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ORIGINAL ARTICLE

Atopic Dermatitis, Urticaria and Skin Disease

Immunoglobulin E autoantibodies in atopic dermatitis associate with Type-2 comorbidities and the atopic march

Inge Kortekaas Krohn[1,2] | Fariza Mishaal Saiema Badloe[1,2] | Nadine Herrmann[3,4] | Laura Maintz[3,4] | Shauni De Vriese[1,2] | Johannes Ring[5] | CK-CARE Study Group† | Thomas Bieber[3,4,6] | Jan Gutermuth[1,2]

[1]Skin Immunology & Immune Tolerance (SKIN) Research Group, Vrije Universiteit Brussel (VUB), Brussels, Belgium

[2]Department of Dermatology, Universitair Ziekenhuis Brussel (UZ Brussel), Vrije Universiteit Brussel (VUB), Brussels, Belgium

[3]Department of Dermatology and Allergy, University Hospital Bonn, Bonn, Germany

[4]Christine Kühne-Center for Allergy Research and Education, Davos, Switzerland

[5]Department Dermatology and Allergy Biederstein, Technical University Munich, Munich, Germany

[6]Davos Biosciences, Davos, Switzerland

Correspondence
Inge Kortekaas Krohn, SKIN Research Group, Department of Dermatology, Vrije Universiteit Brussel, Laarbeeklaan 103, Building D, Room D.148, 1090 Jette, Belgium.
Email: inge.kortekaas@vub.be

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Abstract

Background: Autoreactive immunoglobulin E (IgE) antibodies to self-peptides within the epidermis have been identified in patients with atopic dermatitis (AD). Prevalence, concomitant diseases, patient characteristics, and risk factors of IgE autoantibody development remain elusive. We aimed to determine IgE autoantibodies in serum samples ($n = 672$) from well-characterized patients with AD and controls (1.2–88.9 years).

Methods: Atopic dermatitis patients were sub-grouped in AD with comorbid Type-2 diseases ("AD + Type 2"; asthma, allergic rhinitis, food allergy, $n = 431$) or "solely AD" ($n = 115$). Also, subjects without AD but with Type-2 diseases ("atopic controls," $n = 52$) and non-atopic "healthy controls" ($n = 74$) were included. Total proteins from primary human keratinocytes were used for the immunoassay to detect IgE autoantibodies. Values were compared to already known positive and negative serum samples.

Results: Immunoglobulin E autoantibodies were found in 15.0% (82/546) of all analyzed AD-patients. "AD + Type 2" showed a higher prevalence (16.4%) than "solely AD" (9.6%). "Atopic controls" (9.6%) were comparable with "solely AD" patients, while 2.7% of healthy controls showed IgE autoantibodies. Of those with high levels of IgE autoantibodies, 15 out of 16 were patients with "AD + Type 2". AD patients with IgE autoantibodies were younger than those without. Patients with IgE autoreactivity also displayed higher total serum IgE levels. Factors that affected IgE autoantibody development were as follows: birth between January and June, cesarean-section and diversity of domestic pets.

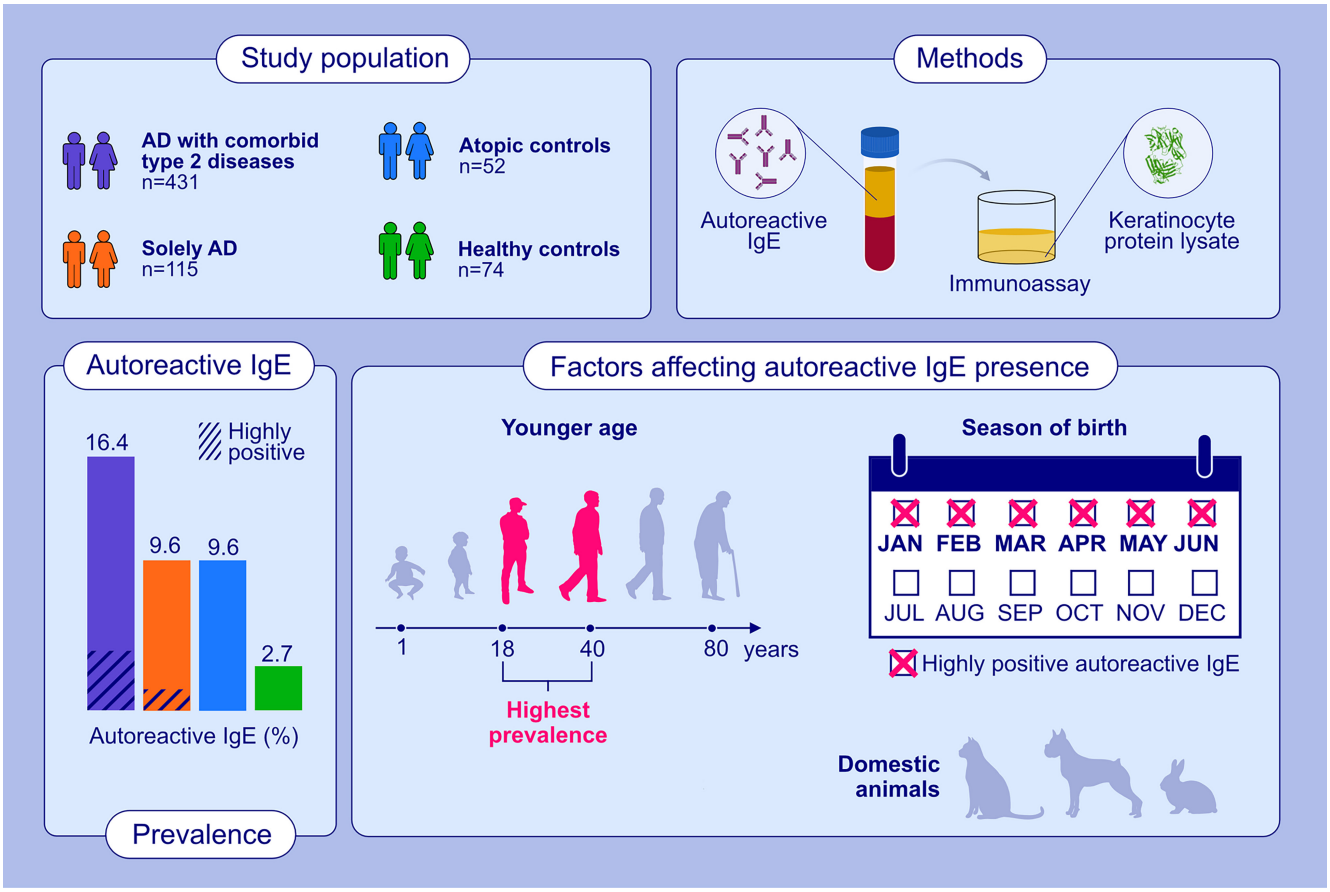
Conclusions: Immunoglobulin E autoantibodies in AD seem to associate with the presence of atopic comorbidities and environmental factors. The potential value of IgE autoantibodies as a predictive biomarker for the course of AD, including the atopic march, needs further exploration.

Abbreviations: AA, Allergic asthma; AD, Atopic Dermatitis; AR, Allergic rhinitis; BCA, Bicinchoninic acid assay; CK-CARE, Christine Kühne-Centre for Allergy Research and Education; EASI, Eczema Area and Severity Index; FA, Food allergy; HEKa, Human epidermal keratinocytes; IgE, immunoglobulin E; IQR, Interquartile range; OD, Optical density; ProRaD, Prospective Longitudinal Observations Research in Atopic Dermatitis; SCORAD, SCOring Atopic Dermatitis; TMB, Tetramethylbenzidine.

Inge Kortekaas Krohn and Fariza Mishaal Saiema Badloe should be considered joint first author.

†Members of the CK-CARE Study Group are listed in Appendix 1.

