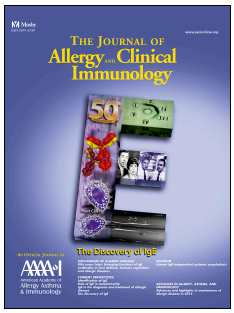
Accepted Manuscript



Latent class analysis reveals clinically relevant atopy phenotypes in two birth cohorts

Alexander J. Hose, MA, MPH, Martin Depner, PhD, Sabina Illi, PhD, Susanne Lau, MD, PhD, Thomas Keil, MD, MScPH, Ulrich Wahn, MD, Oliver Fuchs, MD, PhD, Petra Ina Pfefferle, PhD, DrPH, Elisabeth Schmaußer-Hechfellner, BSc, Jon Genuneit, MD, Roger Lauener, MD, Anne M. Karvonen, PhD, Caroline Roduit, MD, Jean-Charles Dalphin, MD, PhD, Josef Riedler, MD, Juha Pekkanen, MD, PhD, Erika von Mutius, MD, Markus J. Ege, MD, Carl Peter Bauer, Johannes Forster, Fred Zepp, Volker Wahn, Antje Schuster, Renate L. Bergmann, Karl E. Bergmann, Andreas Reich, Linus Grabenhenrich, Bianca Schaub, Georg J. Loss, Harald Renz, Michael Kabesch, Marjut Roponen, Anne Hyvärinen, Pekka Tiittanen, Sami Remes, Charlotte Braun-Fahrländer, Remo Frei, Vincent Kaulek, Marie-Laure Dalphin, Gert Doekes, Nicole Blümer, Urs Frey

PII: S0091-6749(16)31135-6

DOI: 10.1016/j.jaci.2016.08.046

Reference: YMAI 12399

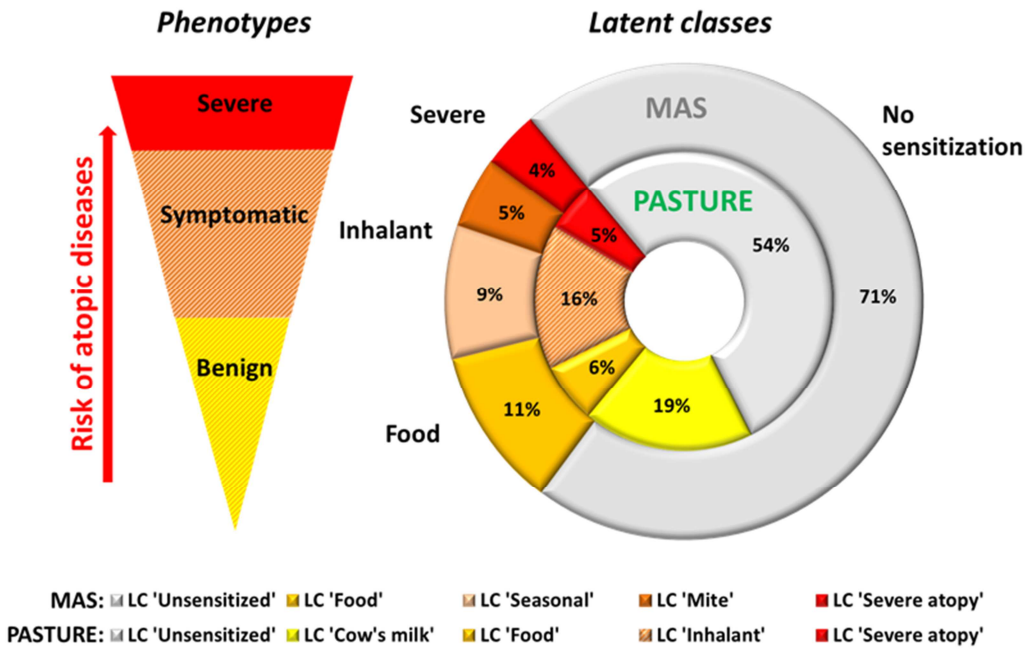
To appear in: *Journal of Allergy and Clinical Immunology*

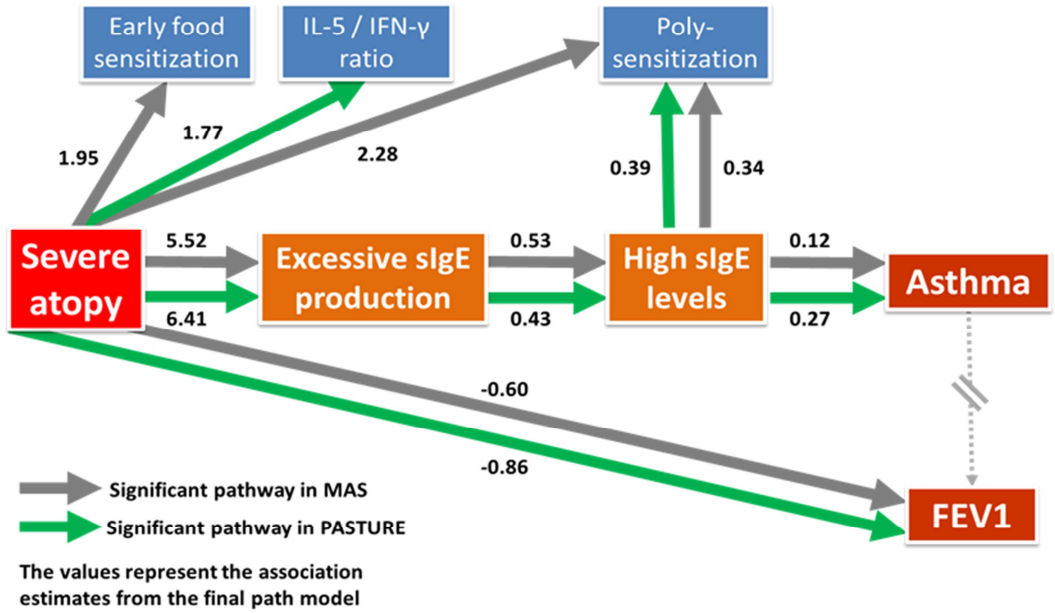
Received Date: 22 January 2016

Revised Date: 11 August 2016

Accepted Date: 22 August 2016

Please cite this article as: Hose AJ, Depner M, Illi S, Lau S, Keil T, Wahn U, Fuchs O, Pfefferle PI, Schmaußer-Hechfellner E, Genuneit J, Lauener R, Karvonen AM, Roduit C, Dalphin J-C, Riedler J, Pekkanen J, von Mutius E, Ege MJ, the MAS, Bauer CP, Forster J, Zepp F, Wahn V, Schuster A, Bergmann RL, Bergmann KE, Reich A, Grabenhenrich L, PASTURE study group members, Schaub B, Loss GJ, Renz H, Kabesch M, Roponen M, Hyvärinen A, Tiittanen P, Remes S, Braun-Fahrländer C, Frei R, Kaulek V, Dalphin M-L, Doekes G, Blümer N, Frey U, Latent class analysis reveals clinically relevant atopy phenotypes in two birth cohorts, *Journal of Allergy and Clinical Immunology* (2016), doi: 10.1016/j.jaci.2016.08.046.


Phenotypes
Latent classes
Severe
Symptomatic
Benign
Risk of atopic diseases
Severe
MAS
No sensitization
Inhalant
Food
PASTURE
4%
5%
5%
54%
9%
16%
71%
6%
11%
19%
MAS: LC 'Unsensitized' LC 'Food' LC 'Seasonal' LC 'Mite' LC 'Severe atopy'
PASTURE: LC 'Unsensitized' LC 'Cow's milk' LC 'Food' LC 'Inhalant' LC 'Severe atopy'


Early food sensitization
IL-5 / IFN-γ ratio
Poly-sensitization
1.95
1.77
2.28
0.39
0.34
Severe atopy
5.52
6.41
Excessive sIgE production
0.53
0.43
High sIgE levels
0.12
0.27
Asthma
-0.60
-0.86
FEV1
Significant pathway in MAS
Significant pathway in PASTURE
The values represent the association estimates from the final path model

Latent class analysis reveals clinically relevant atopy phenotypes in two birth cohorts

Alexander J. Hose, MA, MPH[1]; Martin Depner, PhD[1], Sabina Illi, PhD[1], Susanne Lau, MD, PhD[2], Thomas Keil, MD, MScPH[3,4], Ulrich Wahn, MD[2], Oliver Fuchs, MD, PhD[5,6,7], Petra Ina Pfefferle, PhD, DrPH[8], Elisabeth Schmaußer-Hechfellner, BSc[1], Jon Genuneit, MD[9], Roger Lauener, MD[10,11], Anne M. Karvonen, PhD[12], Caroline Roduit, MD[10,13], Jean-Charles Dalphin, MD, PhD[14], Josef Riedler, MD[15], Juha Pekkanen, MD, PhD[12,16], Erika von Mutius[5], MD; Markus J. Ege[5], MD, and the MAS and PASTURE study groups

MAS study group members:

Carl Peter Bauer[17], Johannes Forster[18], Fred Zepp[19], Volker Wahn[20], Antje Schuster[21], Renate L. Bergmann[22], Karl E. Bergmann[22], Andreas Reich[23], Linus Grabenhenrich[23]

PASTURE study group members:

Bianca Schaub[5], Georg J. Loss[1,24], Harald Renz[25], Michael Kabesch[26,27], Marjut Roponen[28], Anne Hyvärinen[12], Pekka Tiittanen[12], Sami Remes[29], Charlotte Braun-Fahrländer[30,31], Remo Frei[10,32], Vincent Kaulek[14], Marie-Laure Dalphin[33], Gert Doekes[34], Nicole Blümer[35], Urs Frey[7]

[1]Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich, Lindwurmstr. 4, D-80337, Munich, Germany

[2]Department for Pediatric Pneumology and Immunology, Charité–Universitätsmedizin Berlin, Berlin, Germany

[3]Institute of Social Medicine, Epidemiology and Health Economics, Charité–Universitätsmedizin Berlin, Berlin, Germany

[4]Institute for Clinical Epidemiology and Biometry, University of Wuerzburg, Wuerzburg, Germany

[5]Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich, Lindwurmstr. 4, D-80337, Munich, Germany, and Comprehensive Pneumology Center, Munich (CPC-M), Germany; Member of the German Center for Lung Research (DZL)

[6]Division of Respiratory Medicine, Department of Pediatrics, Inselspital, University of Bern, Bern, Switzerland

[7]University Children's Hospital (UKBB), University of Basel, Spitalstr. 33, CH-4056 Basel, Switzerland

[8]Comprehensive Biomaterial Bank Marburg CBBM, Fachbereich Medizin der Philipps Universität Marburg, Zentrum für Tumor und Immunbiologie ZTI, Hans-Meerweinstr. 3, D-35043 Marburg, Germany; Member of the German Center for Lung Research

[9]Ulm University, Institute of Epidemiology and Medical Biometry, Helmholtzstr. 22, D-89081 Ulm, Germany

[10]Christine Kühne Center for Allergy Research and Education (CK-CARE), Davos, Switzerland

[11]Children's Hospital of Eastern Switzerland, St. Gallen, Switzerland

[12]Department of Health Protection, National Institute for Health and Welfare, P.O. Box 95 FIN-70701 Kuopio, Finland

[13]Children's Hospital, University of Zürich, Zürich, Switzerland

[14]University of Besançon, Department of Respiratory Disease, UMR/CNRS6249 Chrono-environment, University Hospital, 25030 Besançon, France

[15]Children's Hospital Schwarzach, Kardinal Schwarzenbergstr. 2-6, A-5620 Schwarzach, Austria, Teaching Hospital of Paracelsus Medical Private University Salzburg

[16]Department of Public Health, University of Helsinki, Helsinki, Finland

[17]Technical University of Munich, Department of Pediatrics, Munich, Germany

[18]Department of Paediatrics, St. Josefs Hospital, Freiburg, Germany

[19]Centre for Paediatric and Adolescent Medicine, University Medical Centre Mainz, Mainz, Germany

[20]University Children's Hospital, Charité, Berlin, Germany

[21]Department of Paediatrics, University Medical Centre Düsseldorf, Düsseldorf, Germany

[22]Department of Obstetrics, Charité-Universitätsmedizin Berlin, Berlin, Germany

[23]Institute of Social Medicine, Epidemiology and Health Economics, Charité-Universitätsmedizin Berlin, Berlin, Germany

[24]University of California, San Diego, School of Medicine, Department of Pediatrics, 9500 Gilman Drive, La Jolla, CA 92093-0831, U.S.A.

