-2.14
Allergic rhinitis, stratified	1.02	0.69-1.51	1.23	0.75-2.02
Severe allergic rhinitis	1.70	1.09-2.66	1.16	0.67-2.02
Severe allergic rhinitis, stratified	1.94	1.09-2.64	0.89	0.41-1.96
Allergic multimorbidity	1.64	1.02-2.64	2.34	1.37-4.00

SE, *S. aureus* enterotoxin; OR, odds ratio; CI, confidence interval.

Analyzed in separate multivariate logistic regression models with each allergic disease entity and multimorbidity as dependent variable and *S. aureus* carriage or SE sensitization as independent variables, both models adjusted for BMI, gender, smoking, use of snuff tobacco, and physical activity. Stratified: Participants with allergic disease other than the disease tested in the model are excluded from the analysis.

<sup>a</sup>Results regarding *S. aureus* carriage are previously published,<sup>6</sup> but not with stratification and with slightly different adjustments in the multivariate models.

carriage on allergic symptoms and specific IgE reactivity. We did not have enough serum stored at  $-70^{\circ}\text{C}$  to analyze specific IgE to SE in all participants who already had analyzed specific IgE values to standard food and inhalant allergens. This may create bias, but we found no difference between the group with (n=656) vs the group without (n=109) specific IgE to SE regarding age, gender, total IgE, asthma, eczema, and allergic rhinitis (data not shown). Finally, allergic disease definitions are based on an established, widely used questionnaire. However, for allergic rhinitis we should expect that all participants were sensitized to an inhalant allergen. In our study, some

participants with allergic rhinitis were not sensitized to any of the measured inhalant allergens. Most likely, these participants are sensitized to other allergens from pets or pollen, but we cannot rule out that some participants interpreted and reported subjective nasal symptoms as allergy in spite of having a nonallergic condition, resembling allergy.

## 4.2 | Interpretation

Allergen sensitization found in the present study corresponds to expected exposures for allergens. The prevalence of sensitization to at least one food or inhalant allergen in our study is in line with findings from the UK,<sup>20</sup> but slightly lower than reported for 16-year-old adolescents in Southern Norway (48.6%)<sup>21</sup> and Sweden (51% being sensitized ever).<sup>22</sup> In general, it is difficult to compare prevalence of sensitizations due to different allergen exposure in different geographical regions. However, allergenic exposure in the Arctic region is probably quite similar to Sweden and UK.

Both *S. aureus* carriage and SE sensitization were previously shown to be associated with allergic disease, but to our knowledge, this has not been studied concomitantly in one single study using the same methods. In our study, nasal *S. aureus* carriage was twice as frequent as, but not associated with, SE sensitization. This may partially be due to carriage of *S. aureus* strains not producing enterotoxins and partially due to SE-sensitized participants no longer being *S. aureus* carriers.

*Staphylococcus aureus* carriage was not associated with inhalant allergen poly-sensitization when SE-sensitized participants were excluded from the analysis. This indicates that SE sensitization is more likely to affect poly-sensitization than *S. aureus* carriage per se. These interpretations should be made with caution due to limited numbers of participants when SE-sensitized participants are excluded. However, the finding is supported by higher inhalant allergen IgE levels in SE-sensitized compared to nonsensitized, but no difference between *S. aureus* carriers and noncarriers was observed. Our finding is also in line with a study in adult patients with allergic rhinitis, where no association was found between *S. aureus* carriage and allergen poly-sensitization measured with skin prick tests.<sup>2</sup> In this study, they could not differentiate between intermittent and

persistent carriage, they observed the same carriage rate as we found in a general population of adolescents, but they found no relevant IgE-dependent systemic immune reaction to SE-A and SE-B using a higher cut-off for specific IgE (0.35 kU<sub>A</sub>/L) than we did.<sup>2</sup> In contrast, we found a strong association between SE sensitization and food and inhalant allergen poly-sensitization, but no difference between *S. aureus* carriers and noncarriers. We also found increasing risk of poly-sensitization to inhalant allergens with increasing number of SE sensitizations, but these data should be interpreted with caution due to low numbers of participants sensitized to three or four SE. In addition, SE sensitization, but not *S. aureus* carriage, was strongly associated with the level of total IgE.