KEYWORDS
atopic dermatitis, autoantibodies, autoreactive IgE, IgE-mediated autoreactivity, Type-2 diseases



GRAPHICAL ABSTRACT

This study investigates the prevalence of immunoglobulin E (IgE)-mediated autoreactivity in serum samples from a well-characterized large cohort of atopic dermatitis (AD) patients and controls and their correlation with clinical characteristics and environmental factors potentially related to the development of IgE autoantibodies in AD. IgE autoantibodies in AD associate with the presence of atopic comorbidities and the atopic march. AD patients with IgE autoantibodies were younger than those without, and higher total serum IgE levels were found. Environmental factors that affected IgE autoantibodies were birth between January and June, c-section and diversity of domestic pets.
Abbreviations: AD, atopic dermatitis; IgE, immunoglobulin E.

1 | INTRODUCTION

Atopic dermatitis (AD) is the most common chronic recurrent inflammatory skin disease and exerts a detrimental impact on the quality of life of patients and their families. AD has a highly complex phenotype with a variety of age of onset and natural courses of the disease. It commonly starts in early childhood and affects up to 25% of children,[1] but many AD patients outgrow the disease before adolescence with a remaining prevalence of 2%–8% in adults. Other patients experience a persistent trajectory, but evidence for relapses, persistence, or onset in adulthood is growing.[2,3] Important pathophysiologic hallmarks are a T helper 2 (Type-2) polarization of the immune system and increased total serum immunoglobulin E (IgE) levels in about 80% of cases with frequent sensitizations against seasonal or perennial allergens.[4,5] Further important mechanistic factors include disturbed skin barrier function, which can be elicited or aggravated by Type-2-immune dysregulation and microbiome alteration with widespread *Staphylococcus aureus* colonization.[6–8]

An association between AD and the development of other atopic diseases during life has been characterized as the "atopic march," describing the concomitant or subsequent emergence of IgE-mediated allergic asthma (AA), food allergies (FA), and allergic rhinoconjunctivitis/allergic rhinitis (AR).[9–12] While very heterogeneous in individual course,[13–16] this progression is likely driven by both genetic and environmental factors resulting in "Type-2 related atopic comorbidities".[17,18]

Aside from AA, FA, or AR, associations were described between AD and non-atopic comorbidities such cardiovascular, inflammatory bowel disease, cardiovascular disease, and others, some of them with autoimmune pathomechanisms sometimes with contradicting results.[19–24] Similar to autoimmune diseases, systemic factors may underly the chronic relapsing course of the disease.[25–27] Immune reactions to self-proteins have been observed in inflammatory skin-related diseases including chronic spontaneous urticaria,[28] bullous pemphigoid,[29,30] systemic lupus erythematosus,[31] and AD,[32] suggesting the development of an autoimmune response against self-peptides. For AD, more than 100 epitopes and ubiquitous self-antigens have been described.[33–38] Several studies have found a positive correlation between IgE-mediated autoreactivity and AD severity.[39,40] However, a recent literature review on autoreactive IgE and AD showed that available studies are of small sample size and that larger population-based and longitudinal studies are needed to elucidate potential clinical relevance of autoallergy in AD.[41] Moreover, only a few studies included children or adolescents, even though AD has a significantly higher prevalence in childhood.[34,39,42] Thus, the pediatric profile of IgE-autoreactivity in AD has not been well characterized. Finally, environmental factors and the role of other Type-2 immunity diseases on the development of IgE autoantibodies are lacking.

Taken together, the contribution of autoreactive IgE to AD-pathophysiology and clinical relevance has not yet been elucidated and the question remains whether the presence of IgE autoantibodies poses a specific disease endotype or an immunological epiphenomenon secondary to the chronic inflammation without clinical relevance. An increased understanding of IgE-autoreactivity in AD may have direct consequences for diagnosis and prevention. Therefore, the aim of this study is to investigate the prevalence of IgE-mediated autoreactivity in serum samples from a well-characterized large cohort of AD patients and controls and their correlation with clinical characteristics and environmental factors potentially related to the development of IgE autoantibodies in AD.

2 | METHODS

2.1 | Study subjects

Cross-sectional data from the baseline visit of the "Prospective longitudinal study investigating the Remission phase in patients with Atopic Dermatitis and other allergy-associated diseases" (=PRORAD) were analyzed. A total of 672 subjects (aged 1.2–88.9 years) were recruited between November 2016 and June 2021 in the Christine Kühne-Centre for Allergy Research and Education (CK-CARE) program[43] at the Department of Dermatology and Allergy, University Hospital Bonn, Germany, after written informed consent. AD patients ($n=546$) fulfilling the Hanifin and Rajka criteria and 126 controls without AD were subdivided based on the presence of self-reported Type-2 diseases (Tables 1 and 2): (i) AD with atopic comorbidities (allergic rhinitis, food allergy, and/or asthma), "AD + Type 2," ($n=431$); (ii) "solely AD" ($n=115$); (iii) subjects without AD but with atopic disorders ("atopic controls," $n=51$); and (iv) non-atopic "healthy controls" ($n=75$). Clinical characteristics of a part of the study cohort and details of the study design have been described previously. Briefly, history of AD course, other personal and familial comorbidities, lifestyle, and epidemiological factors was self-reported and assessed within the standardized ProRaD questionnaire. At the visit, open questions from participants and the information from the questionnaire were re-evaluated with the medical doctor. AD severity (Eczema area and Severity Index [EASI], affected Body surface Area [BSA], SCORing Atopic Dermatitis [SCORAD]) and atopic stigmata were assessed by experienced dermatologists.[44–51] The study was approved by the Ethics Committee of the University of Bonn (ProRaD 232/15) first in 2015 and a last amendment in 2020, and the Universitair Ziekenhuis Brussel (2018-346) in 2018. Samples were registered at the CK-CARE biobank and the local Biobank of the Universitair Ziekenhuis Brussel. The study was conducted in accordance with the Good Clinical Practice guidelines and with the guidelines of the World Medical Association's Declaration of Helsinki.

TABLE 1 Demographics of the study participants.

Characteristics	AD + Type 2 comorbidities ($n=431$)	Solely AD ($n=115$)	Atopic controls ($n=52$)	Healthy controls ($n=74$)	Significance (p-value)
Age (years)[a]	35.6 (25.1–51.2)	37.9 (22.8–59.3)	38.7 (29.5–46.5)	34.4 (28.0–43.2)	.552
Female/male	242/189 (56/44%)	69/45 (60/40%)	39/13 (75/25%)	51/23 (69/31%)	
BMI[a]	23.9 (21.5–27.8)	23.9 (21.0–27.5)	23.3 (20.5–26.3)	22.6 (20.4–24.9)	**.043**
AD active/remission	406/25 (94.2/5.7%)	105/10 (91.3/8.7%)	-	-	
Total disease years[a]	27.8 (17.9–39.8)	19.9 (6.9–34.3)	-	-	**<.0001**
EASI[a]	7 (2.1–16.5)	4.8 (1.6–15.6)	-	-	.175
SCORAD[a]	37.9 (23.8–52.2)	35.7 (18.0–52.4)	-	-	.458
Body surface area	16 (4.5–35.8)	13.5 (2.3–34.5)	-	-	.202
Log total serum IgE (kU/L)[a]	2.8 (2.1–3.5)	1.9 (1.5–2.6)	1.8 (1.4–2.2)	1.4 (0.9–1.7)	**<.0001**

Abbreviations: AD, atopic dermatitis; BMI, body mass index; EASI, eczema area and severity index; IgE, immunoglobulin E; SCORAD, SCORing atopic dermatitis.

[a]Data are given as median (interquartile range).

TABLE 2 Prevalence of IgE autoreactivity and disease comorbidities in atopic dermatitis patients.