[25]Department of Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, Marburg, Germany; Member of the German Center for Lung Research

[26]KUNO Children's University Hospital Regensburg, Department of Pediatric Pneumology and Allergy Campus St. Hedwig, Steinmetzstr. 1-3, D-93049 Regensburg, Germany

[27]Hannover Medical School, Clinic for Pediatric Pneumology and Neonatology, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany

[28]Department of Environmental Science, Inhalation Toxicology Laboratory, University of Eastern Finland, P.O Box 1627, Kuopio, Finland

[29]Department of Pediatrics, Kuopio University Hospital, Kuopio, Finland

[30]Swiss Tropical and Public Health Institute, Socinstr. 57, P.O. Box, CH-4002 Basel, Switzerland

[31]University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland

[32]Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland

[33]University of Besançon, Department of Pediatrics, University Hospital, 25030 Besançon, France

[34]Utrecht University, Institute for Risk Assessment Sciences (IRAS), Division of Environmental Epidemiology, PO Box 80178, 3508TD, Utrecht, Netherlands

[35]Department of Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, Marburg, Germany

Corresponding author:

Alexander Hose, MA MPH

Dr. von Hauner Children's Hospital

Ludwig-Maximilians-University of Munich

Lindwurmstr. 4

D – 80337 München

Phone: +49(0)89 – 4400-57993

Fax: +49(0)89 – 4400-54452

E-mail: Alexander.Hose@med.uni-muenchen.de

Funding (MAS):

The MAS study was funded by grants from the German Federal Ministry of Education and Research (BMBF; reference numbers 07015633, 07 ALE 27, 01EE9405/5, 01EE9406) and the German Research Foundation (DFG; reference number KE 1462/2-1). The funders had no role in the design, management, data collection, analysis or interpretation of the data or in the writing of the manuscript or the decision to submit for publication.

Funding (PASTURE):

This work has been supported by the European Commission (research grants QLK4-CT-2001-00250, FOOD-CT-2006-31708, and KBBE-2007-2-2-06), the European Research Council (Grant 250268), and the German Center for Lung Research (DZL). The funding sources did not influence the study design; the collection, analysis, and interpretation of data; the writing of the manuscript; and the decision to submit the paper for publication. The corresponding author had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements (MAS):

We would like to thank all MAS contributors, especially the PIs of the study centres Carl Peter Bauer (Technische Universität München), Johannes Forster (St. Josefs Krankenhaus Freiburg), Walter Dorsch, Wolfgang Kamin, Fred Zepp (Mainz University Medical Center), Volker Wahn, Antje Schuster (Düsseldorf University Hospital), the co-initiators Karl Bergmann and Renate Bergmann as well as Bodo Niggemann, Petra Wagner and Gabi Schulz (Charité - Universitätsmedizin Berlin).

Abstract

Background:

Phenotypes of childhood-onset asthma are characterized by distinct trajectories and functional features. For atopy, definition of phenotypes during childhood is less clear.

Objective:

To define phenotypes of atopic sensitization over the first 6 years of life by a latent class analysis (LCA) integrating three dimensions of atopy: allergen specificity, time course, and levels of specific IgE.

Methods:

Phenotypes were defined by LCA in 680 children of the MAS and 766 of the PASTURE birth cohorts and compared to classical non-disjunctive definitions of seasonal, perennial, and food sensitization with respect to atopic diseases and lung function. Cytokine levels were measured in PASTURE.

Results:

The LCA classified predominantly by type and multiplicity of sensitization (food versus inhalant), allergen combinations, and sIgE levels. Latent classes were related to atopic disease manifestations with higher sensitivity and specificity than the classical definitions. LCA detected in both cohorts consistently a distinct group of children with *severe atopy* characterized by high seasonal sIgE and a strong propensity for asthma, hay fever, eczema and impaired lung function even in children without an established asthma diagnosis. *Severe atopy* was associated with an elevated interleukin-5/interferon-gamma ratio. A path analysis among sensitized children revealed that among all features of *severe atopy* only excessive sIgE production early in life impacted on asthma risk.

Conclusions:

LCA revealed a set of benign, symptomatic, and *severe atopy* phenotypes. The severe phenotype emerged as a latent condition with signs of a dysbalanced immune response. It determined high asthma risk via excessive sIgE production and directly impacted on impaired lung function.

Clinical Implications:

Atopic sensitization was classified into benign, symptomatic and severe phenotypes. *Severe atopic* children were characterized by a strong propensity for atopic diseases mediated by excessive sIgE production early in life and poor lung function even in those without an established asthma diagnosis.

Capsule summary:

Atopic sensitization was classified with respect to disease relevance in three phenotypes of benign, symptomatic, and *severe atopy*, which impacted on asthma risk via excessive production of specific IgE early in life and on poor lung function.

Key words:

Atopy; IgE; sensitization; asthma; lung function; cytokines; latent class analysis; unsupervised clustering; path analysis; epidemiology

Abbreviation list:

Abbreviations used

IgE: Immunoglobulin E

sIgE: specific Immunoglobulin E

LCA: Latent class analysis

LC: Latent class

MAS: Multizentrische Allergiestudie

PASTURE: Protection against allergy: Study in rural environments

CAP: Carrier polymer system

ISAAC: International Study of Asthma and Allergies in Childhood

AD: Atopic dermatitis

FEV1: Forced expiratory volume in 1 second

ROC: Receiver operating characteristic

AIC: Akaike information criterion

AUC: Area under the ROC curve

IU/ml: International Units/milliliter

ng/ml: Nanogramms/milliliter

µg/ml: Microgramms/milliliter

pg/ml: Picogramms/milliliter

IL-5: Interleukin-5

IFN-γ: Interferon-γ

PI: Phorbol 12-myristate 13-acetate / ionomycin

Introduction

Asthma and atopy often manifest concomitantly before school-age. But the interrelation of both phenomena remains obscure, possibly because both conditions may result from a multitude of individual pathologies, whose complex interferences blur the entire picture. In the case of asthma, wheezing phenotypes have been identified and consolidated by data-driven approaches.[1-3] These approaches, however, are currently only emerging for atopy classification.

Because of co-sensitizations, categorization by allergen specificity or type of sensitization is ambiguous and leads to overlapping groups such as food, inhalant perennial, or inhalant seasonal sensitization.[4] Other approaches applying disjunctive categories mainly rely on temporal patterns, focusing on the age of onset,[5-9] longitudinal trends,[10] persistence of IgE sensitization,[11,12] or consider multiplicity of allergen specificities, i.e. mono- versus polyvalent sensitization.[13-18] However, it has been pointed out that all the above approaches are susceptible to investigator bias.[19] This issue can be overcome by data-driven, unsupervised statistical methods such as latent class analysis (LCA). Until now, these approaches focused on allergen specificities at one[20] or several[19,21,22] time points, but did not consider strength of sensitization as assessed by IgE levels.

We considered this omission a shortcoming given the well-known disease relevance of IgE-levels[23] and therefore included this dimension in our analysis. We applied LCA to two rather different birth cohorts, i.e. the urban MAS cohort (Multizentrische Allergiestudie, MAS) and the rural PASTURE study (Protection against allergy: Study in rural environments). The aim of this analysis was to compare LCA-derived classification to classical definitions of atopy based on carrier polymer system (CAP) classes and to relate both systems to manifestation of asthma, allergic diseases, cytokine expression, and lung function. Finally we sought to integrate the various aspects of atopy in a path model for asthma and lung function.

Methods

Study design and population

Both birth cohorts were set up to study the development of childhood asthma and allergies. MAS recruited 1314 healthy mature infants born in 1990 in five German cities (Berlin, Düsseldorf, Freiburg, Mainz, and Munich).[24] Of those, 499 had risk factors for atopy, i.e. raised cord blood IgE (≥0. 9 kU/L) or at least two atopic family members. PASTURE recruited 1133 children in 2002-2005 from rural areas in 5 European countries: Austria, Finland, France, Germany, and Switzerland.[25] Children of mothers living on family-run livestock farms were assigned to the farm study group. The reference study group comprised children of mothers from the same rural areas but not living on a farm. Both studies were approved by the ethics committees of the participating institutions, and written informed consent was obtained from the children's parents or guardians.

Atopic sensitization (Specific IgE in serum samples)

In MAS, serum samples were obtained from the children at 1, 2, 3, 5, 6 and 7 years of age. Specific IgE antibodies (sIgE) to food allergens (cow's milk, egg white, soy bean, wheat) and inhalant allergens (house dust mites *Dermatophagoides pteronyssinus*, cat dander, mixed grass, birch pollen, and dog dander from age 3 years on) were determined with ImmunoCAP (Phadia, Freiburg, Germany). Soy bean was excluded from the analyses because it was not measured in PASTURE for all time points and dog dander due to the lack of measurements at year 1 and 2. In PASTURE, specific IgE for 6 food and 13 common inhalant allergens was assessed in cord blood samples and at the age of 12, 54 and 72 months in peripheral blood by using the semiquantitative Allergy Screen test panel for atopy (Mediwiss Analytic, Moers, Germany) in a central laboratory.[4] Because of common cross-reactivity and low frequencies of some specificities, the original 19 specificities were combined into 9 categories finally entered in the LCA: grass pollen (rye pollen or grass pollen mix), tree pollen (alder, birch pollen or hazel pollen) cat, dog, mites (*Dermatophagoides pteronyssinus* or *Dermatophagoides farinae*), hen's egg, cow's milk, wheat flour, nuts (peanut or hazelnut). In MAS the categories nuts and dog were not available.

Questionnaires

In MAS, at each follow-up visit at the age of 1, 3, 6, 12, 18, and 24 months and from then on yearly within 4 weeks of the child's birthday up to the age of 7 years, parents were interviewed for asthmatic and atopic symptoms and disease, diet, development, and psychological aspects. From age 5 years onwards, questions relating to wheeze corresponded to the International Study of Asthma and Allergies in Childhood (ISAAC) core questions. In PASTURE, questionnaires were administered at the end of pregnancy and when the children were 2, 12, 18, 24, 36, 48, 60, and 72 months of age to obtain information on frequencies of wheeze, parental atopic status, and environmental exposures with a focus on farming and nutrition.[4] Variable definitions were harmonized between both studies. Lifetime asthma was defined as a physician's diagnosis of asthma at least once per lifetime as reported by the parents at age 6 years, children with no diagnosis of asthma and no current wheeze in the last 12 months served as controls. Hay fever was defined as parental reported rhinitis symptoms ever or a physician's diagnosis of hay fever or allergic rhinitis ever at age 6. Atopic dermatitis (AD) was defined as a physician's diagnosis of atopic eczema at least once per lifetime as reported by the parents at age 6 years, children with no diagnosis of atopic eczema and no atopic eczema in the last 12 months where the control subjects.

Lung function measurements

At the age of 7 years in MAS in 801 children,[6] and at 6 years in PASTURE in 799 children,[3] forced expiratory volume in 1 second (FEV1) was measured and z-standardized.[26]

Cytokine assessment

In PASTURE, whole blood supernatants from 6-year-old children were collected after 48h stimulation with PI (5ng/ml PMA, 1µg/ml Ionomycin). Interleukin (IL)-5 and Interferon (IFN)-γ were measured in the supernatants by multiplexed cytometric bead array (BD Biosciences, San Jose, CA) in Marburg, Germany. The detection limit was 0.01pg/ml and values below were replaced by 0.001 in n=17 (IL-5) and n=11 (IFN-γ) individuals. Cytokine concentrations were standardized to peripheral blood leukocyte counts (Sysmex KX-21N blood cell analyzer; Sysmex Corporation, Kobe, Japan) and z-transformed.

Statistical Analysis

Children with missing sIgE data for at least 3 out of 6 (MAS) or 2 out of 4 (PASTURE) measurement time points were excluded. For all other children, missing sIgE values were imputed by multiple linear imputation of the continuous sIgE values in 20 replicates. Categorical variables were created from the imputed continuous variables for the level of sIgE with following categories (in kU/L): sIgE <0.35; ≥0.35 sIgE <0.7; ≥0.7 sIgE <3.5; sIgE ≥ 3.5 corresponding to CAP classes; in PASTURE the lowest category was again split at 0.2 kU/L because of the comparably lower sIgE values and a lower detection limit of the measurement method. For each imputed dataset an LCA based on categorized sIgE values between birth and year 6 was performed assigning individuals to classes by their highest posterior probabilities,[27] and each subject was assigned to the latent class (LC) it was classified in the majority of the 20 replications (more details see in the methods section of the Online Repository). To enhance recognition the retrieved LCs were arbitrarily labeled according to their key features.

Classical definitions of atopy were defined as being sensitized to a specific allergen or groups of allergens (seasonal, perennial, or food allergens) at a specific CAP class at a specific time point, irrespectively of sensitizations to other allergens. The LCs were compared to these classical definitions with respect to true- and false-positive rates using receiver operating characteristics (ROC) curves. Associations of outcomes with potential determinants were calculated by linear or logistic regression. Effect estimates are given with 95%-confidence intervals as odds ratios (ORs) for dichotomous outcomes and β-estimates for linear continuous outcomes such as lung function parameters. All regression analyses were adjusted for center and in PASTURE additionally for study group. Control subjects used in the regression models for LCA were subjects assigned to LC "*unsensitized*" and for classical definitions children without any sensitization at CAP class 1 at the respective time point. Statistical analyses were performed with SAS 9.4 and MPLUS 7.

