

DR IRENE MITTERMANN (Orcid ID : 0000-0002-6240-6730)

DR JON GENUNEIT (Orcid ID : 0000-0001-5764-1528)

DR CLAUDIA L GRAY (Orcid ID : 0000-0002-7765-1501)

DR RUDOLF VALENTA (Orcid ID : 0000-0001-5944-3365)

PROFESSOR MICHAEL ELIAD LEVIN (Orcid ID : 0000-0003-2439-7981)

Article type : Original

Molecular IgE-sensitisation profiles of urban and rural children in South Africa.

Irene Mittermann¹, Sheron Dzoro¹, Pia Gattinger¹, Maresa Botha², Wisdom Basera³, Heidi E. Facey-Thomas², Ben Gaunt^{4,5}, Jon Genuneit⁶, Claudia L. Gray³, Carol Hlela⁷, Sabine Flicker¹, Nonhlanhla Lunjani², Avumile Mankahla⁴, Jordache Ramjith^{8,9}, Rudolf Valenta^{1,10,11,12}, Michael Eliad Levin²

¹ Division of Immunopathology, Department of Pathophysiology and Allergy Research, Center for Pathophysiology, Infectiology and Immunology, Medical University of Vienna, Vienna, Austria

² Division of Paediatric Allergy, Department of Paediatrics, University of Cape Town, Cape Town, South Africa

³ School of Public Health and Family Medicine, Faculty of Health Sciences, University of Cape Town, Cape Town, South Africa

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the [Version of Record](#). Please cite this article as [doi: 10.1111/pai.13377](https://doi.org/10.1111/pai.13377)

This article is protected by copyright. All rights reserved

⁴ Zithulele Hospital, Eastern Cape Department of Health, South Africa

⁵ Institute of Epidemiology and Medical Biometry, Ulm University, Ulm, Germany

⁶ Pediatric Epidemiology, Department of Pediatrics, Medical Faculty, Leipzig University, Leipzig, Germany

⁷ Department of Dermatology, University of Cape Town, Cape Town, South Africa

⁸ Department for Health Evidence, Biostatistics Research Group, Radboud Institute for Health Sciences, Radboud University Medical Center, Nijmegen, Netherlands

⁹ Division of Epidemiology & Biostatistics, School of Public Health & Family Medicine, University of Cape Town, Cape Town, South Africa

¹⁰ NRC Institute of Immunology FMBA of Russia, Moscow, Russian Federation

¹¹ Sechenov First Moscow State Medical University, Laboratory of Immunopathology, Department of Clinical Immunology and Allergy, Moscow, Russian Federation

¹² Karl Landsteiner University for Health Sciences, Krems, Austria

Corresponding author: Michael Levin

Associate Professor

Division of Allergy

Department of Paediatrics and Child Health

University of Cape Town, South Africa

Email: michael.levin@uct.ac.za

Running title: IgE-sensitization profiles of AD children from South Africa

Word count: 3030

Number of tables and figures: 6

Conflicts of interests: RV has received grants from the Austrian Science Fund (FWF), Viravaxx, Vienna, Austria and is recipient of a Megagrant of the Government of the Russian Federation, grant number 14.W03.31.0024. He serves as a consultant for Viravaxx, Vienna, Austria. All other authors declare that they have no relevant conflict of interest.

Financial support: This work is supported by a research grant from the Medical Research Council of South Africa and the grant P26728, F4605 and F4607 from the Austrian Science Fund (FWF). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Abstract

Background: Allergens can act as disease-triggering factors in atopic dermatitis (AD) patients. The aim of the study was to elucidate the molecular IgE-sensitization profile in children with and without AD living in urban and rural areas of South Africa.

Methods: Specific IgE-reactivity was assessed in 166 Black South African children aged 9-38 months using a comprehensive panel of micro-arrayed allergens. According to clinical characterization children fell in 4 groups, urban AD cases (n=32), urban controls (non-AD, n=40), rural cases (n=49) and rural controls (non-AD, n=45).

Results: IgE-reactivity to at least one of the allergens was detected in 94% of urban and 86% of rural AD children. House dust mite (HDM) (81% urban, 74% rural AD) and animal-derived allergens (50% urban, 31% rural AD) were the most frequently recognized respiratory allergens whereas IgE to pollen allergens was almost absent. Urban AD children showed significantly higher frequency of IgE-reactivity (50%) to mouse lipocalin, Mus m 1, than rural AD children (12%). The most frequently recognized food allergens were from egg (63% urban, 43% rural AD), peanut (31% versus 41%) and soybean (22% versus 27%), whereas milk sensitization was rare. α -gal specific IgE almost exclusively occurred in rural children (AD: 14%, non-AD: 49%).

Conclusion: Molecular allergy diagnosis detects frequent IgE sensitization to HDM, animal but not pollen allergens and to egg, peanut and soy, but not milk allergens in African AD children. Urban AD children reacted more often to Mus m 1, whereas α -gal sensitization is more common in rural children likely due to parasite exposure.

Keywords: Allergy, allergen, atopic dermatitis, aeroallergen sensitisation, component-resolved diagnosis, food sensitization, microarray

Abbreviations used:

AD: Atopic dermatitis

AIT: Allergen-specific Immunotherapy

α -Gal: Galactose-1,3-galactose

HDM: House dust mite

RAD: Rural atopic dermatitis

ISU: ISAC standardized units

SCORAD: Scoring atopic dermatitis

SPT: Skin prick test

UAD: Urban atopic dermatitis

Introduction

Atopic dermatitis (AD) is a common childhood disorder, which is strongly associated with other allergic diseases. Birth cohort studies have shown that IgE-reactivity to food allergens, followed by sensitization to aeroallergens can occur early in life.^{1,2} Allergens may trigger AD exacerbations and early sensitization to food allergens is a risk factor for sensitization to inhalant allergens at an older age.³ Identification of potentially disease-triggering allergens is important, so that allergen-specific treatment can be performed and, if food allergy is diagnosed, an appropriate diet can be prescribed.⁴

In the ISAAC phase 3 study, the prevalence rate of AD for 13-to-14-year old children in South Africa was 13%⁵ and recent studies have shown marked differences in the prevalence of atopic dermatitis in urban and rural settings. Markedly higher rates of allergy exist in urban versus rural children tested in the South African food allergy cohort (asthma 9% vs 1%, eczema 26% vs 2%, rhinitis 25% vs 3%, challenge proven food allergy 2.5% vs 0.5%).^{6,7} Nevertheless, little information is available about the sensitizing allergen molecules associated with AD in African children.

Using the allergen chip technology, allergen-specific antibody responses to a comprehensive panel of micro-arrayed allergen molecules can be determined.⁸ By using purified allergens, genuine- and cross-sensitization can be distinguished in poly-sensitized individuals, which is often the case in AD patients.^{4,9} Molecular allergy diagnosis allows the determination of sensitization profiles to a broad panel of allergens and hence can act as guide for disease management, which may include allergen avoidance or AIT.⁴ In this study, we performed a comprehensive analysis of the IgE reactivity profiles of AD children from South Africa using allergen microarrays.⁸ Serum samples of toddlers aged 9-38 months from urban Cape Town and rural Eastern Cape, South Africa were used to gain information about molecular food- and aeroallergen sensitization profiles in different environmental settings.

Methods

Study population

Children were recruited as part of the South African Food Allergy study (SAFFA) from the City of Cape Town and the rural Eastern Cape, South Africa.^{6,7,10} According to clinical characterization children fell in 4 groups, urban AD cases (n=32), urban controls (non-AD, n=40), rural cases (n=49) and rural controls (non-AD, n=45). Urban as well as rural controls had no history or clinical signs of AD and had no positive skin prick test to 7 common food- and 4 aeroallergens. A detailed description of the study population can be found in the Supporting information.

This study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC REF: 038/2012). Informed consent was obtained from parents or legal guardians of participants.

Micro-array-based IgE-antibody measurements

IgE sensitization was analysed with a customized allergen-chip based on the ISAC technology (ThermoFisher Scientific, Uppsala, Sweden) as described.⁸ Arrays were analyzed by using a confocal laser scanner (LuxScan-10 K microarray scanner, Capital-Bio, Beijing, People's Republic of China) and evaluated by Microarray Image Analyzer v3.1.2 software (Phadia AB). For calibration and detection of background signals a calibrator serum and sample diluent (Phadia AB) were included in each analysis. The results are expressed as ISAC standardized units (ISU) and values ≥ 0.1 ISU were considered positive.

Statistics

For comparison of the four groups of children from rural or urban settings and of children with or without AD, respectively, with respect to frequencies of IgE-reactivities to micro-arrayed allergen molecules, two-sided asymptotic p-values were calculated by Chi-square test. P-values ≤ 0.05 were considered statistically significant.

Results

Demographic and clinical characterization of AD children and controls

The study included 166 toddlers, aged 9-38 months living in the City of Cape Town or the rural Eastern Cape, South Africa. The 72 children from urban settings included 32 children with AD and 40 children without AD. Ninety-four children were recruited from the rural area; 49 with AD and 45 without AD.^{6,7} There were no significant differences with regard to age or gender distribution between the urban and rural groups. The median SCORAD index was 29.5 in the urban and 29 in the rural AD children (Table S1). The parental family history of allergy was similar in urban and rural AD groups (Table S1).

Exclusive breastfeeding for 4 to 6 months was reported in 44% and 30% of the urban AD and control infants, respectively. Thirty percent of rural AD children were exclusively breastfed for 4 to 6 months compared to 38% of rural non-AD children (Table S1).