AD patients ($n=546$)	AD + Type 2 comorbidities ($n=431$)			Solely AD ($n=115$)			Atopic controls ($n=52$)		Healthy controls ($n=74$)	
IgE autoreactivity	High positive ($n=15$)	Positive ($n=56$)	Negative ($n=360$)	High positive ($n=1$)	Positive ($n=10$)	Negative ($n=104$)	Positive ($n=5$)	Negative ($n=47$)	Positive ($n=2$)	Negative ($n=72$)
Atopic dermatitis (AD)	15 (100%)	56 (100%)	360 (100%)	1 (100%)	10 (100%)	104 (100%)	-	-	-	-
Active AD	15 (100%)	53 (94.6%)	338 (93.9%)	1 (100%)	9 (90%)	95 (91.3%)	-	-	-	-
Allergic rhinitis (AR)	15 (100%)	48 (85.7%)	310 (86.1%)	-	-	-	5 (100%)	43 (91.5%)	0 (0%)	0 (0%)
Active AR	14 (93.3%)	46 (82.1%)	294 (81.7%)	-	-	-	5 (100%)	40 (85.1%)	0 (0%)	0 (0%)
Food allergy (FA)	13 (86.7%)	36 (64.3%)	235 (65.3%)	-	-	-	2 (40%)	13 (27.7%)	0 (0%)	0 (0%)
Active FA	12 (80%)	32 (57.1%)	223 (61.9%)	-	-	-	2 (40%)	12 (25.5%)	0 (0%)	0 (0%)
Asthma	10 (66.7%)	31 (55.4%)	194 (53.9%)	-	-	-	2 (40%)	12 (25.5%)	0 (0%)	0 (0%)
Active asthma	8 (53.5%)	28 (50.0%)	157 (43.6%)	-	-	-	2 (40%)	9 (19.1%)	0 (0%)	0 (0%)
Psoriasis	1 (6.7%)	1 (1.8%)	7 (1.9%)	0 (0%)	0 (0%)	2 (1.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Active psoriasis	0 (0%)	0 (0%)	2 (0.6%)	0 (0%)	0 (0%)	2 (1.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Diabetes mellitus (DM)	0 (0%)	1 (1.8%)	10 (2.8%)	0 (0%)	0 (0%)	1 (1.0%)	1 (20%)	1 (2.1%)	0 (0%)	2 (2.8%)
Active DM	0 (0%)	1 (1.8%)	9 (2.5%)	0 (0%)	0 (0%)	1 (1.0%)	0 (0%)	1 (2.1%)	0 (0%)	2 (2.8%)
Multiple sclerosis	0 (0%)	0 (0%)	1 (0.3%)	0 (0%)	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Active MS	0 (0%)	0 (0%)	1 (0.3%)	0 (0%)	0 (0%)	1 (1.0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Abbreviations: AD, atopic dermatitis; AR, allergic rhinitis; DM, diabetes mellitus; FA, food allergy; IgE, immunoglobulin E; MS, multiple sclerosis.

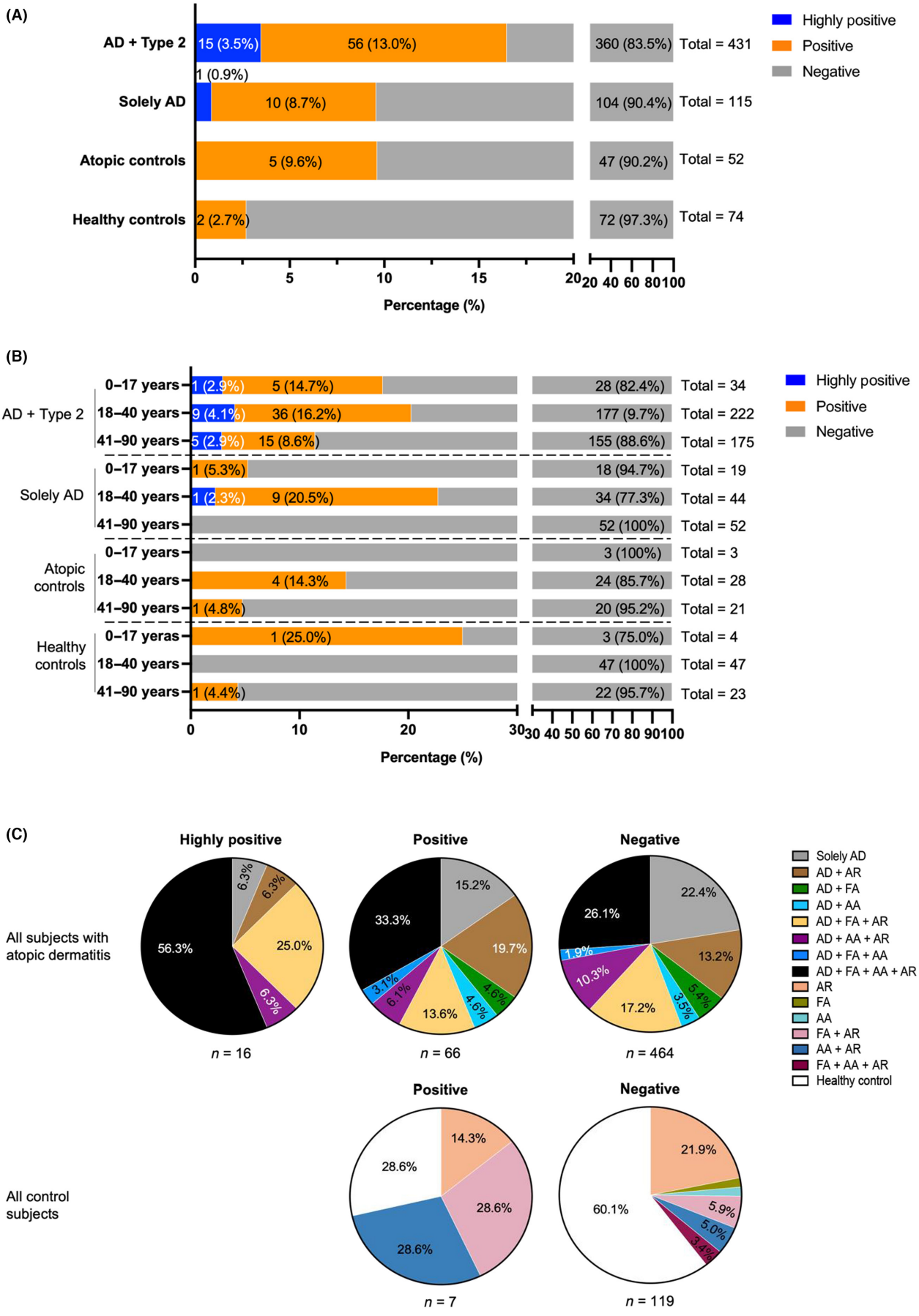


FIGURE 1 Patients with AD + Type 2 showed the highest prevalence of autoreactive IgE antibodies. In total, 672 serum samples were investigated for the presence of IgE autoantibodies using an immunoassay. Plates were coated with total proteins from normal human epidermal keratinocytes of adult origin and incubated with 100 μL undiluted sera, two positive controls (pool of sera from three patients positive for IgE autoantibodies), two negative controls (serum from a healthy subject negative for autoreactive IgE), and two blanks (blocking buffer). The immunoassay was performed in two technical duplicates. Optical density (OD) values were obtained by measurements at 450 and 655 nm. OD values ≤ negative control were considered negative. OD values ≥ positive control were highly positive. A cutoff value was calculated for the values in between by taking the mean of the negative controls +1× standard deviation. These samples were considered positive. Data are shown as percentages. (A) After unblinding, the subjects were sub-grouped for evaluation of the self-reported prevalence of IgE-autoreactivity based on the presence of atopic dermatitis and/or comorbid Type 2 diseases (asthma, food allergy and allergic rhinitis). (B) The samples were sub-grouped based on age groups: 0–17, 18–40, and 41–90 years for the analysis of the prevalence of IgE-autoreactivity. (C) The samples were sub-grouped based on IgE-autoreactivity for analysis of the contribution of atopic dermatitis and/or Type 2 diseases. AA, allergic asthma; AD, atopic dermatitis; AR, allergic rhinitis/rhinoconjunctivitis; FA, food allergy.