Results

The analysis population consisted of 680 MAS children (52% of 1314 at recruitment, **Figure 1A**) and 766 PASTURE children (68% of 1133, **Figure 1B**) with complete or imputed sIgE values which did not differ from the excluded children with respect to sensitization status at any age (**Table E1**). The LCA revealed solutions with 3 to 6 classes with the best AIC-values for the 5-class solutions in both studies (**Table E2**). The distribution of LCs across study centers was rather homogenous in both studies (**Figure E1**).

As illustrated by **Figure 2**, the largest classes containing 71% (MAS) and 54% (PASTURE) of all children were characterized by absence of sensitization and consequently labeled "*unsensitized*". One MAS class and two PASTURE classes included mainly children with sensitization to food allergens. The MAS children in the "*food*" class were predominantly mono-sensitized to cow's milk or hen's egg; in PASTURE the larger class was sensitized only to "*cow's milk*" and the other class to "*food*" allergens beyond cow's milk. The remaining classes represented mainly inhalant sensitization: In PASTURE one class included children with sensitization predominantly to either seasonal or perennial "*inhalant*" allergens. The corresponding MAS children were grouped into two classes with either sensitization to "*seasonal*" or "*mite*" allergens. The smallest class within each study was termed "*severe atopy*" for its specific features explained below.

A hallmark of LC *"severe atopy"* was sensitization predominantly to seasonal allergens up to CAP class 3 with a steep increase in the prevalence of sensitization before year 4 or 5. Food co-sensitization occurred in the majority of this LC (MAS: 88%; PASTURE: 67%) and mite co-sensitization in a relevant proportion (MAS: 31%; PASTURE: 26%) at year 6 and CAP class 2. In the MAS LC "*severe atopy*" food co-sensitization was very common already at year 1 (81%, CAP class 2). In PASTURE, food sensitization at year 1 occurred in 22% when considering a cut-off level of 0.2 kU/L. Taken together, LCA grouped mainly for allergen specificity (food versus inhalant classes), strength of sensitization and partially for temporal patterns.

LCs are mutually exclusive and integrate information across CAP classes and over various time points, whereas classical definitions of sensitization such as sIgE to any inhalant or any food allergens can overlap and depend on the underlying CAP class and the time point of measurement. Though both

systems were comparable at the most suitable time points and CAP cut-off levels as determined by ROC curves (**Figure E2, Table E3)**, their associations with disease manifestations diverged in several instances (**Figure 3, Figure E3**): In both studies, sIgE to any food allergens overestimated the associations with health conditions when compared to the food LCs. Conversely, LC "*severe atopy*" was much stronger associated with asthma-related conditions as compared to sIgE against any inhalant allergens even at CAP class 3. The associations of disease risk with the respective LCs were paralleled by those of parental atopy (**Figure E4**). A sensitivity analysis (**Figures E5 and E6**) revealed that each of the three dimensions allergen specificity, specific IgE levels, and time course importantly contributed to the composition and disease relevance of the respective LCs.

Based on disease relevance, the LCs were grouped within three atopy phenotypes (**Figure 4):** LCs related to food sensitization represented a benign phenotype without any disease relevance, LCs related to inhalant sensitization corresponded to a symptomatic phenotype with risk of asthma, yet normal lung function. In contrast, the LC "*severe atopy"* was characterized by impaired lung function and a much higher propensity of atopic disease.

To better understand the singular phenomenon of *severe atopy* and to contrast it with benign and symptomatic sensitized children we assessed biologically relevant features of atopy. Though the LCA discriminated well between oligo- and polyvalent sensitization, polyvalence was not specific for *severe atopy* but also characterized "*food*" sensitization in PASTURE (**Figure E7**). However, a unique feature of *severe atopy* consisted in high levels of specific IgE to inhalant, particularly seasonal, allergens ($p<0.0001$, **Figure 5A, Figure E8**). This resulted from an excessive increment in sIgE levels in the first 3-4 years (and a milder trend in subsequent years) as compared to the weak rise in symptomatic and benign atopy, particularly for seasonal and food sIgE ($p<0.0001$, **Table 1**). Similarly *severe atopy* differed from the other LCs with respect to the ratio of IL-5 over IFN-γ expression, thereby reflecting the activation of T helper (Th) 2 rather than Th1 subsets ($p<0.01$, **Figure 5B**).

To elucidate the mutual relation between *severe atopy* and its various features differentiating it from the benign and symptomatic phenotypes, we performed a path analysis (**Figure 6**). In both studies, asthma was determined by *severe atopy* via an excessive increment in sIgE to seasonal allergens and high levels of sIgE at 6 years. Though including only 5% of all children, *severe atopy* explained 20%

of all sensitized asthma cases. Early sensitization to food allergens, Th2/Th1-ratio, and poly-sensitization were similarly determined directly or indirectly by *severe atopy*, but not related to asthma.

Similarly as in atopic individuals also in the entire population of both cohorts, the inverse association of sIgE levels and FEV1 was completely explained by *severe atopy* (change-in-estimate: 104%) as held partially true for the association of asthma and FEV1 (change-in-estimate: 38%). This was not unexpected since *severe atopy* contained also a substantial proportion of children without current wheeze or an established asthma diagnosis, but with FEV1 values within the lowest decile (**Figure 7**).

Discussion

Using LCA we classified preschool children for sensitization patterns considering the three dimensions allergen specificity, time course and strength of sensitization. The resulting LCs were related to manifest atopic disease with higher sensitivity and specificity as compared to classical definitions of sensitization. The food LCs of both cohorts emerged as a benign atopy phenotype without individual risk and family history of asthma. A symptomatic phenotype was found in the inhalant LCs with substantial risk of atopic diseases, but without impaired lung function. The LC "*severe atopy*" comprised children with high sIgE levels to seasonal allergens, much stronger associations with atopic diseases and low FEV1-values even in those without an established asthma diagnosis.

A major advantage of this analysis was the comprehensive approach covering the first 6 years of life with detailed information on various major allergen specificities at different levels. Missing values were successfully imputed thereby providing a complete dataset for 1446 children without observable selection from the originally recruited populations. A further strength was the replication of the main findings in two rather different birth cohorts.

Admittedly, not all LCs were fully congruent between the studies: the LC with mono-sensitization to "*cow's milk*" at low sIgE levels was specific for PASTURE and might be explained by the rather common consumption of cow's milk in this rural population. Correspondingly, in MAS a specific "*mite*" class emerged reflecting the relevance of this allergen in an urban cohort. An additional characteristic of MAS was the higher proportion of early sensitization to food allergens in LC "*severe atopy*" possibly resulting from the recruitment focus on children with elevated sIgE levels in cord blood. Nevertheless these peculiarities do not interfere with the core results of this analysis.

The role of specific IgE in the manifestation of atopic diseases has long been discussed controversially. In 1989, Burrows and colleagues suggested a linear relation between total IgE levels and asthma risk.[23] Ten years later, the question arose whether elevated total IgE levels were to some extent determined by specific wheeze phenotypes.[8] Soon thereafter, Illi et al. hypothesized that "an underlying condition drives both a certain pattern of sensitization and the development of childhood asthma."[11] Later-on the concept of multiplicity of sensitizations was introduced as a genuine risk factor for respiratory allergy.[13-19,28]

Against this background of conflicting hypotheses we sought a unifying concept. Without providing any information on atopic disease, an LCA merely based on time course and levels of sIgE against food and inhalant allergens yielded a clear trichotomy with respect to manifestation, severity, and family history of atopic disease in both cohorts. Using classical definitions of atopy such as sIgE to any food or any seasonal allergens, the respective associations were over- or underestimated, and the signals were diluted.

The detection of an innocent or benign atopy phenotype predominantly related to food sIgE is clinically relevant as it suggests that children with asthma allocated to one of the benign food LCs should not be considered 'atopic asthma' in epidemiologic studies. Rather these children might suffer from non-atopic asthma and concomitantly happen to produce irrelevant food sIgE as do many children without asthma.

Also the distinction between symptomatic and severe atopy has vast implications: Children with symptomatic atopy suffer from asthma, hay fever and eczema, though at lower risk and with a less severe phenotype as suggested by rather normal lung function parameters.

Severe atopy was characterized by specific and also unspecific features: With LC "*food*" in PASTURE *severe atopy* shared polyvalent sensitization to five or more allergens (**Figure E7**). Children with early food sensitization were allocated both to *severe atopy* and to the food LCs with similar absolute counts, though at different proportions. While early food sensitization might be seen as the first raised flag of *severe atopy*, it cannot serve as a specific predictor of this condition among sensitized children. A unique hallmark of *severe atopy*, however, was the elevated Th2/Th1 cytokine ratio at the age of 6 years (**Figure 5B**). This emerging dysbalance may result from an initial Th2 cell activation without subsequent resolution into "protective immunologic tolerance'' as suggested by Rowe et al.[29] Besides the specifically strong association with impaired lung function, *severe atopy* harbored a relevant proportion of children with FEV1 values in the lowest decile but without an established asthma diagnosis. In practical terms, this group of children might benefit from further clinical work-up and careful monitoring of sIgE increment within the first 3-4 years.

A further exclusive feature of *severe atopy* was found in high sIgE levels, which followed a steep rise in seasonal sensitization particularly before age 3-4. This sharp increase was the only relevant longitu-

dinal variation among the LCs and distinguished the current LCA for atopy from an earlier LCA for wheeze.[3] This earlier LCA was entirely determined by the time course of symptoms and produced a late-onset wheeze phenotype emerging only beyond age 3-4 years with strong associations to atopic sensitization, particularly *severe atopy* (**Figure E3**). In this context it is noteworthy that the steep increase in sIgE levels within *severe atopy* preceded the first symptoms of the atopic late-onset wheeze phenotype. This temporal relationship in combination with the strength and specificity of the association of *severe atopy* with asthma and impaired lung function and the consistency of the findings between both studies argues in favor of a causal relationship.

To corroborate this assumption we performed a path analysis contrasting *severe atopy* with benign and symptomatic atopy in regard to the above features. According to this analysis the effect of *severe atopy* on asthma was completely mediated through the steep increase in sIgE and the resulting high sIgE levels. As this steep increase was seen for all sIgE specificities in *severe atopy* (**Table 1**), one may hypothesize that excessive sIgE production is a generic phenomenon beyond any specific allergen.

This crucial role of uncontrolled sIgE production is indirectly supported by evidence from clinical studies showing an alleviating effect on childhood asthma symptoms by neutralizing sIgE with an anti-IgE antibody.[30,31] Reversely, the pathway model may provide a suitable explanation for the efficacy of anti-IgE treatment. Additionally *severe atopy,* or in practical terms a steep increase in sIgE until age 3-4, may serve as a selection criterion for children susceptible to anti-IgE therapy. As *severe atopy* explains at least every fifth case of atopic asthma a relevant share may profit from this therapeutic approach.

Moreover, *severe atopy* directly determined low FEV1 values and explained the inverse association of FEV1 and asthma, ultimately implying that poor lung function at age 6 years is not a feature of asthma unless it is related to *severe atopy*. In other words, poor lung function and excessive production of sIgE might result from the same latent phenomenon. This shared pathogenesis may point towards a local process of uncontrolled production of sIgE in the bronchial mucosa[32], which again might be the target of future interventions.

The other features of *severe atopy*, i.e. early food sensitization, an elevated Th2/Th1-ratio, and poly-sensitization emerged from the path analysis as epiphenomena without any proper effects on asthma risk. Rather they might hint at an authentic latent phenomenon, which manifests with many faces.

Integrating temporal patterns, allergen specificity and strength of sensitization in a data-driven approach we found three phenotypes of atopy with respect to disease-relevance. In contrast to benign and symptomatic atopy, *severe atopy* identified a circumscribed group of children with high sIgE values, pronounced disease risk, and poor lung function. *Severe atopy* as a latent phenomenon may thus correspond to the condition underlying both childhood asthma and sensitization patterns as previously postulated by Illi et al.[11] The path analysis performed in atopic individuals now suggests a link between *severe atopy* and asthma via excessive sIgE production particularly to seasonal allergens early in life and may direct further research into the biologic fundamentals of atopy.