Breastfeeding duration of more than 10 months was reported in 34% and 35% of the urban AD and control infants, respectively. Twenty-six percent of the rural AD children and 40% of the rural controls were breastfed for more than 10 months (Table S1).

Median total serum IgE levels in the urban (346 kU/L) and rural AD group (274 kU/L) were significantly higher than in the urban controls (48.2 kU/L). Rural controls had a median total IgE level of 155 kU/L, which was significantly higher than in urban controls.

The majority of AD children showed IgE-reactivity to at least one micro-arrayed allergen

Using the MedALL allergen chip⁸, IgE-sensitization to at least one allergen molecule was detected in 94% of AD children from urban- and in 86% of AD children from rural areas. One child with a negative chip test result showed a positive SPT reactivity with peanut extract and 4 chip test negative children were IgE positive with HDM extract.

Twenty-three % of the urban controls without AD and 53% of the rural controls showed IgE-reactivity to at least one allergen molecule on the chip. From the 168 tested allergens, 88 were recognized by at least one child (Table S2).

IgE-sensitization to HDM allergens is frequent and occurs early in life among South African children with AD

All serum samples were tested for IgE-reactivity to 16 house dust- and storage mite allergens by allergen chip technology. Eighty-one % of AD children from urban areas had IgE antibodies to at least 1 of the HDM molecules compared to 74% of rural AD children (Figure 1A, Table S3). Control sera of infants without AD showed significantly less HDM sensitization (15% urban; 7% rural controls) ($p < 0.05$) (Figure 1A, Table S3). In AD infants from urban and rural communities, Der p 2 (66% urban, 63% rural), Der f 2 (63% urban, 61% rural), Der p 1 (56% urban, 45% rural) and Der p 23 (50% urban, 49% rural) were recognized most frequently (Table S2). Der f 1 sensitization was significantly higher in urban AD children (56% urban, 33% rural) (Table S2). The frequencies of IgE-reactivity to minor HDM allergens were between 9 to 31% in urban AD children, and lower with 2 to 16% in rural AD

infants (Tables S2 and S4). Sixteen % of the urban AD group showed already a broad IgE sensitization to more than 9 HDM allergens compared to 4% of rural AD children.

IgE-reactivity to tropomyosin (Der p 10) was detected in 22% of urban and in 8% of rural AD toddlers (Tables S2 and S4). There were no significant differences in the allergen specific IgE-levels in sera of rural or urban AD children for the individual HDM allergens tested (Figure 2, Table S4).

Egg was the most common food-related sensitisation in children with AD from rural as well as urban settings in South Africa

The urban and rural groups had no significant differences with regard to egg exposure and the median age for first egg ingestion. (Table S3). IgE-sensitization to one or more of the egg allergens was detected in 63% of the urban compared to 43% of the rural AD patients and was significantly higher in AD children with parental history of allergy. Only 1 child of the urban controls showed IgE-reactivity to egg allergens (Figure 1B, Table S3). Gal d 1 (53% urban versus 35% rural AD children) was the most frequently recognized allergen, followed by Gal d 2 (34% versus 27%), Gal d 5 (19% versus 22%) and Gal d 3 (6% versus 12%) (Table S2, Figure 3A). Using fresh raw egg white, the positive rate of SPT was significantly higher in urban (72%) than rural AD (45%) children ($p < 0.05$).

Specific IgE to peanut allergens was detected more frequently in AD children from rural areas, despite the fact that they were less likely to be exposed to peanuts

Exposure to peanut (self-reported) was significantly higher in the urban cohort (79% urban versus 39% rural). Sixty-nine % of the urban AD toddlers consumed peanut, compared to 88% of their counterparts without AD. In the rural group, 45% of AD children versus 33% of children without AD were peanut exposed (Table S3). The median age for the first peanut consumption was 12 months in the rural groups and the urban AD children and 14 months in the urban controls (Table S3). IgE sensitization to at least one of the peanut allergens was observed in 31% of the urban and in 41% of the rural AD children and was significantly higher compared to children without AD (Figure 1B, Table S3). Even though self-reported exposure to peanut was low in the rural cohort, 29% of rural AD children showed IgE-sensitization to peanut. Ara h 2 (19% urban, 29% rural AD children) and Ara h 6

(22% urban, 25% rural AD children) were recognized most frequently (Table S2, Figure 3B). In the urban AD group, 7 out of 10 children, who showed IgE-reactivity to at least one peanut allergen had positive SPT results. Of the 20 rural AD children with IgE sensitization to peanut allergens, 13 had positive SPT results with peanut. Additionally, 5 of them were SPT positive without showing any IgE-reactivity to peanut allergens.

The frequency of IgE-sensitization to soybean allergens was similar in AD children from urban and rural communities

Frequency of exposure to soybean and median age of soy introduction was similar among all four groups of children (Table S3). IgE sensitization to at least one of the soybean allergens Gly m 4, Gly m 5 or Gly m 6 was 27% in the rural and 22% in urban AD children (Figure 3C, Table S3). None of the children without AD showed IgE-reactivity to soy allergens. SPT using soy extract was positive in 9% and 12% of urban and rural AD toddlers, respectively.

Sensitization to cow's milk allergens was low and mainly due to serum albumin cross-reactivity

All children were cow's milk exposed. The median age of cow's milk exposure via cow's milk formula or products was 3 months in urban AD children and rural controls and 2 months in urban controls and rural AD children (Table S3). IgE-sensitization to cow's milk allergens was low in both groups of AD children (urban AD: 16%; rural AD 14%) and especially in the rural group mainly due to Bos d 6 (serum albumin) specific IgE (Table S5). None of the children without AD showed IgE-reactivity to cow's milk allergens (Table S3).

Sensitization to cat or dog allergens was uncommon, but AD children from urban settings showed very high frequency of IgE-reactivity to Mus m 1

Families living in the rural Eastern Cape were significantly more likely to have dogs and cats than families living in the urban settings. Children without AD from rural areas were significantly more likely to have cats than children with AD (Table S3). IgE-sensitization to any of the dog allergens Can f 1 to Can f 5 was low in urban (9%) as well as rural (12%) children (Figure 1A and Figure 4).

Sensitization to cat allergens Fel d 1 to Fel d 4 was detected in 22% of urban compared to 16% of

rural AD children (Figure 1A and Figure 4). Specific IgE to horse allergens Equ c 1 or Equ c 3 was measured in 19% of urban and 10% of rural AD children (Figure 1A and Figure 4).

Interestingly, IgE-reactivity to Mus m 1, a mouse lipocalin, was detected in 50% of the urban AD children, which was significantly higher than in rural AD children (12%) and children without AD (Figure 1A and Figure 4). IgE reactivity to Mus m 1 was not associated with IgE-reactivity to the dog lipocalins Can f 2 or Can f 4 or to the cat lipocalin Fel d 4 or to the horse lipocalin Equ c 1 (Table 1). Nine of 24 children with Mus m 1 specific IgE were also IgE-positive to Fel d 4 and 6 children showed IgE-reactivity to Mus m 1, Fel d 4 and Equ c 1. However, the level of IgE-reactivity to Mus m 1 was higher than the IgE levels to Fel d 4 or Equ c 1 (Table 1).

Children from rural environments showed significantly more sensitization to α -gal than children from urban areas

α -gal specific IgE was detected in 31% of rural and in only 3% of urban children. Forty-nine % of the rural children without AD showed α -gal specific IgE compared to 14% of rural toddlers with AD (Figure 5, Table S2). However, α -gal-specific IgE-reactivity was not associated with symptoms in this cohort.

Discussion

In this study, we analysed the molecular IgE-sensitization profiles of children with or without AD from urban Cape Town and the rural Eastern Cape, South Africa using a comprehensive panel of natural or recombinant purified allergens. The urban and rural children come from the same ethno-linguistic group, eliminating genetic factors as a possible cause of the observed differences in the sensitization profile. We found that children with AD had significantly higher rates of IgE-sensitization to foods and aeroallergens than children without AD. The highest rates of food allergen sensitization among AD children were seen for egg and peanut allergens. It is well known, that egg allergy is associated with AD and delaying introduction of egg until 11-12 months of age, as found in our cohort, may be associated with a higher risk of egg allergy compared to an earlier introduction.¹² However, our study has reported sensitization- rather than allergy rates, and it is well established that

not all food-sensitized patients are truly allergic. In a previous study in children with AD in South Africa 66% were food sensitized, but only 40% were challenge proven to be food allergic.¹³⁻¹⁷

In our cohort, cow's milk sensitization was uncommon, notably, serum albumin (Bos d 6) was recognized most frequently. There is an extensive cross-reaction between albumins from different sources, like milk, meat and dander.¹⁸ The molecular analysis indeed indicated that the genuinely sensitizing allergen was Gal d 5 from egg and that cross-reactivity with Bos d 6 was responsible for the reactions with milk. Since Bos d 6 is sensitive to digestion, it does not cause symptoms of milk allergy and dietary restrictions are not necessary for these children.

A study by Van Niekerk from 1979 reported a prevalence of 65% of HDM-sensitivity in asthmatic children living near Cape Town, but no HDM-sensitization was detected in rural asthmatic children.¹⁹ Later studies showed increasing rates of HDM sensitization in both settings, however, lower rates persisted in rural children.^{6,7} In our study, sensitization to HDM allergens was common among urban and rural AD children. In fact, the higher sensitization rate to Der f 1 in urban AD children was the only significant difference, detected.