2.2 | Autoreactive IgE immunoassay

Serum samples were pseudonymized for detection of autoreactive IgE antibodies using a previously established immunoassay.[52] A detailed description is provided in the Appendix S1. Briefly, total protein lysates (50 μg/mL) from adult human epidermal keratinocytes (HEKa) were used for overnight coating of 96-well plates (Nunc). After washing, 100 μL undiluted sera were added per well and incubated at room temperature for 2 h. A pool of three sera already known to be reactive to human keratinocytes was used as a positive control.[53,54] As negative control, serum from a healthy subject negative for autoreactive IgE was used. Peroxidase-conjugated goat-anti-human IgE antibodies (1:4500, Polyclonal anti-epsilon chain specific [Sigma A9667]) were applied to detect IgE autoantibodies. 3,3′,5,5′-Tetramethylbenzidine (TMB) substrate was added, and optical density (OD) values were obtained with a microplate reader (Bio Rad) at dual wavelength measurements 450 and 655 nm. Technical duplicate values were obtained by performing two individual immunoassays.

All OD-values lower or equal to the negative control were considered as "negative" for autoreactive IgE. Samples with OD-values equal to or higher than the positive control were evaluated as "highly positive." For the values in between, a cutoff value was determined for all individual plates by calculating the mean of the negative controls plus one time the standard deviation of the negative controls. Samples between the cutoff value and the positive control were considered as "positive" (Figure S1). Subsequently, all "positive" sera ($n = 79$) were depleted for total IgG using IgG spin columns (Thermo Fisher), as described previously,[55] to avoid possible epitope competition by autoreactive IgG autoantibodies. All IgG-depleted sera were measured again using the immunoassay for reevaluation.

2.3 | Visualization of IgE antibodies on human skin biopsies and cultivated human keratinocytes

For visualization, an immunofluorescence staining was performed on HEKa cells cultivated on chambered slides (Ibidi). Cells were incubated with sera from patients with IgE autoantibodies ($n = 3$) and from one negative individual. Slides were fixed and treated with 0.2% Triton X-100. After blocking, the slides were incubated with 100 μL patient sera. Then, the slides were incubated with purified mouse anti-human IgE (1:100, Biolegend), overnight at 4°C. Next day, slides were stained with goat anti-mouse IgG AF594 (1:100, Biolegend). RSG4 confocal microscope (VivaScope) was used for imaging. A detailed description is provided in the Appendix S1.

2.4 | Statistical analyses

All variables used in the statistical analyses are listed in Table S1. Detailed information on the statistical method can be found in the Appendix S1. All p values are two-sided, and p values ≤ .05 considered statistically significant.

3 | RESULTS

3.1 | Study population

The median age (and interquartile range [IQR]) in the AD + Type-2 group was 35.6 years (25.1–51.2), in the solely AD group 37.9 (22.8–59.3), in the atopic controls 38.7 (29.5–46.5) and 34.4 (28.0–43.2) years in the healthy control group. Females represented 56.1% of the AD + Type-2 group, 60.0% in the solely AD group, 75.0% in the atopic controls, and 69.0% in the healthy controls. Further characteristics are outlined in Table 1.

3.2 | Patients with AD + Type 2 showed the highest prevalence of autoreactive IgE antibodies

We investigated the prevalence of IgE autoantibodies in patients with AD and controls in serum samples of 672 subjects. All samples that were measured as "positive" were subsequently depleted for total IgG and of those, two turned out to be "highly positive" and 73 remained "positive." IgE autoantibodies were found in 15.0% (82/546) of all analyzed AD-patients. Of the AD + Type-2 patients, 3.5% (15/431) were highly positive, 12.9% (56/431) were positive

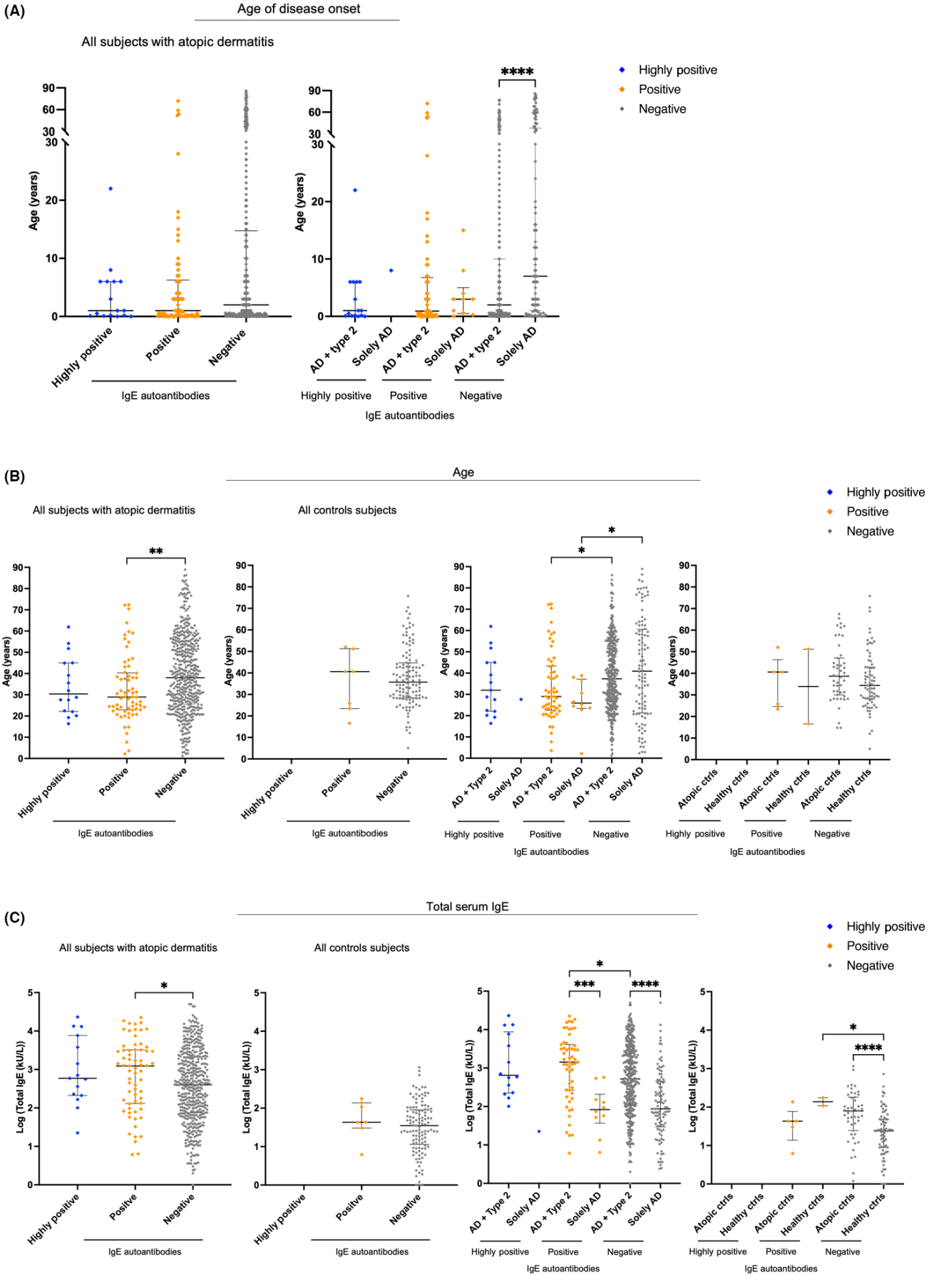


FIGURE 2 Younger age or higher total serum IgE is associated with the prevalence of IgE autoantibodies. For analysis of the data, the subjects were sub-grouped based on the presence or absence of atopic dermatitis or also based on the presence of Type 2 diseases. The presence of highly positive values is given in blue, positive values in orange and negative samples in grey. Data are shown as median with interquartile range. $^{*}p \leq .05$, $^{**}p < .01$, $^{***}p < .001$, $^{****}p < .0001$. (A) Age of disease onset in years. (B) Age of the subjects in years. (C) Log of total serum IgE in kU/L.