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Tables

Table 1: Increment in sIgE production comparing *severe atopy* to the other atopy phenotypes

Time period	Study	Seasonal sIgE		Food sIgE		Perennial sIgE	
		ß (95% CI)	P	ß (95% CI)	P	ß (95% CI)	P
Early increase							
Year 0 – Year 1	**PASTURE**	0.05 (-0.28 - 0.39)	0.7491	0.69 (0.12 – 1.25)	**0.0175**	0.30 (-0.50 - 1.09)	0.4653
Year 1 – Year 3	**MAS**	4.28 (2.97 – 5.60)	**<.0001**	3.29 (1.93 – 4.66)	**<.0001**	0.62 (-0.78 – 2.02)	0.3843
Year 1 – Year 4	**PASTURE**	7.25 (6.32 – 8.18)	**<.0001**	2.45 (1.55 – 3.35)	**<.0001**	1.29 (0.22 – 2.37)	**0.0187**
Late increase							
Year 3 – Year 6	**MAS**	2.47 (0.84 – 4.10)	**0.0030**	3.78 (2.47 - 5.09)	**<.0001**	1.44 (0.01 – 2.87)	**0.0483**
Year 4 – Year 6	**PASTURE**	1.21 (-0.01 - 2.43)	0.0524	-0.27 (-1.09 – 0.55)	0.5193	1.06 (0.11 – 2.00)	**0.0290**
Overall increase							
Year 1 – Year 6	**MAS**	5.01 (3.33 – 6.68)	**<.0001**	4.65 (3.23 – 6.07)	**<.0001**	2.03 (0.09 – 3.97)	**0.0404**
Year 1 – Year 6	**PASTURE**	6.13 (4.99 – 7.27)	**<.0001**	1.17 (0.21 - 2.12)	**0.0163**	1.79 (0.66 - 2.92)	**0.0018**

The beta estimates result from linear regression of the log-transformed sIgE values on *severe atopy* vs. the other two atopy phenotypes within the respective time period, adjusted for baseline sIgE values. The estimates remained stable after mutual adjustment for incremental increase of the other specificities.

Figure Legends

Figure 1: Selection of study populations

Figure 2: Latent classes of atopy as characterized by allergen-specificity, time course, and levels of specific IgE

Figure 3: Associations of asthma-related conditions with latent classes and classical definitions of atopic sensitization at age 6

***** As there was no case of lifetime asthma in this LC, we calculated a conservative estimation of the odds ratio based on one case of asthma in this LC, which was simulated at random. Black point estimates with error bars mark the latent classes as reference, red the classical definitions as comparison.

Figure 4: Atopy phenotypes in relation to the distribution of latent classes in both populations

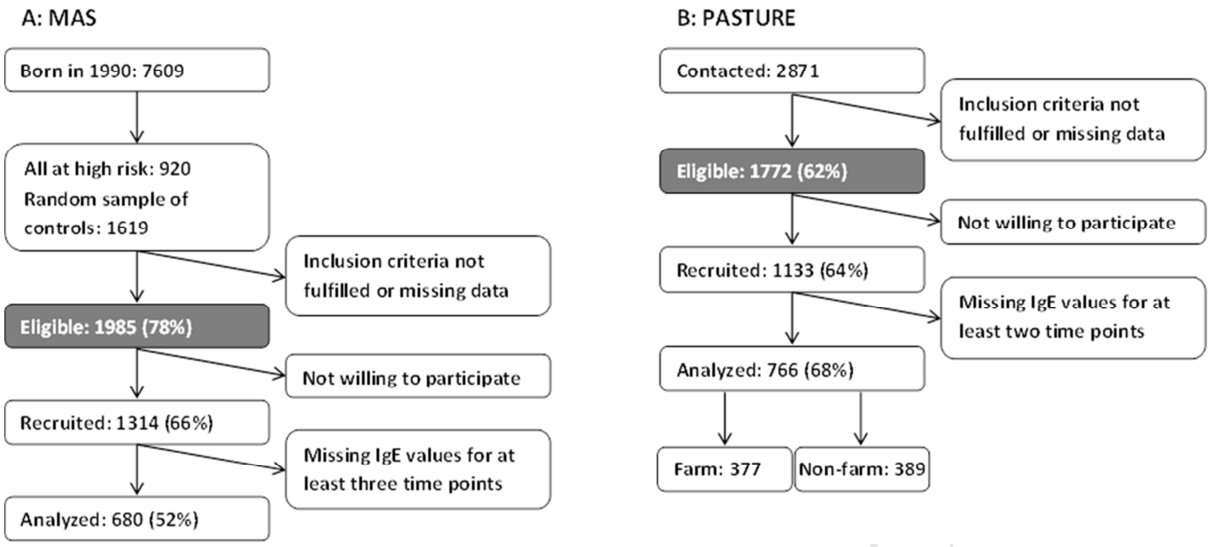
Figure 5: Absolute sIgE levels and ratio of IL-5 to IFN-γ expression at age 6

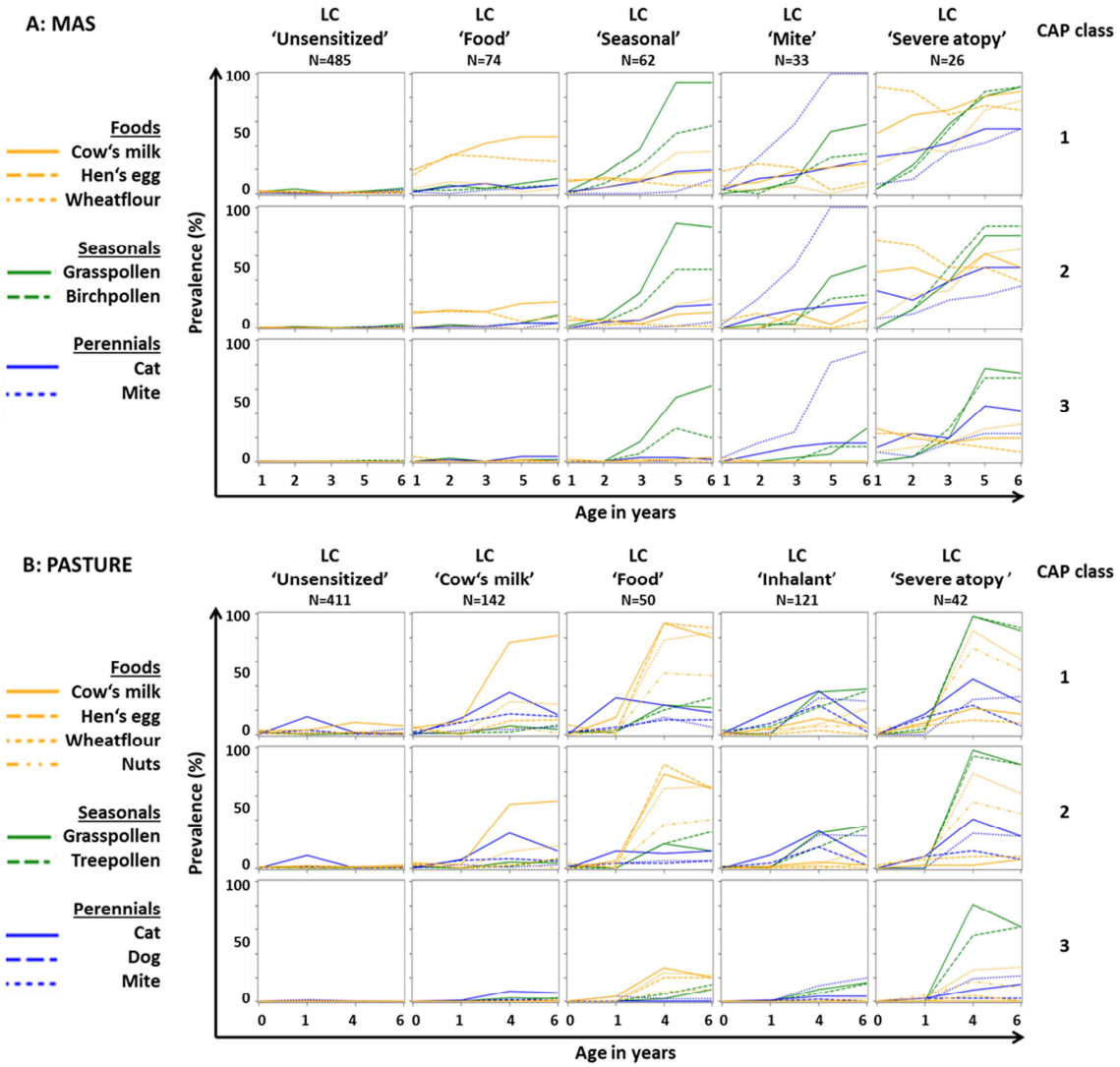
Figure 6: Path diagram comparing *severe atopy* to the other atopy phenotypes, including its features, lung function, and asthma in both populations

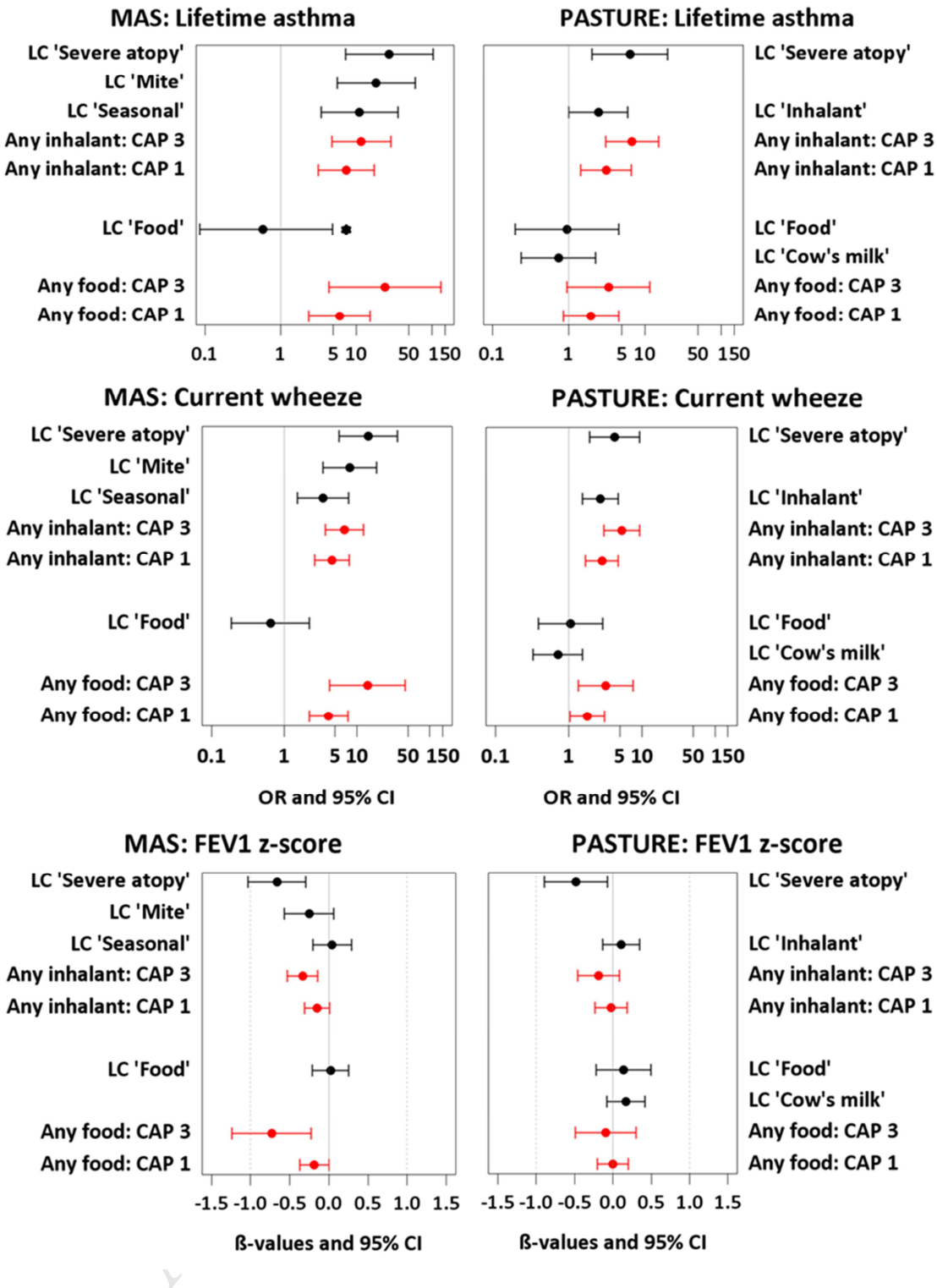
Significant associations are shown by solid arrows. Absent associations are represented by interrupted dotted arrows. The values represent the association estimates from the final model including significant paths only.