The prevalence of sensitization to common pets was low in our study, which is expected at these ages. Interestingly, urban AD children showed significantly more sensitization to the mouse allergen Mus m 1 than rural AD children, which could be due to a more frequent exposure to mice and rats in the urban settings. Mouse sensitization and allergen exposure are associated with asthma severity and mice were identified as the major allergen sources contributing to asthma morbidity in urban children in America.²⁰⁻²²

Interestingly, no sensitization to pollen allergens was detected, which may be due to the young age of the cohort, but it is also possible that they are exposed to low allergenic grasses as was observed in a population from Asia.²³

Nearly 50% of the children without AD from rural communities had IgE antibodies against α -gal. The rural community has been shown to have a high prevalence of α -gal related red meat allergy.^{24,25} The results support the hypotheses that the IgE-response to α -gal is induced by glycosylated salivary proteins following tick bites, which happens more frequent in rural surroundings.^{26,27} On the other hand, it was reported that some African patients with parasitic infections have a high prevalence of anti- α -gal IgE without clinical relevance.²⁸ In our cohort of rural children without AD, the median

total IgE level was significantly higher than in urban controls, which is maybe due to more parasite infections. Only 14% of rural AD children but 49% of rural controls had α -gal-specific IgE. It is likely that the two populations differ with regards to their risk of exposure to the sensitizing factor. Children with eczema maybe less exposed to tick bites, because they may be dressed differently to avoid factors, which can promote skin lesions.²⁹ Whether the children with α -gal-specific IgE will develop symptoms of red meat allergy will be revealed when meat is increasingly introduced into their diet. A limitation of our study is that results may have been influenced by the low amounts of allergen immobilized on the array, potentially leading to competition for allergen binding between IgE and IgG. However, naturally occurring allergen-specific IgG antibodies unlike those induced by AIT seem to target epitopes, which are different from those recognized by IgE, which should mitigate this concern.³⁰ We have elucidated the molecular IgE sensitisation profile of children from 2 selected areas of South Africa: Cape Town and the rural Eastern Cape, which may be not applicable for the entire population.

In summary, our study revealed a particular IgE sensitization profile in children who despite very young age had developed already a very strong sensitization to HDM, in addition to IgE reactivities to egg, peanut and soybean allergens. A strikingly high sensitization rate to the mouse allergen Mus m 1 was noted in urban AD children, but genuine sensitization to pets was low and IgE sensitizations to pollen allergens were basically absent. The precision of molecular allergy diagnosis thus has revealed the nature of the sensitizing allergen molecules, which may form a basis for allergen-specific treatment and prevention strategies for the children consisting of diet and elimination of HDM and/or HDM-specific AIT as exemplified earlier.⁴

Key Message:

Our study is the first molecular analysis of IgE sensitization profiles in an African cohort of children with atopic dermatitis (AD). It reveals a particular IgE sensitization profile to HDM, animal but not pollen allergens already early in life and furthermore IgE to egg, peanut and soy, but not milk allergens and an unusually frequent sensitization against the major mouse allergen Mus m 1 in urban as compared to rural AD children.

References

1. Weidinger S, Novak N. Atopic dermatitis. *Lancet* 2016;387:1109-1122.
2. Chiu CY, Huang YL, Tsai MH, Tu YL, Hua MC, Yao TC, Yeh KW, Huang YL. Sensitization to food and inhalant allergens in relation to atopic diseases in early childhood: a birth cohort study. *PLoS One* 2014;9:e102809.
3. Westman M, Asarnoj A, Hamsten C, Wickman M, van Haage M. Windows of opportunity for tolerance induction for allergy by studying the evolution of allergic sensitization in birth cohorts. *Semin Immunol* 2017;30:61-66.
4. Fedenko E, Elisyutina O, Shtyrbul O, Pampura A, Valenta R, Lupinek C, Khaitov M. Microarray-based IgE-serology improves management of severe atopic dermatitis in two .children. *Pediatr Allergy Immunol* 2016;27:645-649.
5. Asher MI, Montefort S, Björkstén B, Lai CK, Strachan DP, Weiland SK, Williams H; ISAAC Phase Three Study Group. Worldwide time trends in the prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and eczema in childhood: ISAAC Phases One and three repeat multicountry cross-sectional surveys. *Lancet* 2006;368:733-743
6. Botha M, Basera W, Facey-Thomas HE, Gaunt B, Gray CL, Ramjith J, Watkins A, Levin ME. Rural and urban food allergy prevalence from the South African Food Allergy (SAFFA) study. *J Allergy Clin Immunol* 2019;143:662-668.
7. Botha M, Basera W, Facey-Thomas HE, Gaunt B, Genuneit J, Gray CL, Kiragu W, Ramjith J, Watkins A, Levin ME. Nutrition and allergic diseases in urban and rural communities from the South African Food Allergy cohort. *Pediatr Allergy Immunol* 2019; 30:511-521.

8. Lupinek C, Wollmann E, Baar A, Banerjee S, Breiteneder H, Broecker BM, et al. Advances in allergen-microarray technology for diagnosis and monitoring of allergy: the MeDALL allergen-chip. *Methods* 2014;66:106-119.
9. Mittermann I, Wikberg G, Johansson C, Lupinek C, Lundeberg L, Cramer R, Valenta R, Scheynius A. IgE sensitization profiles differ between adult patients with severe and moderate atopic dermatitis. *PLoS One* 2016;11:e0156077.
10. Levin ME, Botha M, Basera W, Facey-Thomas HE, Gaunt B, Gray CL, Kiragu W, Ramjith J, Watkins A, Genuneit J. Environmental factors associated with allergy in urban and rural children from the South African Food Allergy (SAFFA) cohort. *J Allergy Clin Immunol* 2020;145:415-426.
11. Eichenfield LF, Tom WL, Chamlin SL, Feldman SR, Hanifin JM, Simpson EL, et al. Guidelines of care for the management of atopic dermatitis: section 1. Diagnosis and assessment of atopic dermatitis. *J Am Acad Dermatol* 2014;70:338-351.
12. Al-Saud B, Siquardardottir ST. Early introduction of egg and the development of egg allergy in children: a systematic review and meta-analysis. *Int Arch Allergy Immunol* 2018;177:350-359.
13. Gray CL, Levin ME, Zar HJ, Potter PC, Khumalo NP, Volkwyn L, Fenemore B, du Toit G. Food allergy in South African children with atopic dermatitis. *Pediatr Allergy Immunol* 2014;25:572-579.
14. Gray CL, Levin ME, Du Toit G. Egg sensitization, allergy and component patterns in African children with atopic dermatitis. *Pediatr Allergy Immunol* 2016;27:709-715.
15. Gray CL, Levin ME, Du Toit G. Ethnic differences in peanut allergy patterns in South African children with atopic dermatitis. *Pediatr Allergy Immunol* 2015;26:721-730.
16. Gray CL, Levin ME, Du Toit G. Respiratory comorbidity in South African children with atopic dermatitis. *S Afr Med J* 2017;107:904-909.

- Accepted Article
17. Basera W, Botha M, Gray CL, Lunjani N, Watkins ASM, Venter C, Allen KJ, Hlela C, Zar HJ, Levon ME. The South African food sensitization and food allergy population-based study of IgE-mediated food allergy: validity, safety, and acceptability. *Ann Allergy Asthma Immunol* 2015;115:113-119.
 18. Spitzauer S, Pandjaitan B, Söregi G, Mühl S, Ebner C, Kraft D, Valenta R, Rumpold H. IgE-cross-reactivity against albumins in patients allergic to animals. *J Allergy Clin Immunol* 1995;96:951-959.
 19. Van Niekerk CH, Weinberg EG, Shore SC, Heese HV, Van Schalkwyk J. Prevalence of asthma. A comparative study of urban and rural Xhosa children. *Clin Allergy* 1979;9:319-324.
 20. Munera M, Sanchez A, Sanchez J, Nordmann M, Perez M, Aparicio D. Allergy to Mus m 1: A review of structural and immunological features. *Immunol Lett* 2019;209:1-3.
 21. Grant T, Aloe C, Perzanowski M, Phipatanakul W, Bollinger ME, Miller R, Matsui EC. Mouse sensitization and exposure are associated with asthma severity in urban children. *J Allergy Clin Immunol Pract* 2017;5:1008-1014.
 22. Sheehan WJ, Rangsithienchai PA, Wood RA, Rivard D, Chinratanapisit S, Perzanowski MS, Chew GL, Seltzer JM, Matsui EC, Phipatanakul W. Pest and allergen exposure and abatement in inner-city asthma: a work group report of the American Academy of Allergy, Asthma & Immunology Indoor Allergy/Air Pollution Committee. *J Allergy Clin Immunol* 2010;125:575-581.
 23. Cabauatan CR, Lupinek C, Scheiblhofer S, Weiss R, Focke-Tejkl M, Bhalla PL, Singh MB, Knight PA, van Hage M, Ramos JD, Valenta R. Allergen microarray detects high prevalence of asymptomatic IgE sensitizations to tropical pollen-derived carbohydrates. *J Allergy Clin Immunol* 2014;133:910-914.
 24. Mabelane T, Basera W, Botha M, Thomas HF, Ramjith J, Levin ME. Predictive values of alpha-gal IgE: Total IgE ratio and oral food challenge-proven meat allergy in a population

with a high prevalence of reported red meat allergy. *Pediatr Allergy Immunol* 2018; 29:841-849.