for IgE autoantibodies to human keratinocytes (Figure 1A). In the solely AD group, 0.9% (1/115) was highly positive and 8.7% (10/115) positive. Of the atopic controls, no highly positive samples were detected, but 9.6% (5/52) were positive for IgE autoantibodies. In the healthy control group, only 2.7% (2/74) were positive (Figure 1A). For evaluating the prevalence of IgE-mediated autoimmunity during aging, we subdivided the age of the subjects in three age groups: 0–17, 18–40, and 41–90 years (Table S2). The group 0–17 years included 60 children. Of those, 1.7% was highly positive (1/60) and 11.6% were positive (7/60). In children with "AD + Type 2," 2.9% (1/34) were highly positive and 14.7% (5/34) were positive. In the age group 18–40 years with "AD + Type 2," 4.1% (9/222) were highly positive and 16.2% (36/222) were positive. In the age group 41–90 years with "AD + Type 2," 2.9% (5/175) were highly positive and 8.6% (15/175) were positive. A similar age-distribution was found for those with solely AD (Figure 1B, Table S2).

AD + Type-2 patients with highly positive values for IgE autoantibodies were associated with Type-2 immunity-driven diseases (93.8%, 15/16 high positive samples, Table 2). The presence of other diseases (psoriasis, diabetes mellitus, multiple sclerosis) showed no association with IgE autoantibodies (Table 2). When analyzing the most important combination of comorbidities, the presence of AD with the three Type-2 diseases together (AD + FA + AA + AR) represented 56.3% (9/16) in the highly positive group, whereas this proportion was 30.1% (22/73) in the positive group and 20.8% (121/583) in the negative group (Figure 1C, Table S3). Moreover, the combination of AD + FA + AR was associated to 25% (4/16) of the high positive autoreactive compared to 12.3% (9/73) or 13.9% (81/583) in the other groups (Figure 1C, Table S2). Binary logistic regression analysis showed that the presence of allergic rhinitis (OR = 5.89; 95% CI = [1.044–33.239]) increased the likelihood for IgE autoantibody development in the control group (Table S4).

3.3 | Young adults or higher total serum IgE are associated with the prevalence of autoreactive IgE

Next, we evaluated the characteristics of the subjects to evaluate the differences between the highly positive, positive, and negative groups.

Early disease onset was associated with the presence of atopic comorbidities (AD + Type 2) compared to solely AD (Figure 2A, Table S5). IgE autoreactivity did not statistically depend on the age of onset; however, none of the subjects with late disease onset of AD (≥25 years) were highly positive. Further, AD patients with positive values for autoreactive IgE were younger compared to those without ($p < .01$; Figure 2B, Table S5). Binary logistic regression also indicated that young adult AD patients (OR = 1.979; 95% CI = [1.112–3.525]) were more likely to develop IgE autoantibodies compared to the older adults (Table S4).

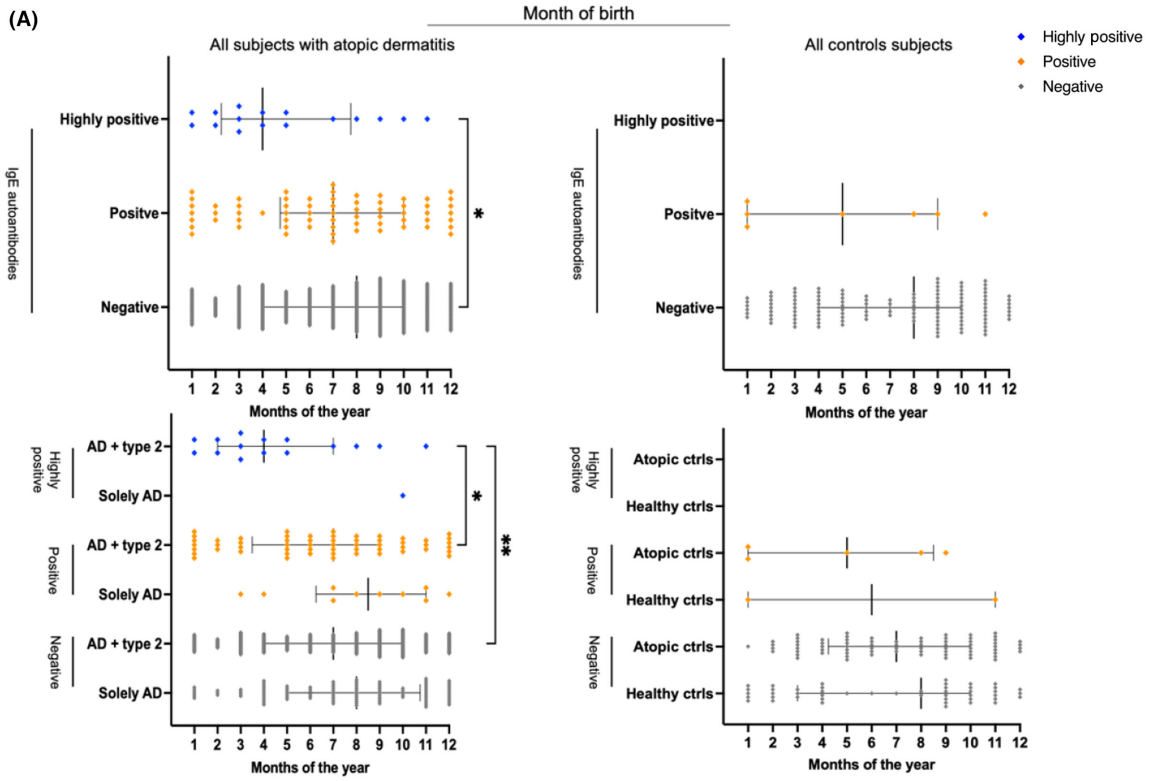
Higher total serum IgE levels were found in patients with AD + Type 2 compared to those with solely AD or control subjects ($p < .001$ and $p < .0001$; Figure 2C, Table 1). Additionally, the presence of autoreactive IgE is accompanied by higher total serum IgE levels compared with those that were negative ($p < .05$; Figure 2C, Tables S3 and S6). This was also confirmed by logistic regression in AD patients (OR = 1.926; 95% CI = [1.043–3.557]; Table S4).

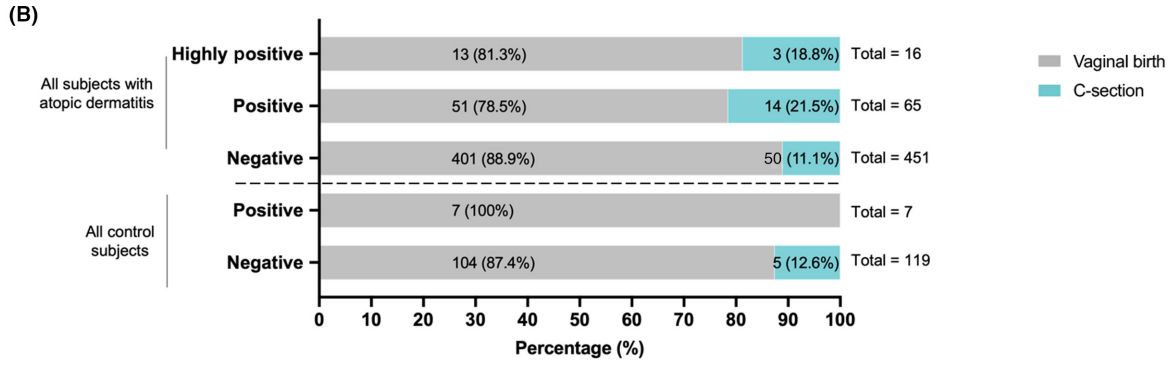
There was no correlation between (highly) positive values of IgE autoantibodies and AD severity scores EASI or SCORAD (Figure S2A,B, Table S5). Total disease years (Figure S3A, Table S5), gender (Figure S3B, Table S5). or body mass index (Figure S3C, Table S5) were not associated with IgE-autoimmunity. Also, median values of the total leukocyte cell counts or individual cell counts (lymphocytes, neutrophils, eosinophils, basophils) did not differ among the groups (Table S7).