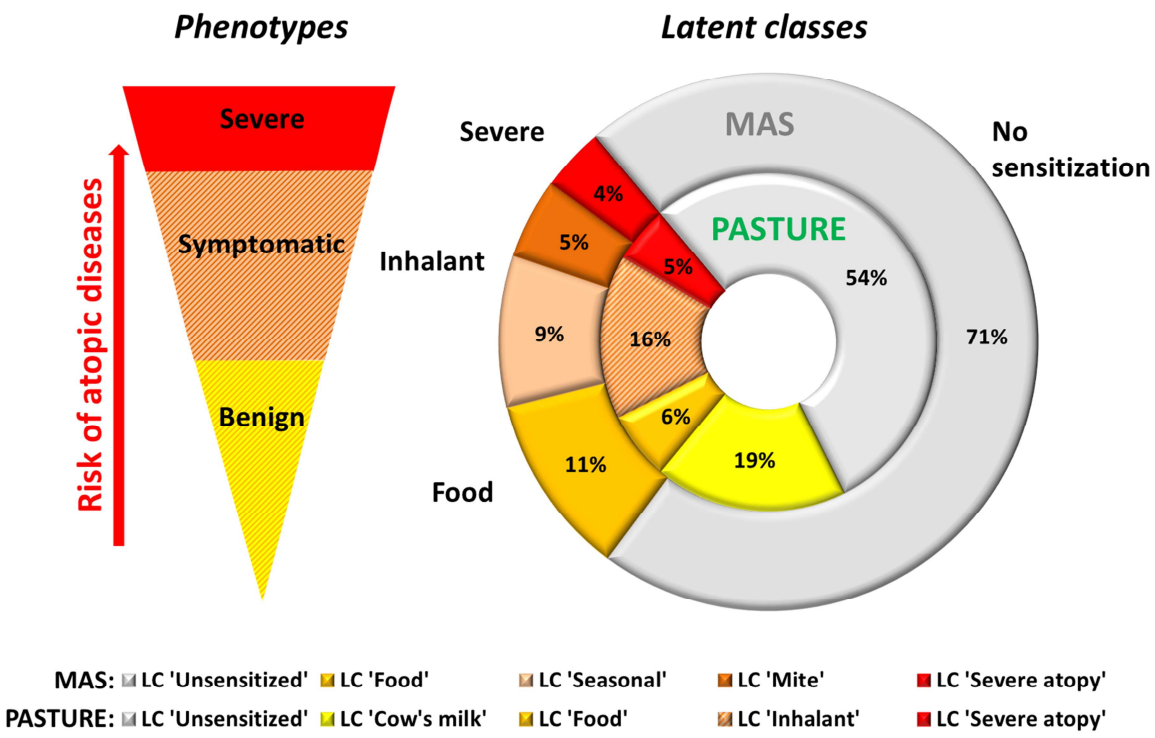
Figure 7: Proportion of non-asthmatic children with reduced lung function by latent classes

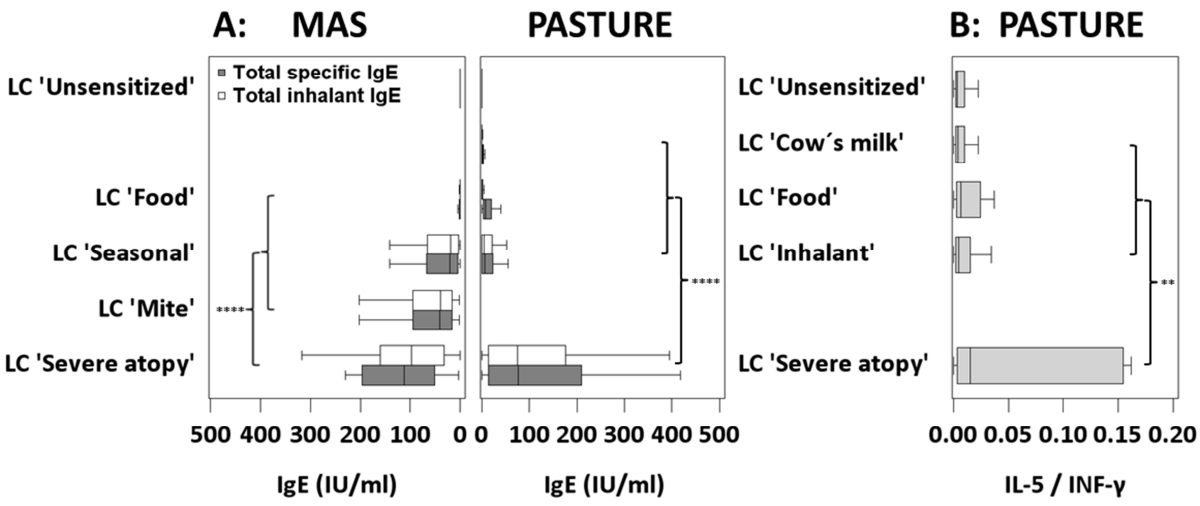
Reduced lung function was defined as values in the lowest decile of the FEV1 distribution.

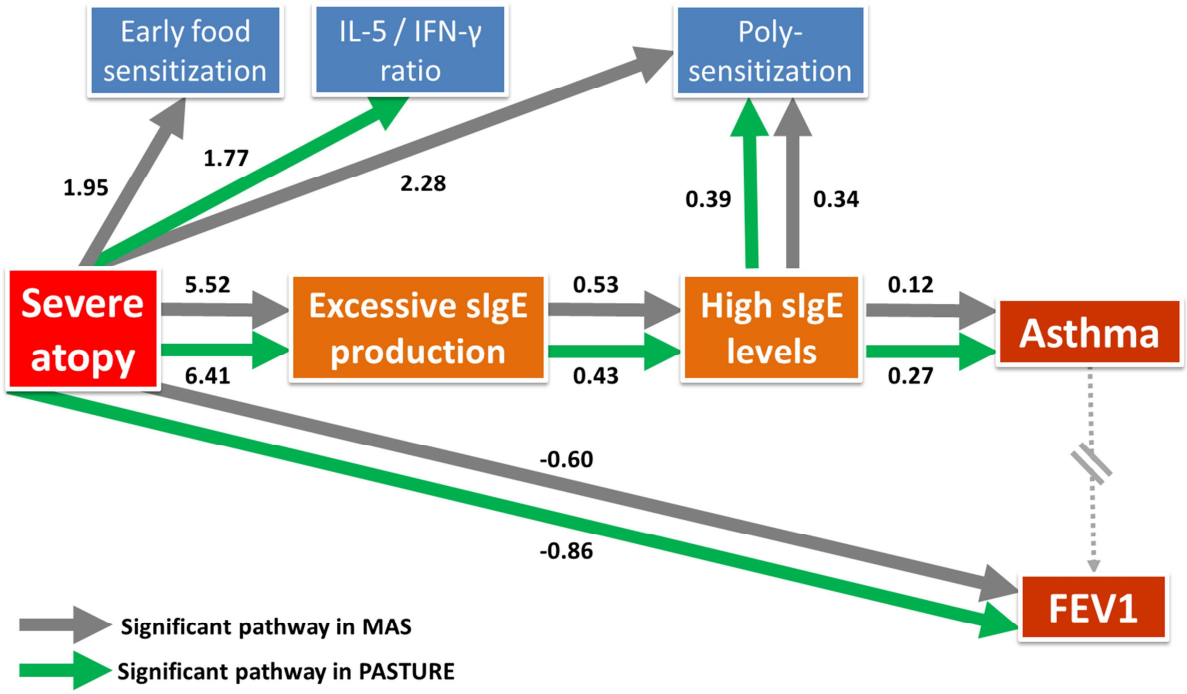


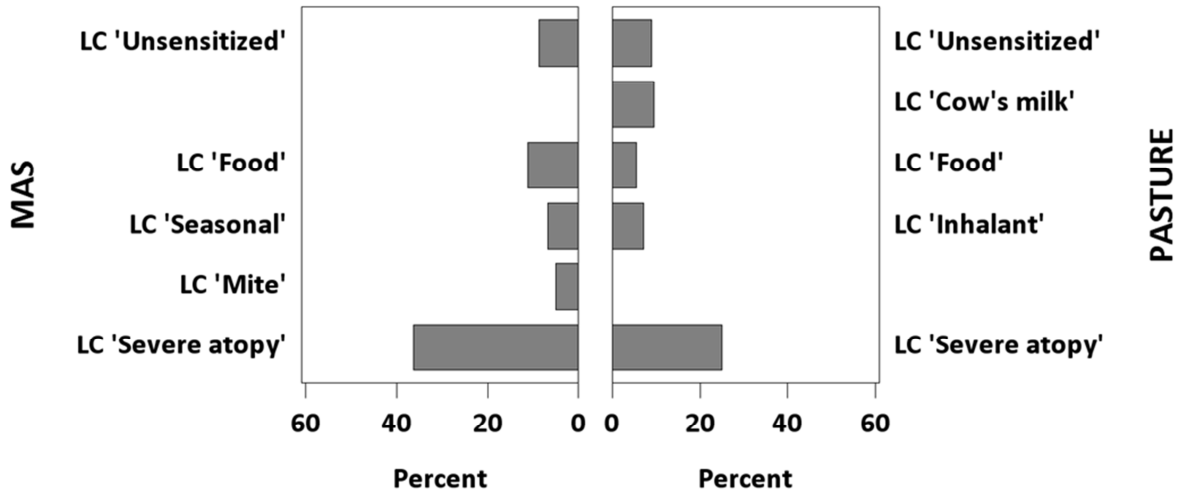

A: MAS
LC 'Unsensitized' N=485
LC 'Food' N=74
LC 'Seasonal' N=62
LC 'Mite' N=33
LC 'Severe atopy' N=26
CAP class
Foods
Cow's milk
Hen's egg
Wheatflour
Seasonals
Grasspollen
Birchpollen
Perennials
Cat
Mite
Prevalence (%)
Age in years
B: PASTURE
LC 'Unsensitized' N=411
LC 'Cow's milk' N=142
LC 'Food' N=50
LC 'Inhalant' N=121
LC 'Severe atopy' N=42
Foods
Cow's milk
Hen's egg
Wheatflour
Nuts
Seasonals
Grasspollen
Treepollen
Perennials
Cat
Dog
Mite
Prevalence (%)
Age in years


MAS: Lifetime asthma
LC 'Severe atopy'
LC 'Mite'
LC 'Seasonal'
Any inhalant: CAP 3
Any inhalant: CAP 1
LC 'Food'
Any food: CAP 3
Any food: CAP 1
0.1 1 5 10 50 150
PASTURE: Lifetime asthma
LC 'Severe atopy'
LC 'Inhalant'
Any inhalant: CAP 3
Any inhalant: CAP 1
LC 'Food'
LC 'Cow's milk'
Any food: CAP 3
Any food: CAP 1
0.1 1 5 10 50 150
MAS: Current wheeze
LC 'Severe atopy'
LC 'Mite'
LC 'Seasonal'
Any inhalant: CAP 3
Any inhalant: CAP 1
LC 'Food'
Any food: CAP 3
Any food: CAP 1
0.1 1 5 10 50 150
OR and 95% CI
PASTURE: Current wheeze
LC 'Severe atopy'
LC 'Inhalant'
Any inhalant: CAP 3
Any inhalant: CAP 1
LC 'Food'
LC 'Cow's milk'
Any food: CAP 3
Any food: CAP 1
0.1 1 5 10 50 150
OR and 95% CI
MAS: FEV1 z-score
LC 'Severe atopy'
LC 'Mite'
LC 'Seasonal'
Any inhalant: CAP 3
Any inhalant: CAP 1
LC 'Food'
Any food: CAP 3
Any food: CAP 1
-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5
ß-values and 95% CI
PASTURE: FEV1 z-score
LC 'Severe atopy'
LC 'Inhalant'
Any inhalant: CAP 3
Any inhalant: CAP 1
LC 'Food'
LC 'Cow's milk'
Any food: CAP 3
Any food: CAP 1
-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5
ß-values and 95% CI









Online Repository

Latent class analysis reveals clinically relevant atopy phenotypes in two birth cohorts

Alexander J. Hose, MA, MPH[1]; Martin Depner, PhD[1], Sabina Illi, PhD[1], Susanne Lau, MD, PhD[2], Thomas Keil, MD, MScPH[3,4], Ulrich Wahn, MD[2], Oliver Fuchs, MD, PhD[5,6,7], Petra Ina Pfefferle, PhD, DrPH[8], Elisabeth Schmaußer-Hechfellner, BSc[1], Jon Genuneit, MD[9], Roger Lauener, MD[10,11], Anne M. Karvonen, PhD[12], Caroline Roduit, MD[10,13], Jean-Charles Dalphin, MD, PhD[14], Josef Riedler, MD[15], Juha Pekkanen, MD, PhD[12,16], Erika von Mutius[5], MD; Markus J. Ege[5], MD, and the MAS and PASTURE study groups

MAS study group members:

Carl Peter Bauer[17], Johannes Forster[18], Fred Zepp[19], Volker Wahn[20], Antje Schuster[21], Renate L. Bergmann[22], Karl E. Bergmann[22], Andreas Reich[23], Linus Grabenhenrich[23]

PASTURE study group members:

Bianca Schaub[5], Georg J. Loss[1,24], Harald Renz[25], Michael Kabesch[26,27], Marjut Roponen[28], Anne Hyvärinen[12], Pekka Tiittanen[12], Sami Remes[29], Charlotte Braun-Fahrländer[30,31], Remo Frei[10,32], Vincent Kaulek[14], Marie-Laure Dalphin[33], Gert Doekes[34], Nicole Blümer[35], Urs Frey[7]

[1]Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich, Lindwurmstr. 4, D-80337, Munich, Germany

[2]Department for Pediatric Pneumology and Immunology, Charité–Universitätsmedizin Berlin, Berlin, Germany

[3]Institute of Social Medicine, Epidemiology and Health Economics, Charité–Universitätsmedizin Berlin, Berlin, Germany

[4]Institute for Clinical Epidemiology and Biometry, University of Wuerzburg, Wuerzburg, Germany