25. Levin ME, Apostolovic D, Biedermann T, Commins SP, Iweala OI, Platts-Mills TAE, Savi E, Van Hage M, Wilson JM. Galactose α -1,3-galactose phenotypes. Lessons from various patient populations. *Ann Allergy Asthma Immunol* 2019;122:598-602.
26. Cabezas-Cruz A, Hodzic A, Roman-Carrasco P, Mateos-Hernandez L, Duscher GG, Sinha DK, Hemmer W, Swoboda I, Estrada-Pena A, de la Fuente J. Environmental and molecular drivers of the α -GAL syndrome. *Front Immunol* 2019;10:1210-1222.
27. Horak IG, Fourie LJ, Heyne H, Walker JB, Needham GR. Ixodid ticks feeding on humans in South Africa: with notes on preferred hosts, geographic distribution, seasonal occurrence and transmission of pathogens. *Exp & Applied Acarology* 2002;27:113-126, Kluwer Acad. Publishers.
28. Arkestal K, Sibanda E, Thors C, Troye-Blomberg M, Mdulaza T, Valenta R, Grönlund H, van Hage M. Impaired allergy diagnostics among parasite-infected patients caused by IgE antibodies to the carbohydrate epitope galactose- α 1,3-galactose. *J Allergy Clin Immunol* 2011;127:1024-1028.
29. Oszukowska M, Michalak I, Gutfreund K, Bienias W, Matych M, Szewczyk A, Kaszuba A. Role of primary and secondary prevention in atopic dermatitis. *Postepy Dermatol Alergol* 2015;32:409–420.
30. Wollmann E, Lupinek C, Kundi M, Selb R, Niederberger V, Valenta R. Reduction in allergen-specific IgE binding as measured by microarray: A possible surrogate marker for effects of specific immunotherapy. *J Allergy Clin Immunol* 2015;136:806-809.

TABLE 1. Hierarchy of sensitization to lipocalins from different sources measured by microarray technology in children with or without AD (controls) from urban and rural areas in South Africa. Shown are sera, which showed IgE-reactivity to at least one of the tested allergens Mus m 1, Fel d 4, Equ c 1, Can f 2 or Can f 4 >0.1 ISU.

FIGURE 1. IgE-recognition frequencies for inhalant and food allergens in children with or without AD (Ctrl) living in urban or rural areas of Cape Town, South Africa. Shown are the percentages of children (y-axes) with IgE ≥ 0.1 ISU to any **A.** HDM, dog, cat, horse, mouse or **B.** egg, peanut, soybean and cow's milk allergens (x-axes). Significant differences are indicated *** $p < 0.001$.

FIGURE 2. Percentages (y-axes) of urban and rural AD children with IgE-reactivity to the major house dust mite allergens Der p 1, Der p 2 and Der p 23 (x-axes). Ranges of allergen-specific IgE levels are indicated.

FIGURE 3. IgE-levels specific for the **A,** egg allergens Gal d 1, Gal d 2, Gal d 3 and Gal d 5 **B,** peanut allergens Ara h 1, Ara h 2, Ara h 3, Ara h 6 and Ara h 9 and **C,** soy bean allergens Gly m 4, Gly m 5 and Gly m 6 (y-axes, ISU) in sera of AD children living in urban or rural areas of South Africa (x-axes). Horizontal dotted lines indicate the cut off for IgE-sensitization (≥ 0.1 ISU). All IgE levels of 0 ISU were substituted by a value of 0.001 to retain these data points in the figure on a logarithmic scale.

FIGURE 4. IgE-levels specific for the dog, horse, cat and mouse allergens Can f 1, Can f 3, Equ c 1, Fel d 1, Fel d 4 and Mus m 1 (y-axes, ISU) in sera of AD children living in urban or rural areas of South Africa (x-axes). Horizontal dotted lines indicate the cut off for IgE-sensitization (≥ 0.1 ISU). All IgE levels of 0 ISU were substituted by a value of 0.001 to retain these data points in the figure on a logarithmic scale.*** $p \leq 0.001$.

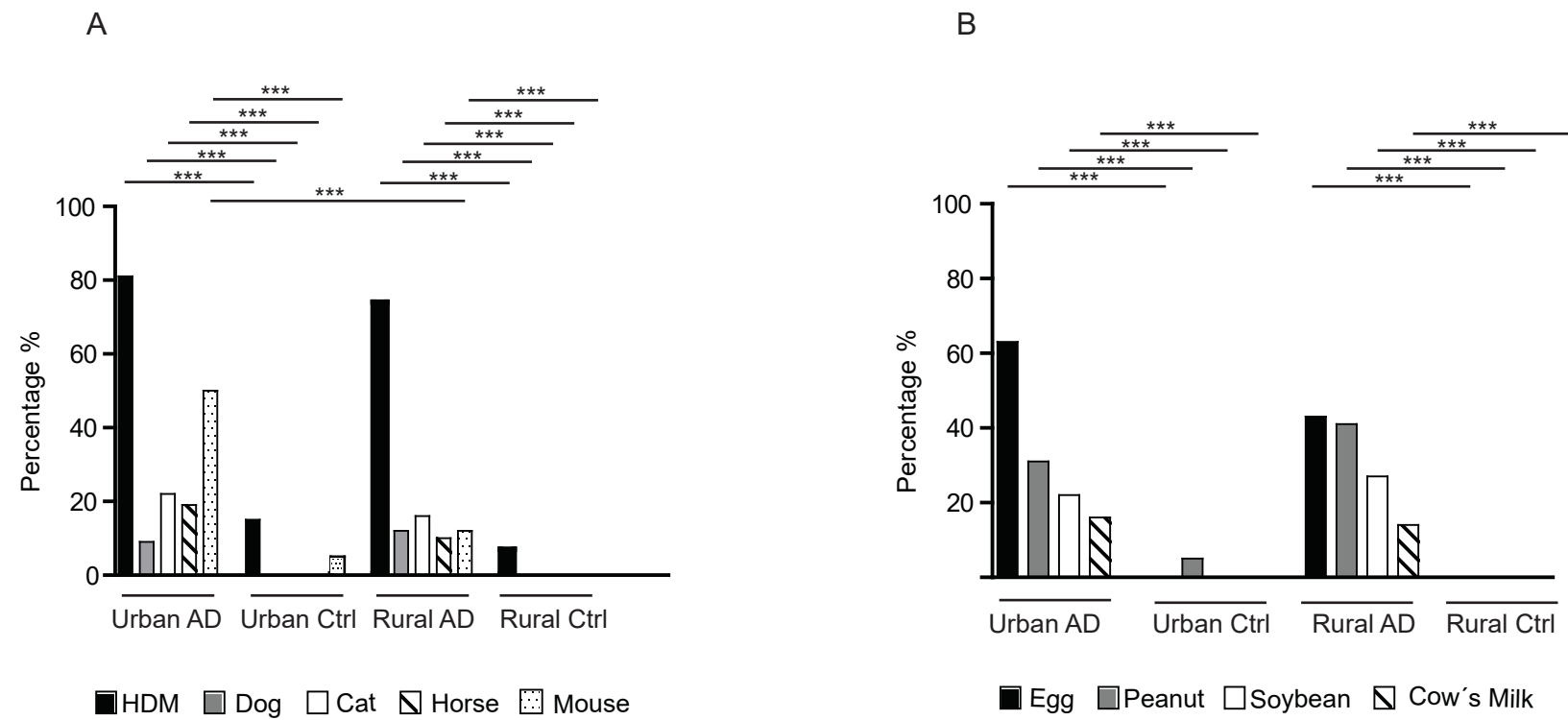
FIGURE 5. IgE-levels specific for α -gal (y-axes, ISU) in samples from children with and without AD (urban and rural cases and controls) (x-axes). Horizontal dotted lines indicate the cut off for IgE-sensitization (≥ 0.1 ISU). All IgE levels of 0 ISU were substituted by a value of 0.001 to retain these data points in the figure on a logarithmic scale.*** $p \leq 0.001$.

TABLE 1. Hierarchy of sensitization to lipocalins from different sources measured by microarray technology in children with or without AD (controls) from urban and rural areas in South Africa. Shown are sera, which showed IgE-reactivity to at least one of the tested allergens Mus m 1, Fel d 4, Equ c 1, Can f 2 or Can f 4 >0.1 ISU.

ID	Mus m 1	Fel d 4	Equ c 1	Can f 2	Can f 4
Urban AD (n=32)					
UAD2	2.54	1.21	0	0	0
UAD3	2.08	0	0	0	0
UAD8	9.01	0	0	0	0
UAD9	6.64	0.84	1.19	0	0
UAD11	0.13	0	0	0	0
UAD12	40.43	0.74	21.57	0	0
UAD14	0.18	0	0	0	0
UAD15	6	0.2	0	0	0
UAD16	1.34	0	0.7	0	0
UAD19	69.2	12.07	13.15	0.35	0
UAD21	34.19	0.95	0.38	0	0
UAD26	1	0	0	0	0
UAD27	17.04	3.28	1.87	0	0
UAD28	0.93	0	0	0	0
UAD29	11.38	0	0	0.16	0
UAD31	3.3	0	0	1.07	0
Urban control (n=40)					
UCO1	1.22	0	0	0	0
UCO30	0.26	0	0	0	0
Rural AD (n=49)					
RAD14	0.33	0	0.66	0	0
RAD22	0.28	0	0	0	0
RAD26	8.2	5.41	6.65	0	0.45
RAD27	0.15	0	0	0	0
RAD29	0.12	0	0.48	15.85	0