3.4 | Month of birth, c-section, and domestic pets can affect IgE autoantibody development

Next, we evaluated the potential correlation to epidemiologic factors and the presence of IgE autoantibodies among patients with AD or the controls. The majority (73.3%) of the patients with AD + Type 2 with highly positive autoreactive IgE-values were born between January and June, whereas those that were negative or in the control group were born throughout the year (Figure 3A, Table S5). Mode of delivery differed significantly among AD with and without IgE autoreactivity ($p = .02$; Figure 3B), but not in controls. The percentage of AD patients born by cesarean section was 18.8% and 21.5% in the high positive and positive groups, respectively, while this was 11.3% in the group negative for autoreactive IgE. Further, AD patients highly positive for IgE autoantibodies had a lower diversity of domestic pets (of which 25% had no pets, 18.8% rodents only, and 12.5% a cat) compared to patients that were positive or negative for autoreactive IgE ($p = .03$; Figure 3C). A similar reduction of diversity in domestic pets was found in the control group positive for IgE autoantibodies compared to the controls negative for IgE autoantibodies ($p = .02$) (Figure 3C). This was confirmed by binary logistic regression for AD patients (OR = 0.554; 95% CI [0.311–0.989]) and controls (OR = 0.183; 95% CI = [0.32–1.035], Table S4).

Furthermore, daily exposure to cigarette smoke may contribute to an increased likelihood to develop IgE autoantibodies in the control subjects without AD. In this group, we found with low statistical





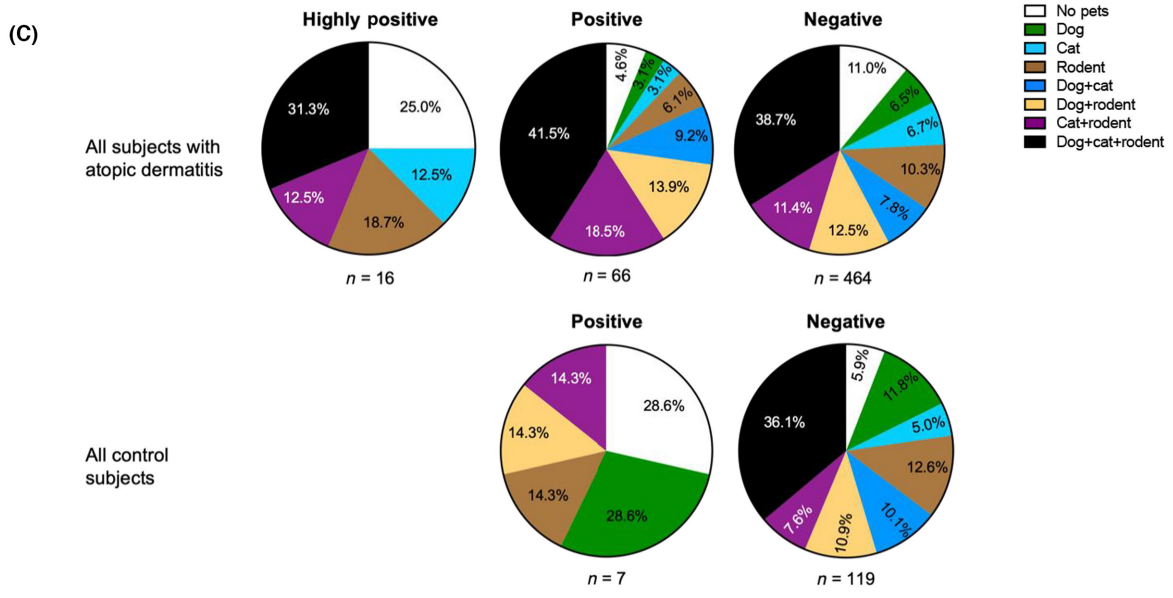


FIGURE 3 Month of birth, c-section, and domestic pets can affect IgE autoantibody development. The prevalence of IgE autoantibodies in patients with atopic dermatitis or control subjects with or without Type 2 diseases. (A) Effect of the month of birth. The presence of highly positive values is given in blue, positive values in orange, and negative samples in grey. Data are shown as median with interquartile range. $^{*}p \leq .05$, $^{**}p < .01$. (B) Effect of the mode of delivery. Subjects were sub-grouped based on the presence of atopic dermatitis and on IgE autoreactivity in relation to vaginal birth (grey) or cesarian section (turquoise). Data are shown as total numbers and percentages. Samples that were not known were excluded from the analysis (for patients with AD [$n = 14$] and controls [$n = 15$]). (C) Effect of keeping domestic pets at home. Subjects were subdivided based on the presence of atopic dermatitis and on IgE autoreactivity. Data are shown as total numbers and percentages.