[5]Dr. von Hauner Children's Hospital, Ludwig-Maximilians-University of Munich, Lindwurmstr. 4, D-80337, Munich, Germany, and Comprehensive Pneumology Center, Munich (CPC-M), Germany; Member of the German Center for Lung Research (DZL)

[6]Division of Respiratory Medicine, Department of Pediatrics, Inselspital, University of Bern, Bern, Switzerland

[7]University Children's Hospital (UKBB), University of Basel, Spitalstr. 33, CH-4056 Basel, Switzerland

[8]Comprehensive Biomaterial Bank Marburg CBBM, Fachbereich Medizin der Philipps Universität Marburg, Zentrum für Tumor und Immunbiologie ZTI, Hans-Meerweinstr. 3, D-35043 Marburg, Germany; Member of the German Center for Lung Research

[9]Ulm University, Institute of Epidemiology and Medical Biometry, Helmholtzstr. 22, D-89081 Ulm, Germany

[10]Christine Kühne Center for Allergy Research and Education (CK-CARE), Davos, Switzerland

[11]Children's Hospital of Eastern Switzerland, St. Gallen, Switzerland

[12]Department of Health Protection, National Institute for Health and Welfare, P.O. Box 95 FIN-70701 Kuopio, Finland

[13]Children's Hospital, University of Zürich, Zürich, Switzerland

[14]University of Besançon, Department of Respiratory Disease, UMR/CNRS6249 Chrono-environment, University Hospital, 25030 Besançon, France

[15]Children's Hospital Schwarzach, Kardinal Schwarzenbergstr. 2-6, A-5620 Schwarzach, Austria, Teaching Hospital of Paracelsus Medical Private University Salzburg

[16]Department of Public Health, University of Helsinki, Helsinki, Finland

[17]Technical University of Munich, Department of Pediatrics, Munich, Germany

[18]Department of Paediatrics, St. Josefs Hospital, Freiburg, Germany

[19]Centre for Paediatric and Adolescent Medicine, University Medical Centre Mainz, Mainz, Germany

[20]University Children's Hospital, Charité, Berlin, Germany

[21]Department of Paediatrics, University Medical Centre Düsseldorf, Düsseldorf, Germany

[22]Department of Obstetrics, Charité-Universitätsmedizin Berlin, Berlin, Germany

[23]Institute of Social Medicine, Epidemiology and Health Economics, Charité-Universitätsmedizin Berlin, Berlin, Germany

[24]University of California, San Diego, School of Medicine, Department of Pediatrics, 9500 Gilman Drive, La Jolla, CA 92093-0831, U.S.A.

[25]Department of Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, Marburg, Germany; Member of the German Center for Lung Research

[26]KUNO Children's University Hospital Regensburg, Department of Pediatric Pneumology and Allergy Campus St. Hedwig, Steinmetzstr. 1-3, D-93049 Regensburg, Germany

[27]Hannover Medical School, Clinic for Pediatric Pneumology and Neonatology, Carl-Neuberg-Str. 1, D-30625 Hannover, Germany

[28]Department of Environmental Science, Inhalation Toxicology Laboratory, University of Eastern Finland, P.O Box 1627, Kuopio, Finland

[29]Department of Pediatrics, Kuopio University Hospital, Kuopio, Finland

[30]Swiss Tropical and Public Health Institute, Socinstr. 57, P.O. Box, CH-4002 Basel, Switzerland

[31]University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland

[32]Swiss Institute of Allergy and Asthma Research (SIAF), University of Zurich, Davos, Switzerland

[33]University of Besançon, Department of Pediatrics, University Hospital, 25030 Besançon, France

[34]Utrecht University, Institute for Risk Assessment Sciences (IRAS), Division of Environmental Epidemiology, PO Box 80178, 3508TD, Utrecht, Netherlands

[35]Department of Clinical Chemistry and Molecular Diagnostics, Philipps University of Marburg, Marburg, Germany

Corresponding author:

Alexander Hose, MA MPH

Dr. von Hauner Children's Hospital

Ludwig-Maximilians-University of Munich

Lindwurmstr. 4

D – 80337 München

Phone: +49(0)89 – 4400-57993

Fax: +49(0)89 – 4400-54452

E-mail: Alexander.Hose@med.uni-muenchen.de

Funding (MAS):

The MAS study was funded by grants from the German Federal Ministry of Education and Research (BMBF; reference numbers 07015633, 07 ALE 27, 01EE9405/5, 01EE9406) and the German Research Foundation (DFG; reference number KE 1462/2-1). The funders had no role in the design, management, data collection, analysis or interpretation of the data or in the writing of the manuscript or the decision to submit for publication.

Funding (PASTURE):

This work has been supported by the European Commission (research grants QLK4-CT-2001-00250, FOOD-CT-2006-31708, and KBBE-2007-2-2-06), the European Research Council (Grant 250268), and the German Center for Lung Research (DZL). The funding sources did not influence the study design; the collection, analysis, and interpretation of data; the writing of the manuscript; and the decision to submit the paper for publication. The corresponding author had full access to all of the data in this study and takes complete responsibility for the integrity of the data and the accuracy of the data analysis.

Acknowledgements (MAS):

We would like to thank all MAS contributors, especially the PIs of the study centres Carl Peter Bauer (Technische Universität München), Johannes Forster (St. Josefs Krankenhaus Freiburg), Walter Dorsch, Wolfgang Kamin, Fred Zepp (Mainz University Medical Center), Volker Wahn, Antje Schuster (Düsseldorf University Hospital), the co-initiators Karl Bergmann and Renate Bergmann as well as Bodo Niggemann, Petra Wagner and Gabi Schulz (Charité - Universitätsmedizin Berlin).

Methods

Statistical Analysis

Multiple imputation was based on continuous sIgE values of at least 4 of 6 time points in MAS (age 1-7 years) and 3 of 4 time points in PASTURE (age 0-6 years). Multiple linear imputation was performed in 20 runs resulting in 20 datasets for each cohort, then the continuous values for each dataset were transformed for MAS into 4 (<0.35, <0.7, <3.5, or ≥ 3.5 kU/L), for PASTURE into 5 (one additional CAP class <0.2 kU/L) ordinal CAP classes. At this step, data up to age 6 years were used in both studies for comparability. Finally, in MAS 35 4-staged variables representing 7 allergen specificities at 5 time points and in PASTURE 36 5-staged variables representing 9 allergen specificities at 4 time points were entered in the LCAs, which were performed for each of the 20 imputed dataset per cohort. For each LCA, individuals were assigned to classes by their highest posterior probabilities. Each subject was assigned to its definite latent class by the majority of the class memberships in 20 repeats. In addition, class membership was confirmed by visualizing sIgE prevalences in analogy to **Figure 2**.

Supplemental tables

Table E1: Selection of study population

		MAS						PASTURE					
		Not included		Included				Not included		Included			
Variable		**N**	**%**	**N**	**%**	**P**		**N**	**%**	**N**	**%**	**P**
Center 1	**Berlin**	316	49.84	278	40.88	**0.0011**	**Austria**	94	25.61	126	16.45	**0.0003**
Center 2	**Düsseldorf**	52	8.20	109	16.03	**0.0000**	**Switzerland**	84	22.89	158	20.63	0.3847
Center 3	**Mainz**	101	15.93	111	16.32	0.8466	**France**	46	12.53	157	20.50	**0.0011**
Center 4	**Freiburg**	100	15.77	108	15.88	0.9567	**Germany**	94	25.61	160	20.89	0.0743
Center 5	**Munich**	65	10.25	74	10.88	0.7106	**Finland**	49	13.35	165	21.54	**0.0010**
High risk group		229	37.06	253	37.76	0.7936		--	--	--	--	--
Farming		--	--	--	--	--		153	41.69	377	49.22	**0.0175**
Sex (female)		303	47.79	327	48.09	0.9144		161	49.39	369	48.30	0.7421
Family history of allergic disease		309	50.08	345	50.88	0.7726		174	51.63	417	54.72	0.3431
Maternal history of allergic disease		207	33.33	226	33.28	0.9850		110	30.05	261	34.07	0.1779
High parental education		362	88.08	531	87.05	0.6264		307	87.46	701	91.87	**0.0198**
At least 2 older siblings		76	11.99	89	13.11	0.5406		112	30.52	267	34.86	0.1475
Breastfeeding in 1st year		547	88.37	612	90.94	0.1292		256	89.20	689	90.90	0.4048
Environmental tobacco smoking		201	58.94	315	53.48	0.1062		32	17.88	56	8.20	**0.0001**
Doctor's diagnosed asthma at age 6		13	4.48	28	4.61	0.9305		4	2.27	36	5.28	0.0917
Sensitized to any allergen at birth (CAP class 1)		--	--	--	--	--		31	12.20	81	11.91	0.9024
Sensitized to any allergen at age 1 (CAP class 1)		28	13.66	90	16.70	0.3107		60	28.17	204	28.10	0.9841
Sensitized to any allergen at age 2 (CAP class 1)		36	24.49	131	25.49	0.8063		--	--	--	--	--
Sensitized to any allergen at age 3 (CAP class 1)		29	24.79	133	26.71	0.6713		--	--	--	--	--
Sensitized to any allergen at age 4 (CAP class 1)		--	--	--	--	--		19	59.38	396	57.81	0.8609
Sensitized to any allergen at age 5 (CAP class 1)		48	40.34	180	34.16	0.2026		--	--	--	--	--
Sensitized to any allergen at age 6 (CAP class 1)		42	42.42	160	37.74	0.3883		25	54.35	376	53.79	0.9415

Given are absolute numbers and percentages (in brackets). P-values are derived from chi-square tests. The two columns represent the entire population and the analysis population with complete sIgE data for the selected time points.

Table E2: Model fit of latent class analysis

Number of classes	AIC	Entropy
MAS		
3	7384 (7361 - 7406)	0.96 (0.95 - 0.96)
4	7172 (7151 - 7192)	0.95 (0.95 - 0.96)
5	**7064 (7044 - 7084)**	**0.97 (0.97 - 0.97)**
6	7067 (7047 – 7088)	0.96 (0.96 - 0.97)
PASTURE		
3	14632 (14474 - 14791)	**0.95 (0.94 - 0.96)**
4	14444 (14290- 14597)	0.92 (0.91- 0.94)
5	**14357 (14202 - 14511)**	0.93 (0.92 - 0.94)
6	14382 (14234 - 14530)	0.93 (0.91 - 0.95)

Mean values of AIC and entropy are given with 95%-confidence intervals for 20 imputed data sets.

Table E3: Prediction of latent sensitization classes by classical definitions of sensitization at age 6 – AUC of ROC analyses with 95% CIs

Latent classes	Any inhalant sensitization	Any food sensitization
MAS		
LC 'Food'	49.52 (44.34 - 54.69)	74.13 (68.58 - 79.68)
LC 'Seasonal'	90.10 (86.74 - 93.45)	65.48 (58.92 - 72.04)
LC 'Mite'	93.22 (91.55 - 94.89)	54.87 (47.00 - 62.73)
LC 'Severe atopy'	90.70 (86.20 - 95.20)	94.89 (90.56 - 99.23)
PASTURE		
LC 'Cow's milk'	53.92 (49.40 - 58.43)	81.78 (78.74 - 84.82)
LC 'Food'	57.85 (50.26 - 65.44)	91.98 (89.72 - 94.25)
LC 'Inhalant'	77.49 (72.87 - 82.11)	55.45 (50.75 - 60.15)
LC 'Severe atopy'	90.67 (87.17 - 94.16)	76.08 (68.24 - 83.91)

Supplemental figure legends

Figure E1: Distribution of latent classes across study centers

Figure E2: Prediction of latent classes by classical definitions of sensitization at age 6

The dots mark the sensitization statuses (from right to left: Unsensitized, CAP class 1-3)

Figure E3: Associations of health conditions with latent classes and classical definitions of atopic sensitization at age 6

***** As there was no case of late onset wheeze in this LC, we calculated a conservative estimation of the odds ratio based on one case of late onset wheeze in this LC, which was simulated at random.

Black point estimates with error bars mark the latent classes as reference, red the classical definitions as comparison.

"Late onset wheeze" was defined as described at Depner M, Fuchs O, Genuneit J, Karvonen AM, Hyvarinen A, Kaulek V, et al. Clinical and epidemiologic phenotypes of childhood asthma. Am J Respir Crit Care Med 2014; 189:129-38.