RAD33	0	0.16	0	0	0
RAD34	0.62	0.98	0	0	0

Fig 1



HDM sensitization

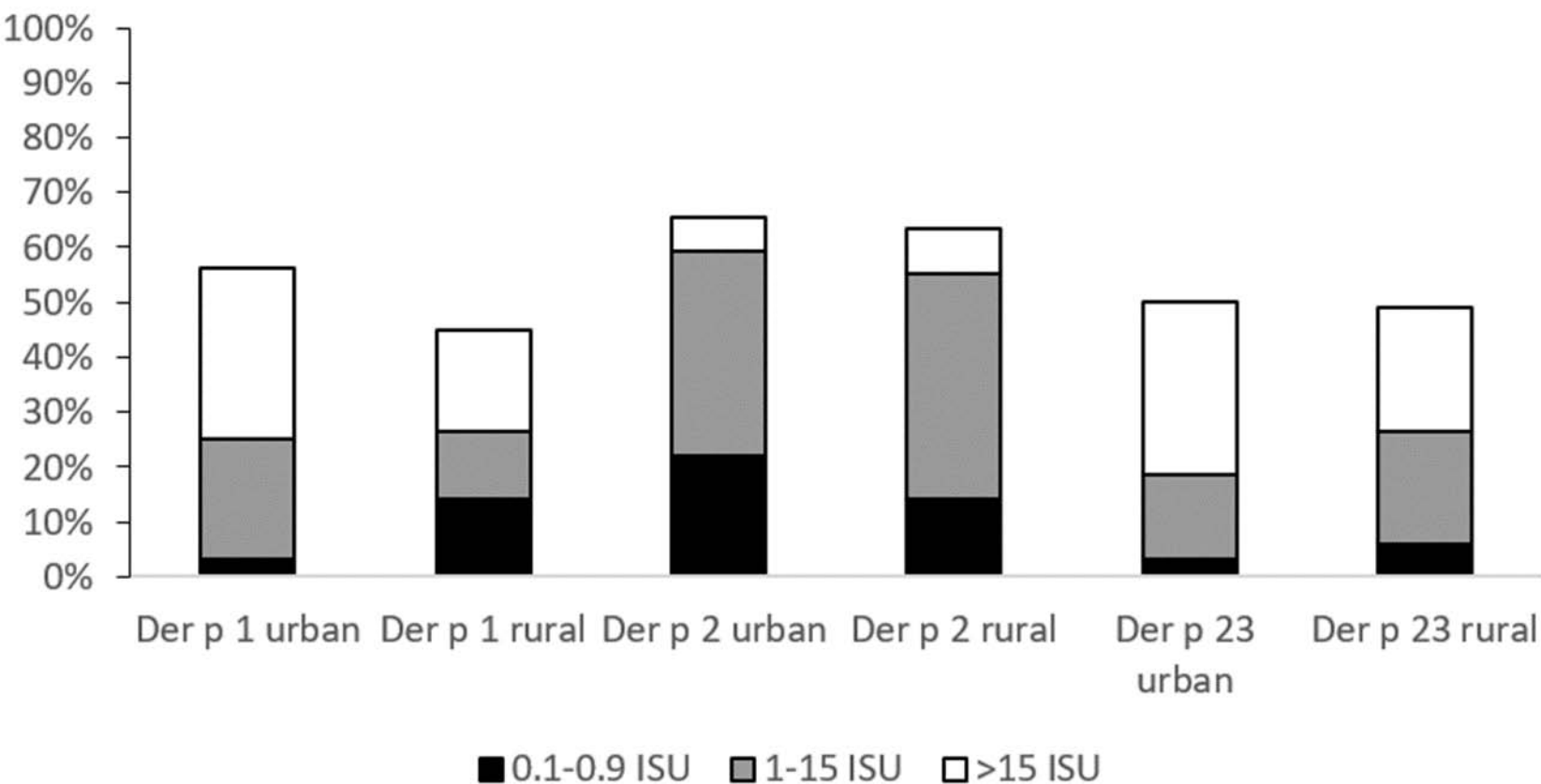


Fig 3

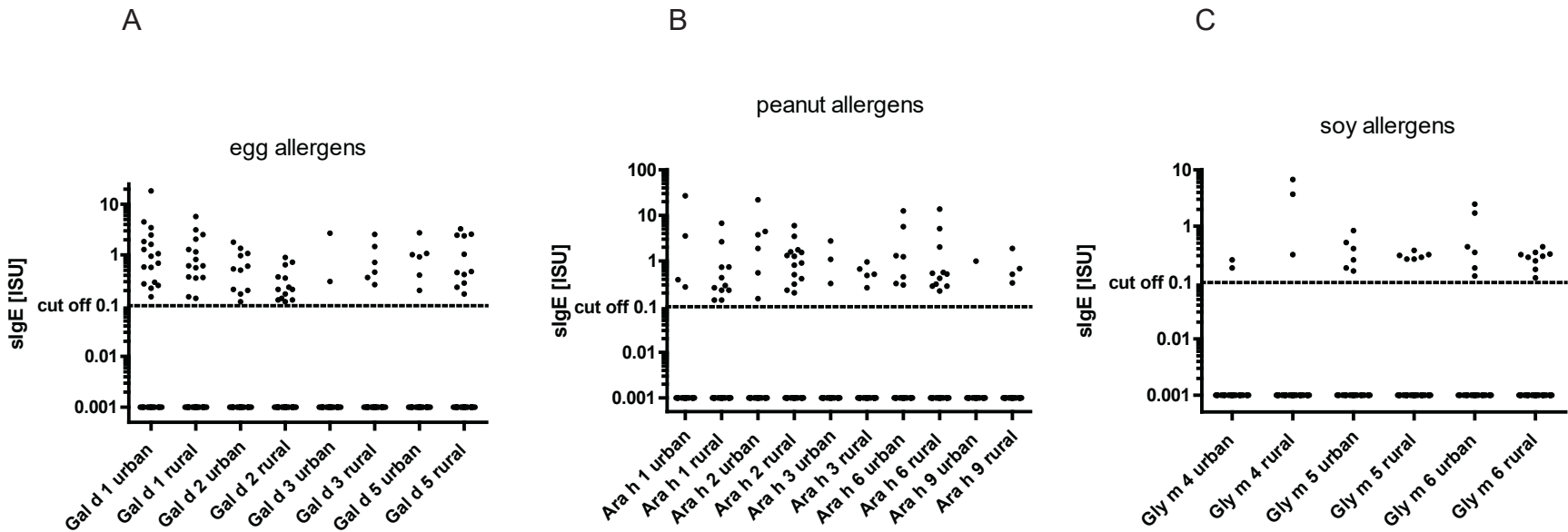


Fig 4

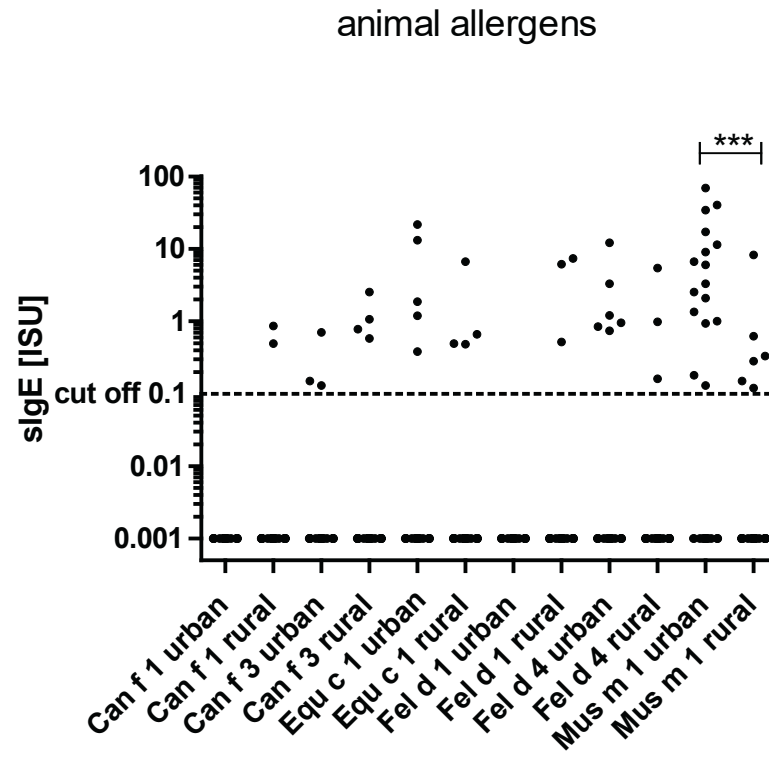
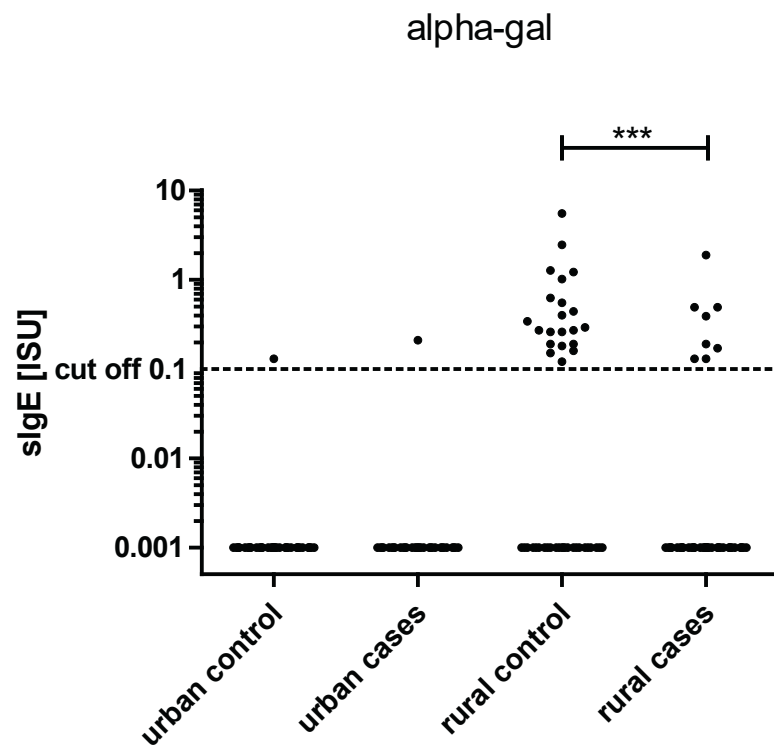


Fig 5



Appendix S1

Supplementary Information

Study population

Controls

Urban controls (n=40), without AD and allergic symptoms were recruited as part of the South African Food Allergy (SAFFA) study at randomly picked creches (registered early childhood development centers) in the Cape Town metropole. As creches are rarely found in the rural district, rural controls (n=46) were recruited from children of eligible age from the areas surrounding 10 district community health care clinics in the rural Mqanduli district of the Eastern Cape.