SE sensitization is strongly associated with eczema and skin colonization.<sup>23-25</sup> However, any causality needs to be confirmed in a longitudinal study. In contrast to other studies, we found no association between SE sensitization and asthma or allergic rhinitis. In the German MAS study, there was a moderate relation between SE sensitization and asthma at age 20 years, and in a GA<sup>2</sup>LEN study in adults, SE sensitization was associated with asthma in the general population.<sup>7,8</sup> Stronger associations between allergic diseases and SE sensitization have been shown for adult<sup>13</sup> and elderly<sup>12</sup> patients with asthma and severe asthma, indicating that the influence of SE sensitization may increase with age. However, in the US population, nasal *S. aureus* carriage was associated with increased risk of asthma prevalence, symptoms, and exacerbations in children and young adults (age 6-30 years), but not among adults aged 31-85 years.<sup>5</sup> The observed association suggests that SE sensitization may play a role in inducing allergic multimorbidity in addition to allergic poly-sensitization.

We have previously reported (in the same study) associations between *S. aureus* carriage and severe asthma, eczema, severe eczema, severe allergic rhinitis, and multimorbidity.<sup>6</sup> Our data indicate that *S. aureus* carriage may affect the severity of allergic diseases in adolescents, rather than inducing polyclonal sensitization. However, these data should be interpreted with caution, due to few participants with severe disease. Possibly, *S. aureus* carriage may induce inflammation through other pathways than SE sensitization, which may explain the associations with severe allergic diseases and other airway diseases such as chronic rhinosinusitis. A link has been shown between *S. aureus* biofilms and skewing of the T-cell response toward the type 2 pathway that is independent of superantigen activities.<sup>26</sup> It is possible that *S. aureus* induce release of epithelial derived cytokines, which might contribute to the inflammation.

Finally, our study supports the MeDALL hypothesis that *S. aureus* may induce re-occurrence of fetal type 2 signaling, resulting in polyclonal allergen sensitization and allergic multimorbidity.<sup>14</sup> The association between SE sensitization and poly-sensitization was known and attributed to the polyclonal activation of IgE. However, the association with allergic multimorbidity<sup>27,28</sup> is a new finding, and the association between poly-sensitization and multimorbidity was not previously identified in subjects sensitized to SE. Type 2 immunity is involved in IgE production, polyclonal activation, the cellular

inflammation of eczema, asthma, and allergic rhinitis<sup>29</sup> as well as in the regulation of the epithelial barrier function in the skin,<sup>30</sup> the airways,<sup>31</sup> and type 2 responses.<sup>32</sup> *S. aureus* can induce IgE class switching in nasal polyps.<sup>33</sup> Interleukin (IL)-33 may be of great importance in the understanding of multimorbidity and poly-sensitization<sup>34</sup> as it modulates the expression of human  $\beta$ -defensin 2 in human primary keratinocytes and may influence the susceptibility to bacterial superinfection in acute atopic dermatitis. Similar mechanisms involving IL-33 release by *S. aureus* have recently been demonstrated in upper airway mucosa (unpublished data).

### 4.3 | Generalizability

Although the study was performed in the Arctic region, its results can be generalized to other allergic environments. Sensitization patterns are comparable to northern Europe,<sup>20-22</sup> and *S. aureus* carriage and infections are common worldwide.

## 5 | CONCLUSIONS

We suggest that sensitization to *S. aureus* enterotoxins may play a role in the development of poly-sensitization to food and inhalant allergens and allergic multimorbidity in adolescents.

### ACKNOWLEDGMENTS

The authors would like to thank all the participants in The Tromsø Study Fit Futures, and the Clinical Research Unit, University Hospital of North Norway, and the Tromsø Study Fit Futures for conducting the study.

### CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

### REFERENCES

1. Brussow H. Turning the inside out: the microbiology of atopic dermatitis. *Environ Microbiol*. 2016;18:2089-2102.
2. Riechelmann H, Essig A, Deutschle T, Rau A, Rothermel B, Weschta M. Nasal carriage of *Staphylococcus aureus* in house dust mite allergic patients and healthy controls. *Allergy*. 2005;60:1418-1423.
3. Shiomori T, Yoshida S, Miyamoto H, Makishima K. Relationship of nasal carriage of *Staphylococcus aureus* to pathogenesis of perennial allergic rhinitis. *J Allergy Clin Immunol*. 2000;105:449-454.
4. van de Kant KD, Klaassen EM, van Aerde KJ, et al. Impact of bacterial colonization on exhaled inflammatory markers in wheezing preschool children. *J Breath Res*. 2012;6:046001.
5. Davis MF, Peng RD, McCormack MC, Matsui EC. *Staphylococcus aureus* colonization is associated with wheeze and asthma among US children and young adults. *J Allergy Clin Immunol*. 2015;135:811-813.
6. Sorensen M, Wickman M, Sollid JU, Furberg AS, Klingenberg C. Allergic disease and *Staphylococcus aureus* carriage in adolescents in the Arctic region of Norway. *Pediatr Allergy Immunol*. 2016;27:728-735.