Figure E4: Associations of latent classes with parental atopy

Figure E5: Sensitivity analyses omitting single dimensions of LCA

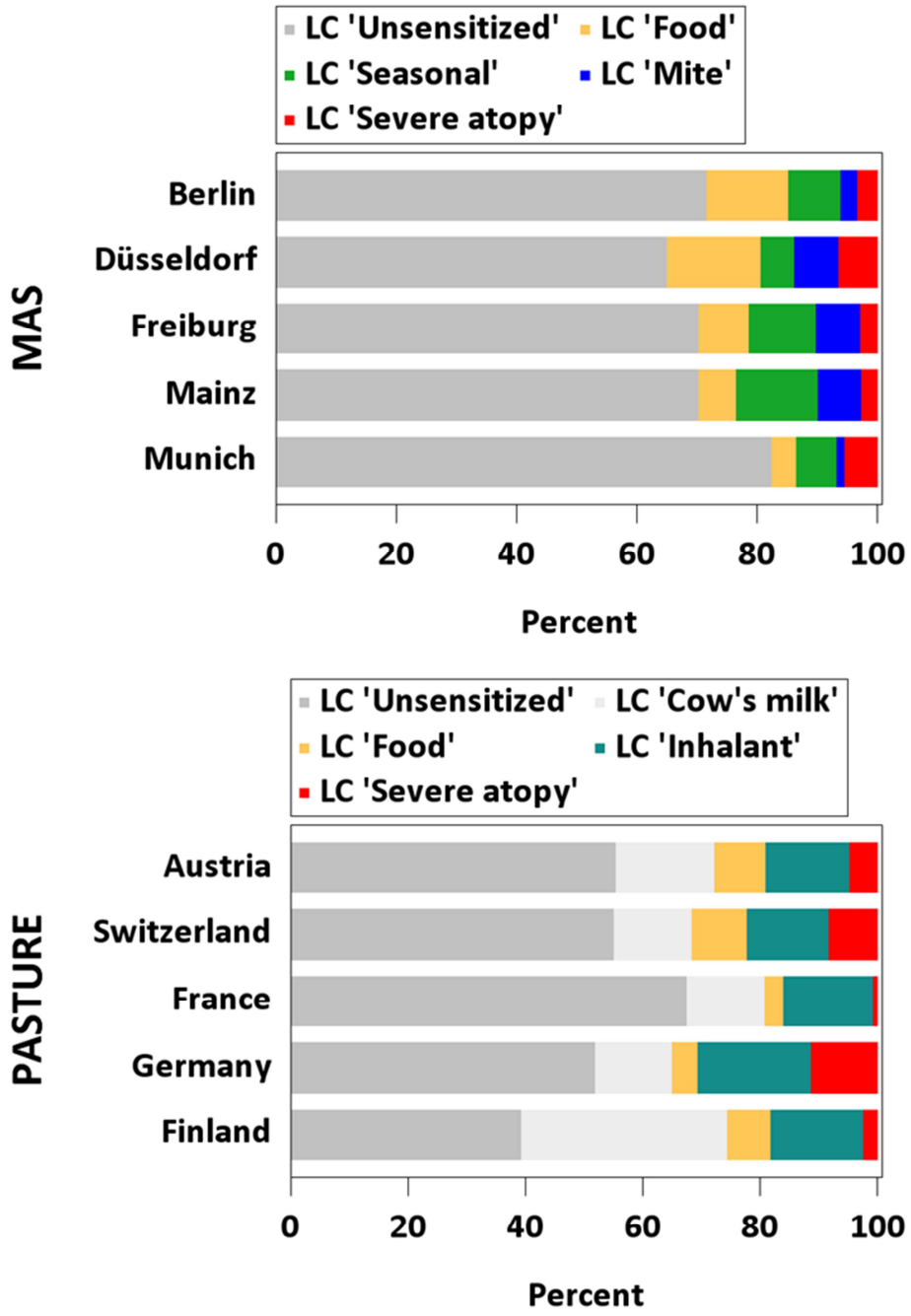
Figure E6: Comparing disease associations across all sensitivity analyses

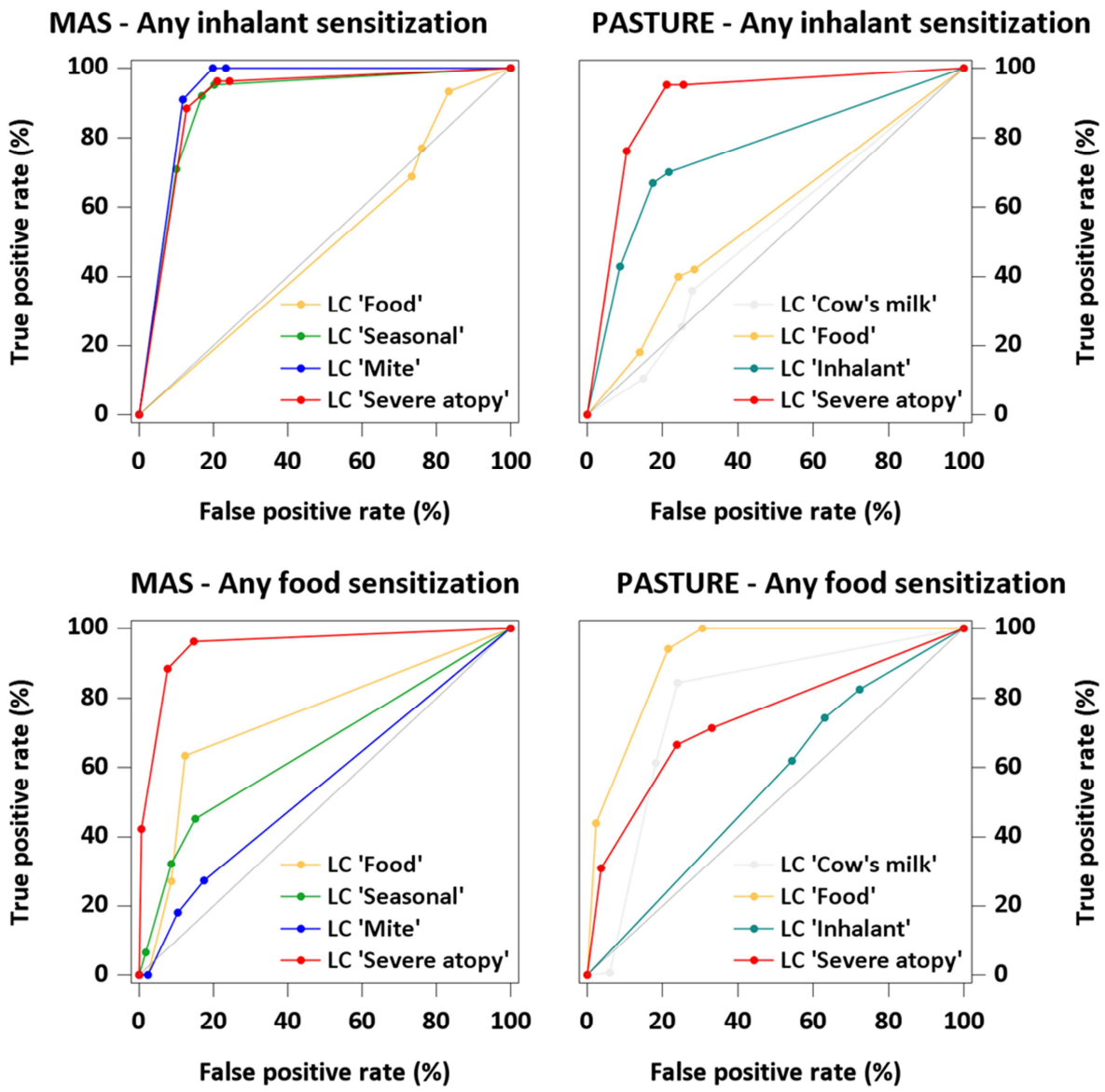
***** As there was no case of lifetime asthma in this LC, we calculated a conservative estimation of the odds ratio based on one case of asthma in this LC, which was simulated at random.

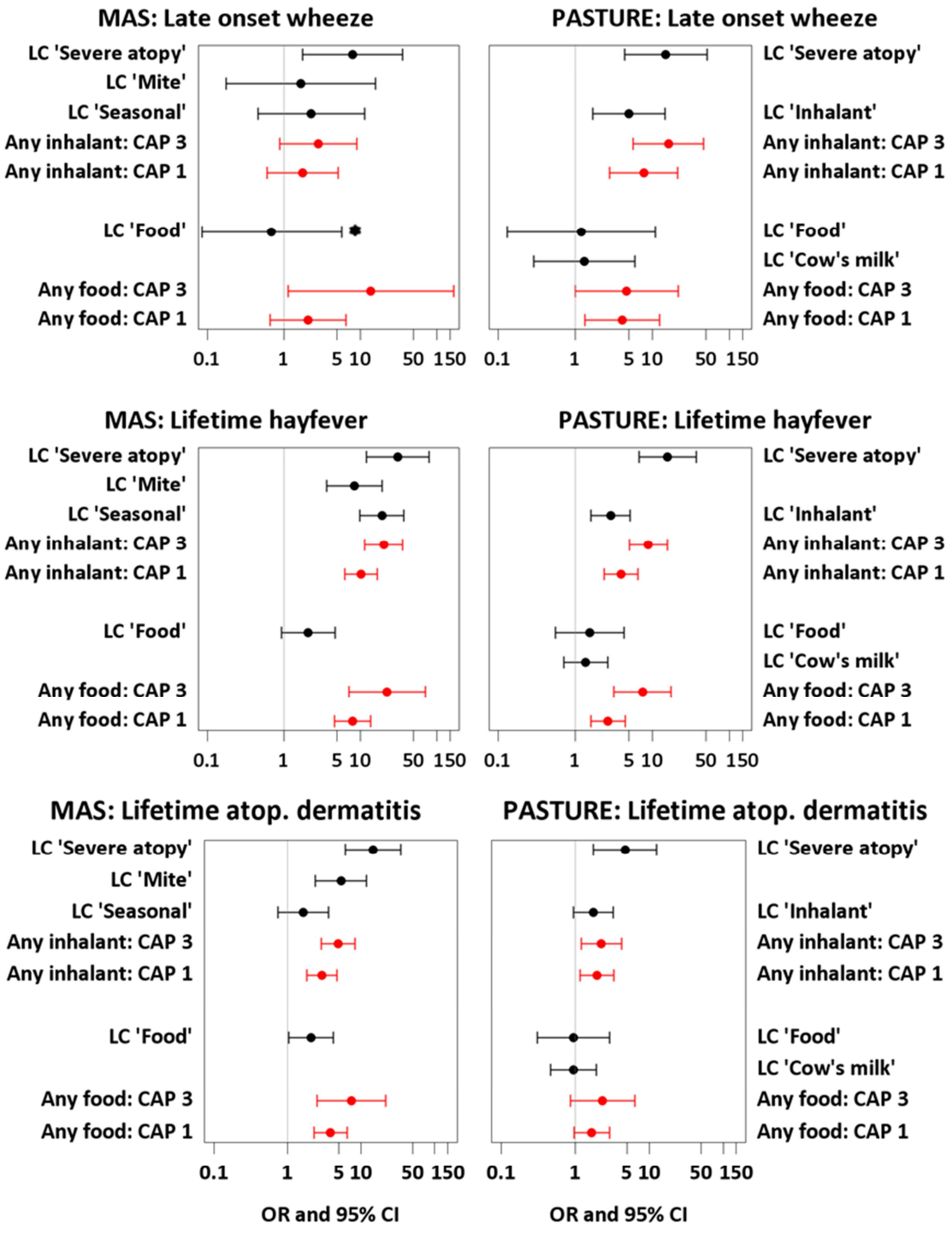
Black point estimates with error bars mark the latent classes as reference, red the classical definitions as comparison.