Atopic dermatitis (AD) children

Children with AD (n=32) were recruited from the paediatric dermatology department of the Red Cross War Memorial Children's Hospital in Cape Town and rural AD cases (n=49) from the dermatology department of the Nelson Mandela Academic Hospital in the Eastern Cape.^{6,7,10} AD was diagnosed by a dermatologist based on diagnostic criteria proposed by the American Academy of Dermatology. The severity of the eczema was assessed using the scoring atopic dermatitis (SCORAD) index.¹¹

For all children, a questionnaire captured information on family history of allergic diseases, family size, breastfeeding, infant diet, household number, parental education and parental employment. Participants' allergic diseases (allergic rhinitis, asthma, food allergy) were determined by self-reported symptoms according to modified ISAAC criteria and through clinical examination for signs of allergic rhinitis and atopic dermatitis. Skin prick tests (SPT) were performed to 7 common food allergens (egg white extract, peanut, cow's milk, cod-fish, soy, wheat and hazelnut) and 4 aero-allergens (*Dermatophagoides pteronyssinus*, *Blomia tropicalis*, *Blatella germanica*) and Grass Mix containing *Dactylis glomerata*, *Festuca elatior*, *Lolium perenne*, *Phleum pratense* and *Poa pratensis*) (ALK-Abello Ltd, Reading, UK). In addition, fresh egg white, peanut butter and fresh cow's milk as well as positive (10mg/mL histamine) and negative (saline) controls were used. SPT results were read at 15 minutes and recorded as the mean wheal diameter in millimetres. A mean wheal diameter of ≥ 3 mm for aeroallergens and ≥ 1 mm for food allergens was considered as positive sensitization but all food allergy was proven by open food challenge.¹⁷

TABLE S1. Demographic and clinical data of children with and without AD, including parental history of allergy and breastfeeding data.

35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52

TABLE S2. Frequencies of IgE-reactivity to purified allergens in AD children and controls from urban and rural areas of Cape Town, South Africa.

TABLE S3. Exposure, IgE-reactivity and SPT-reactivity in children with and without AD (controls) living in urban or rural areas in Cape Town, South Africa.

TABLE S4. Sensitization to HDM allergens measured by microarray technology in children with or without AD from urban and rural areas in South Africa. Serum samples, which showed IgE-reactivity to at least one of the tested minor HDM allergens >0.1 ISU are displayed.

TABLE S5. Hierarchy of sensitization to serum albumin measured by microarray technology in children with AD from urban and rural areas in South Africa. Shown are sera, which showed IgE-reactivity to at least one of the tested allergens Gal d 5, Bos d 6, Fel d 2, Can f 3 or Equ c 3 >0.1 ISU.

TABLE S1. Demographic and clinical data of children with or without (controls) AD, including parental history of allergy and breastfeeding data

	n	Sex F/M n	Age (Month) median (range)	SCORAD ^{a)} median (range)	Breast feeding exposed n (%)	Exclusive breast feeding <4 month n (%)	Exclusive Breast feeding 4-6 month n (%)	Breast feed duration >6 month n (%)	Breast feed duration >10 month n (%)	Mother Allergy n (%)	Father Allergy n (%)	Parental history of allergy n (%)	Total serum IgE (kU/L) n median (range)
AD urban	32	14/18	23 (11-35)	29.5 (0-100)	23 (72)	18 (56)	14 (44)	12 (38)	11 (34)	6 (19)	7 (22) ^{b)*}	12 (38)	N=23 346 ^{b)*} (29-5073)
Controls urban	40	22/18	27.5 (12-36)	NA	31 (78)	27 (68)	12 (30)	14 (35)	14 (35)	8 (20) ^{f)*}	2 (5)	9 (23)	N=38 48.2 ^{f)*} (2-1215)
AD rural	49	21/27 ^{c)}	21 (9-38)	29 ^{d)} (0-102)	34 (72) ^{e)}	32 (70) ^{e)}	14 (30) ^{e)}	13 (28) ^{e)}	12 (26) ^{e)}	9 (20) ^{b)*}	6 (13)	13 (27) ^{b)*}	N=23 274 (19.2-2440)
Controls rural	45	16/29	20 (12-35)	NA	39 (87)	26 (58)	17 (38)	20 (44)	18 (40)	1 (2)	1 (2)	2 (4)	N=29 155 (5.7-1134)

- a) The severity of the eczema was assessed using the SCORAD index.
b) Significant differences between AD children and controls are indicated * p<0.05.
c) There is no information available about the gender of 1 child.
d) There is no information available about the SCORAD of 1 child.
e) There is no information available for 3 children.
f) Significant differences between urban and rural control are indicated * p<0.05.

AD=atopic dermatitis, F=female, M=male, n=number of positive individuals, NA=not applicable

Table S2. Frequencies of IgE-reactivity to purified allergens in AD children and controls from urban and rural areas of Cape Town, South Africa

No.	Allergen	Urban AD n=32	Rural AD n=49	Urban controls n=40	Rural controls n=45	Urban vs rural p values	Urban AD vs Rural AD p values	Urban AD vs Urban controls p values	Rural AD vs Rural controls p values	Allergen family, Function, CCD
1	nDer p 1	18 (56%)	22 (45%)	1 (3%)	1 (2%)	0.778	0.317	<0.001	<0.001	Group 1 mite allergen, Cysteine protease
2	rDer p 2	21 (66%)	31 (63%)	4 (10%)	2 (4%)	0.959	0.827	<0.001	<0.001	Group 2 mite allergen, NPC2 family
3	rDer p 4	10 (31%)	8 (16%)	0%	0%	0.269	0.114	<0.001	0.005	Group 4 mite allergen, α -Amylase
4	rDer p 5	3 (9%)	4 (8%)	0%	1 (2%)	0.731	0.852	0.048	0.200	Group 5 mite allergen
5	rDer p 7	7 (22%)	8 (16%)	1 (3%)	0%	0.746	0.531	0.009	0.005	Group 7 mite allergen
6	rDer p 10	7 (22%)	4 (8%)	0%	0%	0.161	0.079	0.002	0.050	Group 10 mite allergen, Tropomyosin
7	rDer p 11	6 (19%)	4 (8%)	0%	0%	0.274	0.157	0.004	0.050	Group 11 mite allergen, Paramyosin
8	rDer p 14	4 (13%)	2 (4%)	0%	0%	0.241	0.157	0.021	0.171	Vitellogenin, Apolipophorins
9	rDer p 15	6 (19%)	6 (12%)	0%	0%	0.631	0.420	0.004	0.015	Chitin-binding domain
10	rDer p 18	3 (9%)	1 (2%)	0%	0%	0.196	0.136	0.048	0.335	Chitin-binding domain
11	rDer p 21	3 (9%)	5 (10%)	0%	0%	0.731	0.287	0.048	0.028	Group 21 mite allergen
12	rDer p 23	16 (50%)	24 (49%)	2 (5%)	0%	0.938	0.928	<0.001	<0.001	Chitin-binding domain
13	nDer f 1	18 (56%)	16 (33%)	1 (3%)	0%	0.207	0.035	<0.001	<0.001	Group 1 mite allergen, Cysteine protease
14	rDer f 2	20 (63%)	30 (61%)	6 (15%)	3 (7%)	0.893	0.907	<0.001	<0.001	Group 2 mite allergen, NPC2 family
15	rLep d 2	2 (6%)	1 (2%)	0%	0%	0.411	0.330	0.109	0.335	Group 2 mite allergen, NPC2 family
16	rBlo t 5	1 (3%)	1 (2%)	0%	0%	0.849	0.758	0.260	0.335	Group 5 mite allergen
17	nGal d 1	17 (53%)	17 (35%)	0%	0%	0.382	0.100	<0.001	<0.001	Ovomucoid
18	nGal d 2	11 (34%)	13 (27%)	0%	1 (2%)	0.945	0.449	<0.001	0.001	Ovalbumin
19	nGal d 3	2 (6%)	6 (12%)	0%	0%	0.283	0.377	0.109	0.015	Ovotransferrin, Conalbumin
20	nGal d 5	6 (19%)	11 (22%)	0%	0%	0.478	0.688	0.004	0.001	Serum Albumin
21	rAra h 1	4 (13%)	11 (22%)	0%	0%	0.171	0.259	0.021	0.001	Storage protein, 7S globulin
22	rAra h 2	6 (19%)	14 (29%)	0%	0%	0.198	0.317	0.004	<0.001	Storage protein, 2S albumin