7. Tomassen P, Jarvis D, Newson R, et al. Staphylococcus aureus enterotoxin-specific IgE is associated with asthma in the general population: a GA(2)LEN study. *Allergy*. 2013;68:1289-1297.
8. Sintobin I, Keil T, Lau S, et al. Is immunoglobulin E to staphylococcus aureus enterotoxins associated with asthma at 20 years? *Pediatr Allergy Immunol*. 2015;26:461-465.
9. Bachert C, Gevaert P, Howarth P, Holtappels G, van Cauwenberge P, Johansson SG. IgE to Staphylococcus aureus enterotoxins in serum is related to severity of asthma. *J Allergy Clin Immunol*. 2003;111:1131-1132.
10. Huvenne W, Hellings PW, Bachert C. Role of staphylococcal superantigens in airway disease. *Int Arch Allergy Immunol*. 2013;161:304-314.
11. Tanaka A, Suzuki S, Ohta S, et al. Association between specific IgE to Staphylococcus aureus enterotoxins A and B and asthma control. *Ann Allergy Asthma Immunol*. 2015;115:191-197.
12. Song WJ, Sintobin I, Sohn KH, et al. Staphylococcal enterotoxin IgE sensitization in late-onset severe eosinophilic asthma in the elderly. *Clin Exp Allergy*. 2016;46:411-421.
13. Bachert C, van Steen K, Zhang N, et al. Specific IgE against Staphylococcus aureus enterotoxins: an independent risk factor for asthma. *J Allergy Clin Immunol*. 2012;130:376-381.
14. Bousquet J, Anto JM, Wickman M, et al. Are allergic multimorbidities and IgE polysensitization associated with the persistence or re-occurrence of foetal type 2 signalling? The MeDALL hypothesis. *Allergy*. 2015;70:1062-1078.
15. Winther A, Dennison E, Ahmed LA, et al. The Tromso Study: Fit Futures: a study of Norwegian adolescents' lifestyle and bone health. *Arch Osteoporos*. 2014;9:185.
16. Hohmann C, Pinart M, Tischer C, et al. The Development of the MeDALL Core Questionnaires for a Harmonized Follow-Up Assessment of Eleven European Birth Cohorts on Asthma and Allergies. *Int Arch Allergy Immunol*. 2014;163:215-224.
17. Miguères M, Davila I, Frati F, et al. Types of sensitization to aeroallergens: definitions, prevalences and impact on the diagnosis and treatment of allergic respiratory disease. *Clin Transl Allergy*. 2014;4:16.
18. Olsen K, Sangvik M, Simonsen GS, et al. Prevalence and population structure of Staphylococcus aureus nasal carriage in healthcare workers in a general population. The Tromso Staph and Skin Study. *Epidemiol Infect*. 2013;141:143-152.
19. Bousquet J, Anto JM, Akdis M, et al. Paving the way of systems biology and precision medicine in allergic diseases: The MeDALL success story. *Allergy*. 2016;71:1513-1525.
20. Ziyab AH, Karmaus W, Zhang H, et al. Allergic sensitization and filaggrin variants predispose to the comorbidity of eczema, asthma, and rhinitis: results from the Isle of Wight birth cohort. *Clin Exp Allergy*. 2014;44:1170-1178.
21. Skrindo I, Lupinek C, Valenta R, et al. The use of the MeDALL-chip to assess IgE sensitization: a new diagnostic tool for allergic disease? *Pediatr Allergy Immunol*. 2015;26:239-246.
22. Ballardini N, Bergstrom A, Wahlgren CF, et al. IgE-antibodies in relation to prevalence and multimorbidity of eczema, asthma and rhinitis from birth to adolescence. *Allergy*. 2015;71:342-349.
23. Mittermann I, Wikberg G, Johansson C, et al. IgE Sensitization Profiles Differ between Adult Patients with Severe and Moderate Atopic Dermatitis. *PLoS ONE*. 2016;11:e0156077.
24. Orfali RL, Sato MN, Santos VG, et al. Staphylococcal enterotoxin B induces specific IgG4 and IgE antibody serum levels in atopic dermatitis. *Int J Dermatol*. 2015;54:898-904.
25. Szakos E, Lakos G, Aleksza M, et al. Association between the occurrence of the anticardiolipin IgM and mite allergen-specific IgE antibodies in children with extrinsic type of atopic eczema/dermatitis syndrome. *Allergy*. 2004;59:164-167.
26. Foreman A, Holtappels G, Psaltis AJ, et al. Adaptive immune responses in Staphylococcus aureus biofilm-associated chronic rhinosinusitis. *Allergy*. 2011;66:1449-1456.
27. Garcia-Aymerich J, Benet M, Saews Y, et al. Phenotyping asthma, rhinitis and eczema in MeDALL population-based birth cohorts: an allergic comorbidity cluster. *Allergy*. 2015;70:973-984.
28. Pinart M, Benet M, Annesi-Maesano I, et al. Comorbidity of eczema, rhinitis, and asthma in IgE-sensitized and non-IgE-sensitized children in MeDALL: a population-based cohort study. *Lancet Respir Med*. 2014;2:131-140.
29. Chanez P, Vignola AM, Vic P, et al. Comparison between nasal and bronchial inflammation in asthmatic and control subjects. *Am J Respir Crit Care Med*. 1999;159:588-595.
30. Brown SJ, McLean WH. One remarkable molecule: filaggrin. *J Invest Dermatol*. 2012;132:751-762.
31. Saatian B, Rezaee F, Desando S, et al. Interleukin-4 and interleukin-13 cause barrier dysfunction in human airway epithelial cells. *Tissue Barriers*. 2013;1:e24333.
32. Hammad H, Lambrecht BN. Barrier Epithelial Cells and the Control of Type 2 Immunity. *Immunity*. 2015;43:29-40.
33. Gevaert P, Nouri-Aria KT, Wu H, et al. Local receptor revision and class switching to IgE in chronic rhinosinusitis with nasal polyps. *Allergy*. 2013;68:55-63.
34. Alase A, Seltmann J, Werfel T, Wittmann M. Interleukin-33 modulates the expression of human beta-defensin 2 in human primary keratinocytes and may influence the susceptibility to bacterial superinfection in acute atopic dermatitis. *Br J Dermatol*. 2012;167:1386-1389.

## SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

**How to cite this article:** Sørensen M, Klingenberg C, Wickman M, et al. Staphylococcus aureus enterotoxin sensitization is associated with allergic poly-sensitization and allergic multimorbidity in adolescents. *Allergy*. 2017;00:1-8. <https://doi.org/10.1111/all.13175>