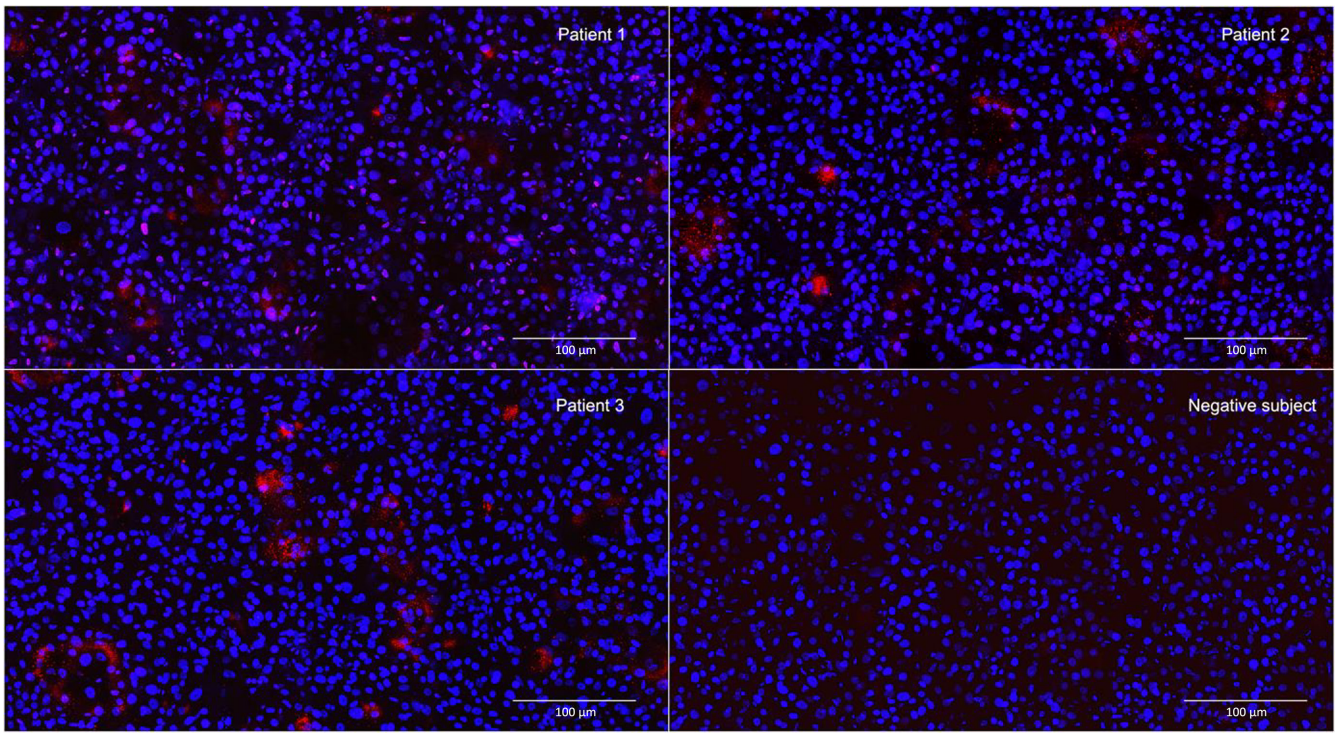


FIGURE 4 Visualization of IgE antibodies in HEKa cultivated cells. Immunofluorescence staining of HEKa cultivated cells incubated with serum from patients ($n = 3$) positive for IgE autoreactivity and a control staining HEKa cells incubated with serum negative for IgE autoantibodies. Patient 1, female, age 30 years, total serum IgE 383.48 kU/L; Patient 2, female, age 21 years, total serum IgE 188.95 kU/L; Patient 3, female, age 22 years, total serum IgE 746.49 kU/L; Negative subject, female, age 37 years, total serum IgE 227.12 kU/L. Cell-bound IgE was stained with mouse anti-human IgE and goat anti-mouse IgG AF594 (red). Nuclei were stained with DAPI (blue). Magnification 400× and the bar is set on 100 µm.

power that 14.3% (1/7) was positive versus 5.9% (7/119) of the active smokers and 28.6% (2/7) were positive compared to 10.9% (12/119) that were negative of the passive smokers (Figure S4A). Breastfeeding or the number of persons in a household did not show significant relations with the development of IgE autoantibodies (Figure S4B,C).

3.5 | IgE autoantibodies in cultivated HEKa can be visualized using immunofluorescence staining

The presence of IgE autoantibodies was visualized in monolayers of cultivated keratinocytes. When incubated with a serum sample from patients ($n = 3$) positive for IgE autoantibodies, some cells and nuclei were stained. Keratinocytes incubated with serum negative for IgE autoantibodies showed no staining for IgE (Figure 4).

4 | DISCUSSION

This study investigated IgE autoantibodies against keratinocytes-derived proteins in AD patients and control subjects with focus on the prevalence, characteristics, and influence of environmental factors. Overall, the prevalence of IgE autoantibodies in patients with AD was 15%. More specifically, 16.4% of AD patients with atopic comorbidities were positive for IgE autoantibodies, 8.7% of patients with solely AD, 9.6% of atopic controls, and 2.7% of healthy controls. Several other studies investigated the prevalence of IgE autoreactivity in AD.[33,35,37,39,40,42,56–58] A review article including eight studies on IgE autoantibodies described that the prevalence of IgE autoantibodies ranged from 23% (40/174) to 91% (11/12) in patients with eczema and 0 to 12% in controls. Although, when studies with a sample size greater than 100 were analyzed in the review article, the prevalence ranged from 23% to 28% in

eczema patients.[59] In the present study, the overall prevalence of 15% of IgE autoantibodies in AD patients is lower than described in the review.[59] This discrepancy could be explained based on the distinct study design and the epitope selection. Here, we addressed IgE autoantibodies against normal primary human keratinocytes, which present the main cell type in the epidermis, while some studies used defined protein epitopes. Another possible explanation may be the inclusion of a larger number of participants in our study ($n = 672$) covering the whole severity spectrum which might be less subjected to bias in contrary to studies that were based on much smaller patient populations. Additionally, this study also included children (<18 years; $n = 60$). The prevalence of IgE autoantibodies within this population group was 13.3% (1/60 high positive and 7/60 positive, of those, 6 had an AD + Type 2-profile). Evidence for development of autoantibodies already in early childhood was found previously.[34,39,42] With only a few studies on IgE autoantibodies in children, including the present study, the question remains when these antibodies start to develop and how this development progresses in early life (0–4; 5–11; 12–16; 17–25 of age). Here, we demonstrated the link between the presence of IgE autoantibodies and younger age in patients with AD compared to those without or control subjects. The age group 18–40 years had the highest prevalence and 41–90 years the lowest; this may suggest a lower likelihood for autoantibody development in late adulthood. In contrast to previously described, we firstly show evidence for an association of IgE autoantibodies and younger age.[59] Despite these findings, we found no significant association with "time of disease onset" or "number of disease years."

To evaluate risk factors for IgE autoantibody development, patient characteristics and environmental factors were analyzed. Interestingly, highly positive values were exclusively found in patients with AD, of which 93.8% (15/16) had one or more Type-2 comorbidities. Further analysis clarified that the presence of four Type-2 diseases (AD, FA, AA, and AR) accounted for 56.3% (9/16) in the highly positive group, suggesting a direct association of IgE autoantibodies and the "atopic march." In agreement with previous studies,[33,40,58] IgE autoantibodies were linked to higher total serum IgE levels in patients with AD + Type 2. Also, the majority (73.3%) of the "AD + Type 2 group" with highly positive values were born between January and June, whereas those that were negative or in the control group were born throughout the year. Being born in the pollen season, may contribute to an "AD + Type 2 profile" resulting in a higher likelihood to develop IgE autoantibodies. Previously, evidence was found for a higher prevalence of IgE autoantibodies in the allergen-season.[36] Other studies showed an association between the season of birth and the risk of AD development or allergic diseases in childhood,[60,61] but studies have been contradicting.[62–64] A systematic review on the association between season of birth and AD concluded that being born in fall or winter is positively associated with the development of AD.[65] The combination of a barrier defect (atopic dermatitis/scratching) and the presence of allergens in the environment may drive or enhance the development of IgE autoantibodies. In allergy studies, samples are commonly taken outside the pollen season as this may influence study outcomes. In the present study, samples were collected all-year-round, but we could not relate IgE autoantibodies with the month of sampling (data not shown).

Contradicting evidence has been reported on the delivery mode[66,67] on the development of Type-2 diseases. Here, we found a higher likelihood for IgE autoantibody development in AD patients born with c-section. Further, keeping several different domestic pets might have a protective effect for IgE autoreactivity, which is in line with the prevention of allergic diseases.[68–70] In our analysis, we did not consider the exact number of pets, duration of having pets, pets during childhood or present, and outdoor versus indoor pets. It might be of interest studying this in further detail. Also, active or passive daily exposure to cigarette smoke seems to be associated with IgE autoantibodies in control subjects, but we found no statistically significant differences in AD-patients. Previously, we demonstrated that daily and former smokers had a higher probability of moderate and severe AD, while never-smokers had a higher probability of mild AD mirrored by lower EASI scores.[44] Several studies also described that smoking could lead to an increased risk of AD and atopic diseases[44,71–73] and one study found a link between smoking and autoimmunity in AD.[20]

Our findings suggest that environmental factors are likely to affect development of IgE autoantibodies. This is, at least partly, in agreement with the "epithelial barrier hypothesis," which proposes exposure to epithelial barrier–impairing components, chronic epithelial inflammation and genetic predisposition as key drivers of allergic and autoimmune disease development.[74,75] It is likely that tissue damage, due to chronic inflammation and/or scratching, leads to release of intracellular peptides and subsequent production of IgE autoantibodies to self-peptides resulting in sensitization to autoantigens. Enhanced knowledge of IgE autoantibody development may lead to preventive actions. Patients at risk could be treated for pruritus to prevent scratching, and thereby, sensitization to autoantigens. They could also be advised to avoid environmental factors that are associated with IgE autoantibodies by modifying their lifestyle factors. A correlation between disease severity[59] and the presence of IgE autoantibodies remains unclear. Previous studies found evidence for the relation between AD severity and presence of autoantibodies,[33,36,37,39,40,42,76,77] while others did not.[42,58,78] Therefore, we investigated whether the latter was linked with disease severity. Patients with "solely AD" had lower EASI scores compared to the "AD + Type 2" patients, but we did not find a correlation between AD severity (EASI or SCORAD) and autoreactive IgE.