23	rAra h 3	3 (9%)	5 (10%)	0%	0%	0.731	0.903	0.048	0.028	Storage protein, 11S globulin
24	nAra h 6	7 (22%)	12 (25%)	1 (3%)	0%	0.746	0.771	0.009	<0.001	Storage protein, 2S albumin
25	rAra h 9	1 (3%)	4 (8%)	1 (3%)	0%	0.613	0.355	0.873	0.050	nsLTP, type 1
26	rGly m 4	2 (6%)	3 (6%)	0%	0%	0.877	0.985	0.109	0.092	PR-10
27	nGly m 5	6 (19%)	6 (12%)	0%	0%	0.631	0.420	0.004	0.015	Storage protein, 11S globulin
28	nGly m 6	6 (19%)	9 (18%)	0%	0%	0.782	0.967	0.004	0.003	Storage protein, 11S globulin
29	nBos d 4	1 (3%)	1 (2%)	0%	0%	0.849	0.758	0.260	0.335	α -Lactalbumin
30	nBos d 5	1 (3%)	0%	0%	0%	0.252	0.218	0.260	-	Lipocalin, β -Lactoglobulin
31	nBos d 6	3 (9%)	6 (12%)	0%	0%	0.532	0.686	0.048	0.015	Serum Albumin
32	nBos d 8	3 (9%)	0%	0%	0%	0.046	0.029	0.048	-	Caseins (α -, β -, κ -caseins)
33	rTri a 14	2 (6%)	2 (4%)	0%	0%	0.787	0.660	0.109	0.171	nsLTP, type 1
34	Tri aA	2 (6%)	3 (6%)	0%	0%	0.877	0.985	0.109	0.092	Alpha-Amylase
35	Tri a 36	0%	1 (2%)	0%	0%	0.380	0.411	-	0.335	Low molecular weight Glutenin
36	Tri a 37	0%	1 (2%)	0%	0%	0.380	0.411	-	0.335	Alpha-Purethionin
37	nAct d 2	3 (9%)	3 (6%)	0%	0%	0.739	0.585	0.048	0.092	Thaumatococcus-like protein
38	nAct d 5	0%	1 (2%)	0%	0%	0.380	0.411	-	0.335	Kiwelling
39	rPru p 3	3 (9%)	4 (8%)	1 (3%)	0%	0.698	0.852	0.206	0.050	nsLTP, type 1
40	nPen m 1	1 (3%)	0%	1 (3%)	0%	0.104	0.218	0.873	-	Tropomyosin
41	nPen m 2	2 (6%)	1 (2%)	0%	0%	0.411	0.330	0.109	0.335	Arginine kinase-like protein
42	nPen m 4	0%	0%	0%	1 (2%)	0.380	-	-	0.294	Calcium-binding protein
43	nJug r 2	3 (9%)	2 (4%)	0%	0%	0.446	0.336	0.048	0.171	Storage protein, 7S globulin, CCD
44	rJug r 3	2 (6%)	3 (6%)	1 (3%)	0%	0.739	0.985	0.429	0.092	nsLTP, type 1
45	rFel d 1	0%	3 (6%)	0%	0%	0.126	0.152	-	0.092	Fel d 1 family, Uteroglobulin
46	nFel d 2	0%	5 (10%)	0%	0%	0.047	0.062	-	0.028	Serum Albumin
47	rFel d 4	7 (22%)	3 (6%)	0%	0%	0.080	0.061	0.002	0.092	Lipocalin
48	rCan f 1	0%	2 (4%)	0%	0%	0.213	0.247	-	0.171	Lipocalin

49	rCan f 2	1 (3%)	1 (2%)	0%	0%	0.849	0.758	0.260	0.335	Lipocalin
50	nCan f 3	3 (9%)	5 (10%)	0%	0%	0.731	0.287	0.048	0.028	Serum Albumin
51	rCan f 4	0%	1 (2%)	0%	0%	0.380	0.411	-	0.335	Lipocalin
52	rCan f 5	1 (3%)	0%	0%	1 (2%)	0.849	0.218	0.260	0.294	Arginine Esterase
53	rEqu c 1	5 (16%)	3 (6%)	0%	0%	0.263	0.375	0.010	0.092	Lipocalin
54	nEqu c 3	1 (3%)	3 (6%)	0%	0%	0.453	0.543	0.260	0.092	Serum Albumin
55	nMus m 1	16 (50%)	6 (12%)	2 (5%)	0%	0.001	<0.001	<0.001	0.015	Lipocalin
56	rBla g 1	1 (3%)	0%	0%	0%	0.252	0.218	0.260	-	Cockroach group 1
57	rBla g 2	6 (19%)	6 (12%)	0%	0%	0.631	0.420	0.004	0.015	Aspartic protease
58	rBla g 5	1 (3%)	0%	0%	0%	0.252	0.218	0.260	-	Glutathione S-transferase
59	nBla g 7	1 (3%)	0%	0%	0%	0.252	0.218	0.260	-	Tropomyosin
60	α-Gal	1 (3%)	7 (14%)	1 (3%)	22 (49%)	<0.001	0.100	0.873	<0.001	Galactose-1,3-galactose
61	CCD	3 (9%)	3 (6%)	0%	1 (2%)	0.978	0.585	0.048	0.349	Carbohydrate-marker
62	rPhl p 1	1 (3%)	0%	0%	0%	0.252	0.218	0.260	-	Grass group 1, β-Expansin
63	rPhl p 2	1 (3%)	0%	0%	0%	0.252	0.218	0.260	-	Grass group 2/3
64	nPhl p 4	3 (9%)	3 (6%)	0%	1 (2%)	0.978	0.585	0.048	0.349	Grass group 4, CCD
65	rPhl p 5b	0%	1 (2%)	1 (3%)	0%	0.849	0.411	0.368	0.335	Grass group 5
66	rPhl p 6	0%	0%	1 (3%)	0%	0.252	0.686	0.386	-	Grass group 5/6
67	rPhl p 11	0%	1 (2%)	0%	0%	0.380	0.411	-	0.335	Ole e 1-related protein
68	nCyn d 1	3 (9%)	6 (12%)	0%	1 (2%)	0.379	0.686	0.048	0.065	Grass group 1, β-Expansin
69	nArt v 1	0%	1 (2%)	0%	0%	0.380	0.411	-	0.335	Defensin-like protein
70	nArt v 3	1 (3%)	3 (6%)	1 (3%)	0%	0.877	0.543	0.873	0.092	nsLTP, type 1
71	nAmb a 1	1 (3%)	0%	0%	0%	0.252	0.218	0.260	-	Pectate Lyase
72	nCup a 1	3 (9%)	4 (8%)	0%	0%	0.978	0.686	0.048	0.050	Pectate Lyase, CCD
73	nPla a 2	1 (3%)	1 (2%)	0%	0%	0.849	0.758	0.260	0.335	Polygalacturonase, CCD
74	nCry j 1	1 (3%)	0%	0%	1 (2%)	0.849	0.218	0.260	0.294	Pectate Lyase, CCD

75	rMer a 1	0%	3 (6%)	0%	0%	0.126	0.152	-	0.092	Profilin
76	rBet v 2	2 (6%)	3 (6%)	0%	0%	0.877	0.985	0.109	0.092	Profilin
77	rOle e 1	1 (3%)	0%	0%	0%	0.252	0.218	0.260	-	Ole e 1-related protein
78	rOle e 5	2 (6%)	2 (4%)	0%	0%	0.787	0.660	0.109	0.171	Superoxide dismutase
79	rOle e 6	1 (3%)	1 (2%)	0%	0%	0.849	0.758	0.260	0.335	unknown
80	nOle e 7	1 (3%)	2 (4%)	1 (3%)	0%	0.787	0.819	0.873	0.171	nsLTP, type 1
81	rOle e 9	0%	1 (2%)	0%	0%	0.380	0.411	-	0.335	Glucanase
82	rOle 10	1 (3%)	1 (2%)	0%	0%	0.849	0.758	0.260	0.335	Glycosyl hydrolase
83	rHev b 1	1 (3%)	0%	0%	0%	0.252	0.218	0.260	-	Rubber elongation factor
84	rHev b 3	2 (6%)	0%	0%	0%	0.104	0.076	0.109	-	Small rubber particle protein
85	rHev b 5	2 (6%)	0%	0%	0%	0.104	0.076	0.109	-	Acidic protein
86	rAlt a 1	3 (9%)	1 (2%)	0%	0%	0.196	0.136	0.048	0.335	Acidic glycoprotein
87	rAlt a 6	1 (3%)	1 (2%)	0%	0%	0.849	0.758	0.260	0.335	Enolase
88	rVes v 5	0%	0%	0%	1 (2%)	0.380	-	-	0.294	Insect venom, Antigen 5

Detected by
MeDALL
allergen chip,
reference
range ≥ 0.1
ISU
AD = atopic
dermatitis,

r=recombinant, n=natural, CCD=Cross-reactive carbohydrate determinants

TABLE S3. Exposure, IgE-reactivity and skin-reactivity in children with AD and children without AD (controls), living in urban or rural areas in Cape Town South Africa.

		Urban AD N=32	Urban controls N=40	Urban AD vs urban controls p values	Rural AD N=49	Rural controls N=45	Rural AD vs rural controls p values	Rural AD vs urban AD
House dust mite	Any Der p n (%) ^{a)}	26 (81)	6 (15)	<0.001 ^{c)} *	36 (74)	3 (7)	<0.001*	0.418
	Any Der f n (%) ^{b)}	24 (75)	6 (15)	<0.001*	30 (61)	3 (7)	<0.001*	0.198
Egg	Egg exposed n (%)	24 (75)	39 (98)	0.004*	42 (86)	40 (89)	0.647	0.226
	Egg exposed age (month) median (range)	12 (5-18)	12 (3-24)		11 (4-24)	12 (5-24)		
	Any Gal d n (%) ^{d)}	20 (63)	0 (0)	<0.001*	21 (43)	1 (2)	<0.001*	0.084
	SPT egg positive n (%)	16 (50)	NA	NA	15 (31)	NA	NA	0.08
	SPT fresh egg positive n (%)	23 (72)	NA	NA	22 (45)	NA	NA	0.017*
Peanut	Peanut exposed n (%)	22 (69)	35 (88)	0.052	22 (45)	15 (33)	0.252	0.035*
	Peanut exposed age (month) median (range)	12 (4-24)	14 (6-30)		12 (6-36)	12 (7-24)		
	Any Ara h n (%) ^{g)}	10 (31)	2 (5)	<0.001*	20 (41)	0 (0)	<0.001*	0.383
	SPT peanut positive n (%)	7 (22)	NA	NA	18 (37)	NA	NA	0.156
	SPT fresh peanut positive n (%)	6 (19)	NA	NA	16 (33)	NA	NA	0.169
Soybean	Soybean exposed n (%)	28 (88)	36 (90)	0.74	45 (92)	44 (98)	0.74	0.522
	Soybean exposed age (month) median (range)	12 (5-24)	12 (6-24)		10 (3-36)	9 (3-14)		
	Any Gly m n (%) ^{h)}	7 (22)	0 (0)	0.002*	13 (27)	0 (0)	<0.001*	0.634
	SPT soybean positive n (%)	3 (9)	NA	NA	6 (12)	NA	NA	0.686
Cow's milk	Cow milk exposed n (%)	32 (100)	40 (100)		49 (100)	45 (100)		
	Cow's milk exposed age (month) median (range)	3 (0-8)	2 (0-8)		2 (0-10)	3 (0-9)		
	Any Bos d n (%) ⁱ⁾	5 (16)	0 (0)	0.009*	7 (14)	0 (0)	0.008*	0.863
	SPT cow milk positive n (%)	4 (13)	NA	NA	5 (10)	NA	NA	0.686
	SPT fresh cow milk positive n (%)	5 (16)	NA	NA	8 (16)	NA	NA	0.931
Animal	Own dog n (%)	6 (19)	11 (28)	0.383	27 (55)	33 (73)	0.067	0.001*
	Any Can f n (%) ^{k)}	3 (9)	0 (0)	0.047*	6 (12)	0 (0)	0.015*	0.686
	Own cat n (%)	2 (6)	5 (13)	0.374	9 (18)	18 (40)	0.021*	0.119
	Any Fel d n (%) ^{m)}	7 (22)	0 (0)	0.002*	8 (16)	0 (0)	0.005*	0.531
	Any Equ c n (%) ⁿ⁾	6 (19)	0 (0)	0.004*	5 (10)	0 (0)	0.028*	0.274
	Mus m 1 n (%)	16 (50)	2 (5)	<0.001*	6 (12)	0 (0)	0.015*	<0.001*

AD=atopic dermatitis, n=number of positive individuals, Na=not applicable

- a) Individuals positive to one or more of the tested Der p allergens Der p 1, Der p 2, Der p 4, Der p 5, Der p 7, Der p 10, Der p 11, Der p 14, Der p 15, Der p 18, Der p 21, Der p 23
- b) Individuals positive to one or more of the tested Der f allergens Der f 1, Der f 2
- c) Significant differences are indicated * $p < 0.05$
- d) Individuals positive to one or more of the tested Gal d allergens, Gal d 1, Gal d 2, Gal d 3, Gal d 5
- e) Individuals positive to one or more of the tested Ara h allergens, Ara h 1, Ara h 2, Ara h 3, Ara h 6, Ara h 9
- f) Individuals positive to one or more of the tested Gly m allergens, Gly m 4, Gly m 5, Gly m 6
- g) Individuals positive to one or more of the tested Bos d allergens, Bos d 4, Bos d 5, Bos d 6, Bos d 8
- h) Individuals positive to one or more of the tested Can f allergens, Can f 1, Can f 2, Can f 3, Can f 4, Can f 5
- i) Individuals positive to one or more of the tested Fel d allergens, Fel d 1, Fel d 2, Fel d 4
- j) Individuals positive to one or more of the tested Equ c allergens, Equ c 1, Equ c 3

TABLE S4. Sensitization to HDM allergens measured by microarray technology in children with or without AD (CO) from urban and rural areas in South Africa. Serum samples, which showed IgE-reactivity to at least one of the minor HDM allergens >0.1 ISU are displayed.

ID	Der p 1 ISU	Der p 2 ISU	Der p 23 ISU	Der p 4 ISU	Der p 5 ISU	Der p 7 ISU	Der p 10 ISU	Der p 11 ISU	Der p 14 ISU	Der p 15 ISU	Der p 18 ISU	Der p 21 ISU
Urban AD (n=32)												
UAD2	22.68	23.81	31.55	0.23	68.2	5.76	9.74	0.15	0	0.17	0	0.65
UAD3	14.33	0.48	14.32	0.21	0	0	0	0	0	0	0	0
UAD8	34.68	1.3	0	0.47	0	0.29	0.38	0	0	0	0	0
UAD9	62.93	0.82	53.25	0.8	0	0.42	0.27	0.19	0.13	0.13	0	0
UAD11	49.4	8.76	0	0.67	0	0.33	0.35	0.14	0	0.14	0	0
UAD12	81.48	5.11	29.93	2.52	58	6.99	1.37	0.58	0.51	0.5	0.27	0.31
UAD14	113.32	35.86	0	2.13	0.2	0.8	1.17	0.42	0.33	0.29	0.19	0.15
UAD24	17.18	0.3	21.58	0.18	0	0	0	0	0	0	0	0
UAD27	46.01	1.4	84.74	0.85	0	8.81	0.32	0.25	0.5	0.3	0.2	0
UAD32	30.36	1.4	21.73	0.18	0	0	0	0	0	0	0	0
Urban Control (n=40)												
UCO18	0	0	28.18	0	0	14.19	0	0	0	0	0	0
Rural AD (n=49)												
RAD1	16.17	2.97	10.88	0.3	0	2.18	0	0	0	0	0	0
RAD4	63.56	32.85	48.93	0.75	0	0.35	0.33	0.21	0	0.27	0	0
RAD10	33.22	9.32	18.34	0.32	0	0	0	0	0	0	0	0
RAD11	53.21	9.36	38.13	0.39	0	0.16	0	0	0	0	0	0
RAD12	21.72	3.75	34.32	0.31	0	0	0	0	0	0	0	0
RAD14	0.3	7.55	0.12	0	0.26	0	0	0	0	2.32	3.25	0.11
RAD18	143.05	79.72	130.51	1.36	0	0.33	0.29	0.17	0.27	0.24	0	0
RAD19	23.5	9.93	17.89	0	0	2.01	0	0	0	0	0	0
RAD21	43.26	0.77	23.69	0.36	41.55	12.24	0.45	0	0	0	0	35.84
RAD27	0	0	0	0	0	0	0	0.19	0	0	0	0
RAD29	39.72	33.18	57.24	0.71	0.16	0.19	0.22	0.15	0.17	0.17	0	89.74
RAD32	0	2.24	2.87	0	0	0	0	0	0	0.86	0	0
RAD38	1.66	3.12	0.32	0	0	0	0	0	0	0	0	0.3
RAD39	5.49	2.3	5.36	0	0	0	0	0	0	0	0	9.27
RAD42	4.44	7.34	11.09	0	0.81	0.2	0	0	0	0	0	0
Rural Control (n=45)												
RCO12	0	0	0	0	0.21	0	0	0	0	0	0	0

TABLE S5. Hierarchy of sensitization to serum albumin measured by microarray technology in children with AD from urban and rural areas in South Africa. Shown are sera, which showed IgE-reactivity to at least one of the tested allergens Gal d 5, Bos d 6, Fel d 2, Can f 3 or Equ c 3 >0.1 ISU.

ID	Gal d 5	Bos d 6	Fel d 2	Can f 3	Equ c 3
Urban AD (n=32)					
UAD5	0.92	0	0	0	0
UAD14	0.4	0.12	0	0	0
UAD16	1.01	0.25	0	0.13	0.38
UAD19	0	0.13	0	0.15	0
UAD27	2.73	0	0	0.7	0
UAD29	1.07	0	0	0	0
UAD31	0.2	0	0	0	0
Rural AD (n=49)					
RAD1	0.41	0	0	0	0
RAD4	3.27	0	0.7	2.53	0
RAD6	0.45	0	0	0	0
RAD10	0.23	0.18	0	0	0
RAD12	0.28	0	0	0	0
RAD18	0	0	0.51	0	0.21
RAD19	2.57	0.19	0	0	0
RAD21	1.03	0	0	0.16	0
RAD26	2.46	2.43	2.56	1.07	0.24
RAD31	0	0.21	0	0	0
RAD33	0	0	0.5	0.78	0
RAD36	0.17	0	0	0	0
RAD37	0.47	0.61	0	0	0.13
RAD43	0	0.26	0.57	0.58	0
RAD47	2.38	0	0	0	0