This study also had some limitations and strengths. Our participants were mainly adult Caucasians recruited in the area of Bonn, Germany. Outcomes might differ in other parts of the world based on environment, lifestyle, ethnicity, age, and others. Also, IgE autoantibodies against skin structures other than human keratinocytes were not investigated in this study and therefore cannot be

excluded. The specific targets of IgE autoantibodies were not identified, but we visualized IgE antibodies from serum samples bound to HEKa cultivated cells in line with previous work.[33] The presence of IgG autoantibodies against keratinocyte proteins can cause epitope competition with IgE as shown in a previous study.[79] Therefore, all samples that were positive for IgE autoantibodies were depleted for total IgG and reanalyzed, but this was not performed for the negative or highly positive samples. Functional assays demonstrating IgE autoreactivity in a prospective study were not included in the study, but the latter is being investigated in ongoing studies. Our ongoing studies may also confirm the results demonstrated in the present study. Despite the limitations, the results of the present study were based on extensive and well-characterized patients with AD and control subjects. This large sample size represents a broader study population than studied thus far. Importantly, the assessors were blinded for any of the clinical data at the time of analysis. After calculation of the IgE-autoreactivity all information was unblinded and the participants were sub-grouped. We also included 60 children, which gives new insights into this important patient group despite the limited number. In-depth analyses of this certain group with high statistical power remain future perspectives. In conclusion, our results strongly suggest a link between the occurrence of IgE autoantibodies with AD, especially in combination with comorbid Type-2 diseases. In patients with AD, IgE autoantibodies may possibly be seen as a biomarker to predict development of comorbid Type-2 diseases; however, this warrants further investigation.

AUTHOR CONTRIBUTIONS

Inge Kortekaas Krohn: designed the protocol, performed experimental work, revised the manuscript, analyzed the data, designed the tables and figures, and approved the final version and obtained funding for the analysis of IgE autoreactivity. Fariza Mishaal Saiema Badloe: performed experimental work, wrote the first version of the manuscript, analyzed the data, designed the tables and figures, revised and approved the final version. Nadine Herrmann: contributed to the PRoRAD study concept and design, organization of the biobank, critical revision of the manuscript for important intellectual content and approved the final version. Laura Maintz: contributed to the PRoRAD study concept and design, recruitment and clinical characterization of the patients, critical revision of the manuscript for important intellectual content and approved the final version. Shauni De Vriese: performed experimental work, contributed to the revised version and approved the final version. Johannes Ring: contributed to the design of the protocol, discussed the content of the manuscript, contributed to the revised version and approved the final version. Thomas Bieber: obtained funding for the PRoRaD study, contributed to the PRoRAD study concept and design, critical revision of the manuscript for important intellectual content and approved the final version. Jan Gutermuth: contributed to the design of the protocol, discussed the content, critical revision of the manuscript for important intellectual content and approved the final version.

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CONFLICT OF INTEREST STATEMENT

Thomas Bieber was speaker, and/or consultant and/or investigator for AbbVie, Allmiral, AnaptysBio, Arena, Asana Biosciences, ASLAN pharma Bayer Health, BioVerSys, Böhringer-Ingelheim, Connect Pharma, Dermavant/Roivant, Domain Therapeutics, Eli Lilly, Galderma, Glenmark, GSK, Incyte, Innovaderm, IQVIA, Janssen, Kirin, Kymab, LEO Pharma, LG Chem, L'Oréal, MSD, Novartis, Numab, OM Pharma, Pfizer, Pierre Fabre, Q32bio, RAPT, Sanofi/Regeneron, UCB. Thomas Bieber is founder and chairman of the non-profit biotech company "Davos Biosciences." Laura Maintz is or was investigator for AbbVie, Almirall, Bristol-Myers Squibb, Eli Lilly, Galderma, LEO Pharma, OM Pharma, Pfizer, Sanofi/Regeneron, advisor for Eli Lilly and received speaker honoraria from AbbVie and LEO pharma outside the submitted work. Jan Gutermuth was speaker and/or consultant and/or investigator for AbbVie, Allmiral, Eli-Lilly, Galderma, LEO-Pharma, Pfizer, Sanofi/Regeneron. Johannes Ring participated in the Advisory Board of Leo Pharma and of Benvard allergy therapeutics. He received speaker honoraria from AbbVie, Allergika, ALK, L'Oreal, Sanofi, and Pfizer. All other authors have no conflict of interest to declare within the scope of the submitted work.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Inge Kortekaas Krohn https://orcid.org/0000-0003-3649-1131
Fariza Mishaal Saiema Badloe https://orcid.org/0000-0002-4881-6450

Nadine Herrmann https://orcid.org/0000-0003-4924-2281
Laura Maintz https://orcid.org/0000-0001-6053-1530
Shauni De Vriese https://orcid.org/0000-0003-2666-238X
Johannes Ring https://orcid.org/0000-0001-8236-3152
Thomas Bieber https://orcid.org/0000-0002-8800-3817
Jan Gutermuth https://orcid.org/0000-0002-5805-3784

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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APPENDIX 1

CK-CARE Study Group

Thomas Bieber, MD, PhD, MDRA (Department of Dermatology and Allergy, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany; Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Davos Biosciences, Herman-Burchard-Str. 1, 7265 Davos, Switzerland); Peter Schmid-Grendelmeier, MD (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Allergy Unit, Department of Dermatology, University Hospital of Zürich, Rämistrasse 100, 8091 Zürich, Switzerland); Claudia Traidl-Hoffmann, MD (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Department of Environmental Medicine, Faculty of Medicine, University of Augsburg, Stenglinstraße 2, 86156 Augsburg, Germany; Institute of Environmental Medicine, Helmholtz Zentrum Muenchen, Augsburg, Germany); Cezmi Akdis, PhD (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Herman-Burchard-Str. 9, 7265 Davos, Switzerland); Roger Lauener, MD (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Children's Hospital of Eastern Switzerland, Claudiusstrasse 6, 9006 St. Gallen, Switzerland); Marie-Charlotte Brüggen, MD, PhD (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Allergy Unit, Department of Dermatology, University Hospital of Zürich, Rämistrasse 100, 8091 Zürich, Switzerland; Hochgebirgsklinik Davos, Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Faculty of Medicine, University of Zürich, Switzerland); Claudio Rhyner, PhD (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Davos Biosciences, Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Swiss Institute of Allergy and Asthma Research (SIAF), Davos, Herman-Burchard-Str. 9, 7265 Davos, Switzerland); Nadine Herrmann, PhD (Department of Dermatology and Allergy, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany; Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland); Laura Maintz, MD (Department of Dermatology and Allergy, University Hospital Bonn, Venusberg-Campus 1, 53127 Bonn, Germany; Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland); Eugen Bersuch, MD (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Allergy Unit, Department of Dermatology, University Hospital of Zürich, Rämistrasse 100, 8091 Zürich, Switzerland); Anita Dreher, MSc (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland); Gertrud Hammel, MA (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Department of Environmental Medicine, Faculty of Medicine, University of Augsburg, Stenglinstraße 2, 86156 Augsburg, Germany; Institute of Environmental Medicine, Helmholtz Zentrum Muenchen, Augsburg, Germany); Daria Luschkova, MD (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Department of Environmental Medicine, Faculty of Medicine, University of Augsburg, Stenglinstraße 2, 86156 Augsburg, Germany; Institute of Environmental Medicine, Helmholtz Zentrum Muenchen, Augsburg, Germany); Claudia Lang, MD (Allergy Unit, Department of Dermatology, University Hospital of Zürich, Rämistrasse 100, 8091 Zürich, Switzerland); Matthias Reiger, PhD (Christine Kühne-Center for Allergy Research and Education Davos (CK-CARE), Herman-Burchard-Str. 1, 7265 Davos, Switzerland; Department of Environmental Medicine, Faculty of Medicine, University of Augsburg, Stenglinstraße 2, 86156 Augsburg, Germany; Institute of Environmental Medicine, Helmholtz Zentrum Muenchen, Augsburg, Germany).