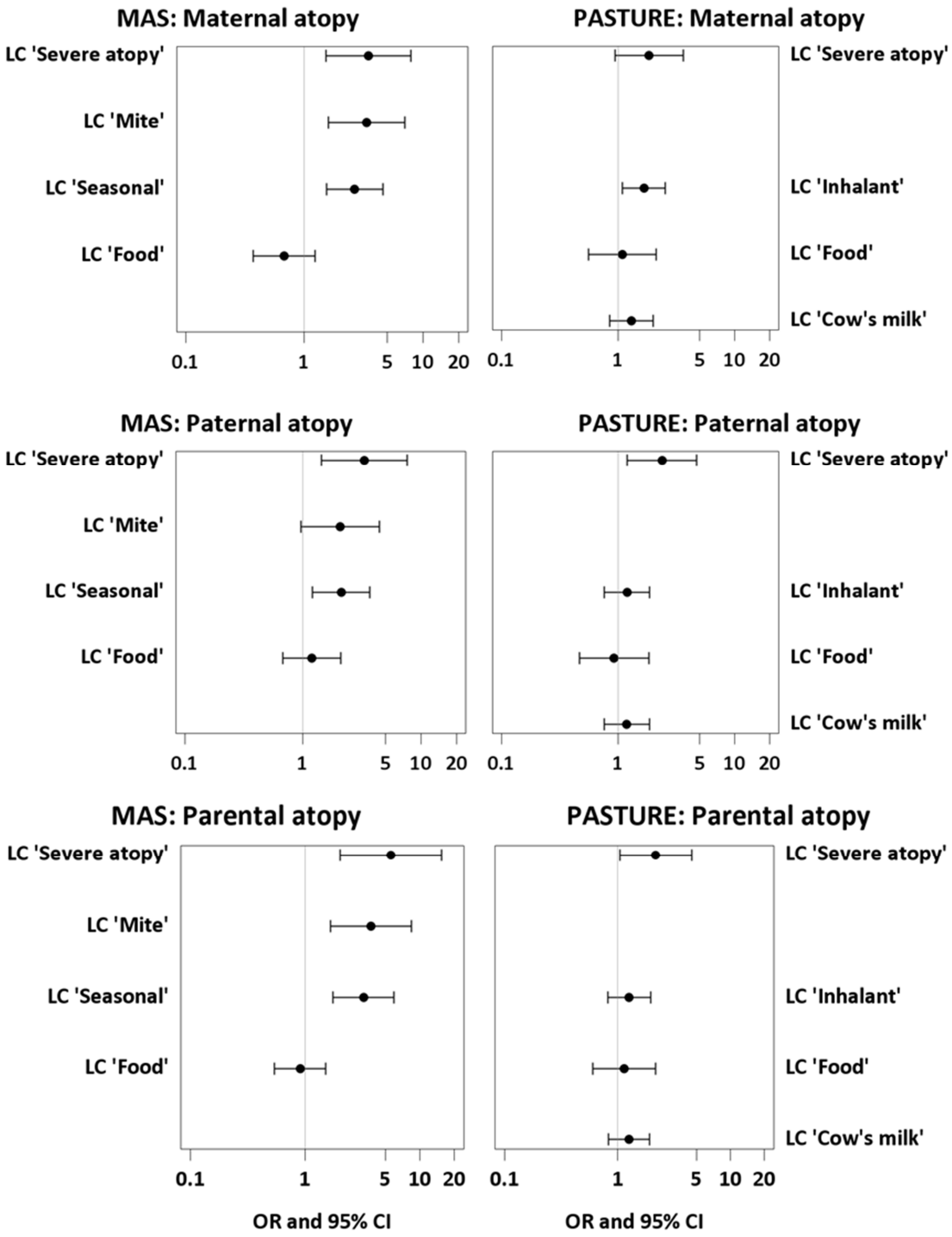
Figure E7: Number of sensitizations to different allergen specificities across latent classes (CAP classes 1-3)

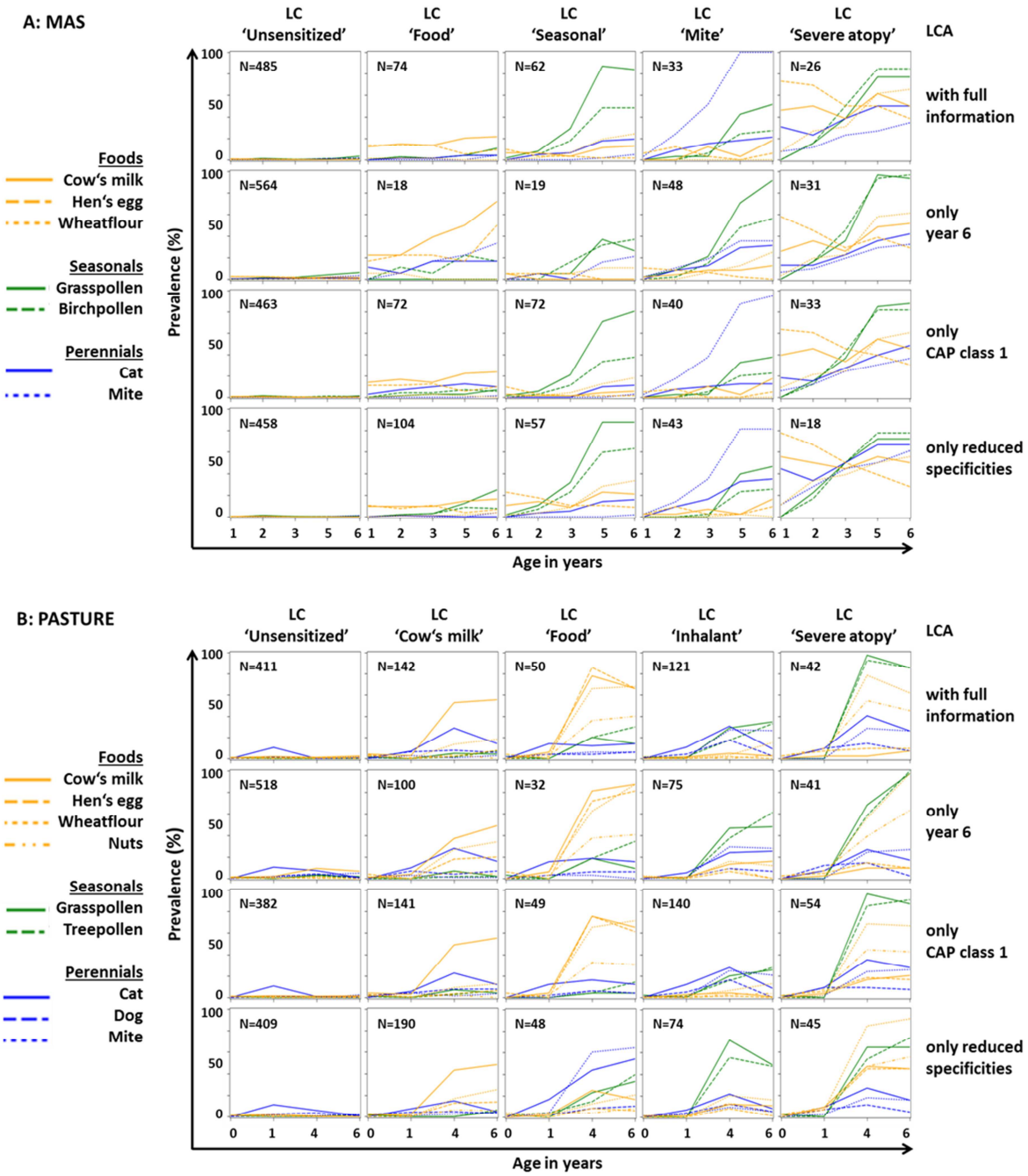
Figure E8: Absolute sIgE levels at age 6

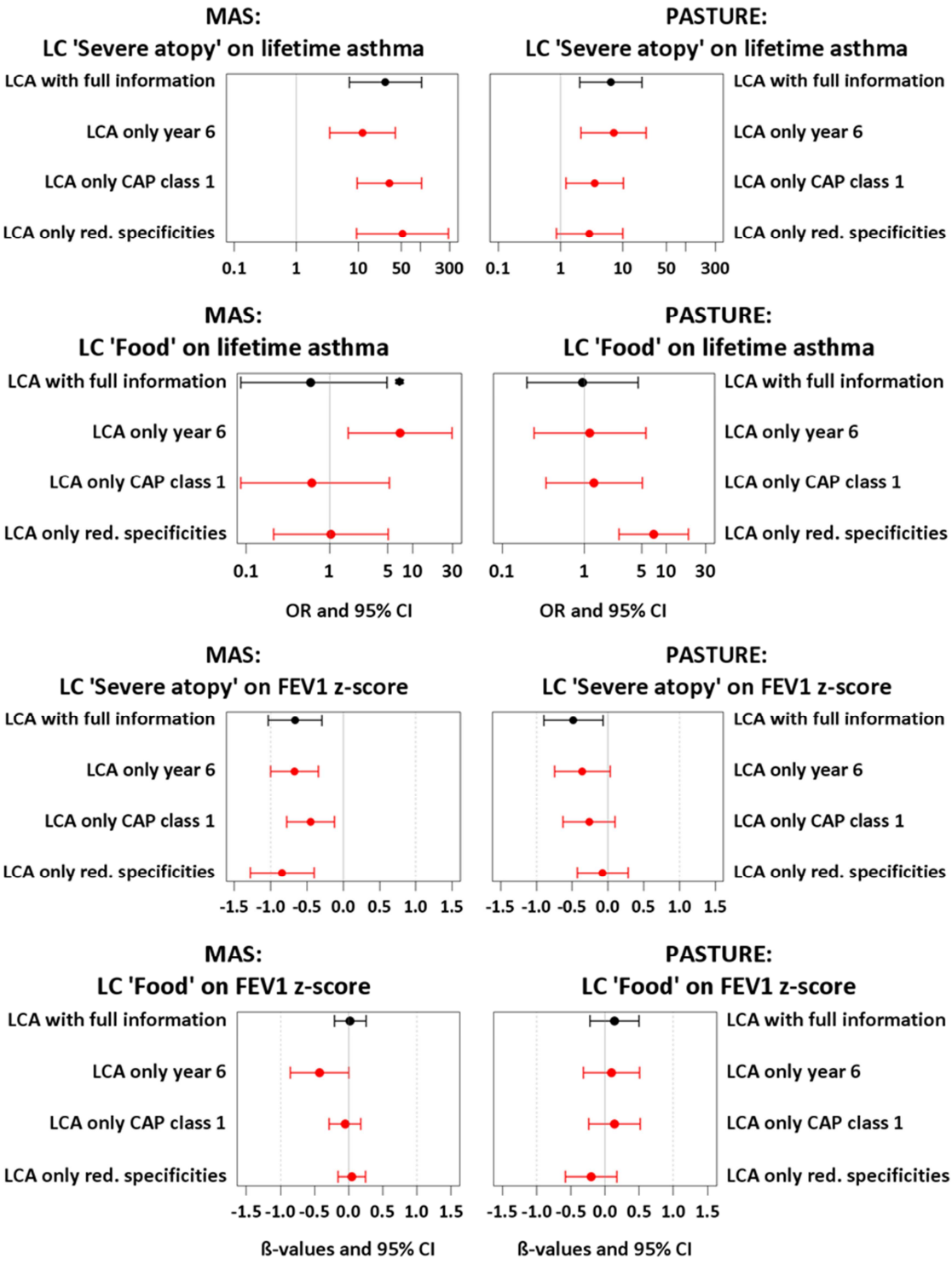

LC 'Unsensitized'
LC 'Food'
LC 'Seasonal'
LC 'Mite'
LC 'Severe atopy'
MAS
Berlin
Düsseldorf
Freiburg
Mainz
Munich
0
20
40
60
80
100
Percent
LC 'Unsensitized'
LC 'Cow's milk'
LC 'Food'
LC 'Inhalant'
LC 'Severe atopy'
PASTURE
Austria
Switzerland
France
Germany
Finland
0
20
40
60
80
100
Percent

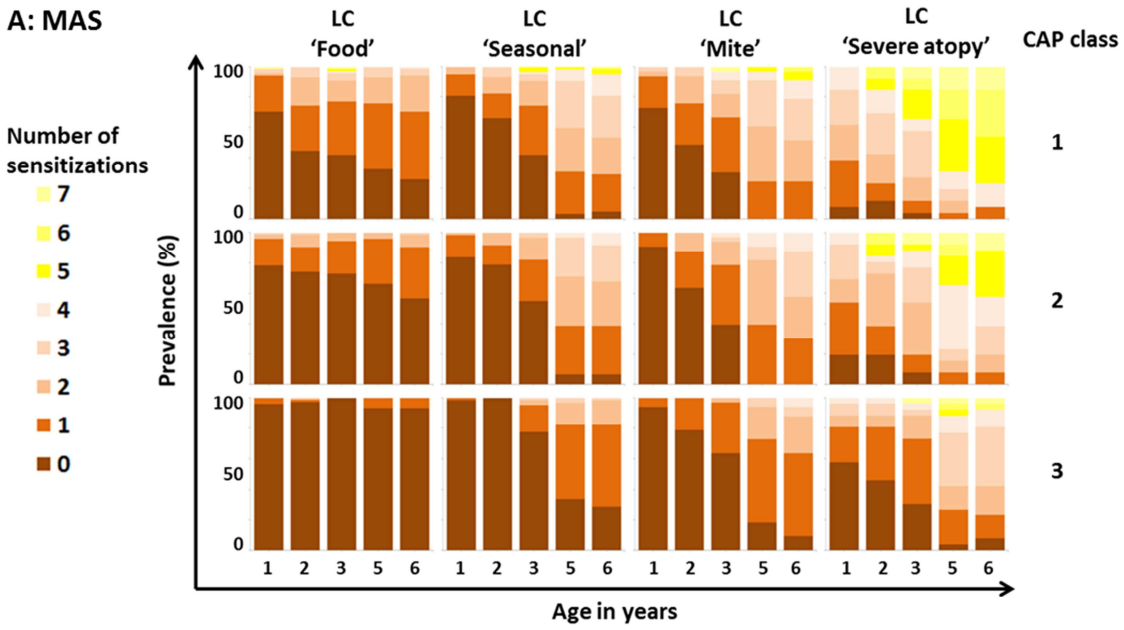

MAS - Any inhalant sensitization
True positive rate (%)
False positive rate (%)
LC 'Food'
LC 'Seasonal'
LC 'Mite'
LC 'Severe atopy'
PASTURE - Any inhalant sensitization
LC 'Cow's milk'
LC 'Food'
LC 'Inhalant'
LC 'Severe atopy'
MAS - Any food sensitization
PASTURE - Any food sensitization
0
20
40
60
80
100

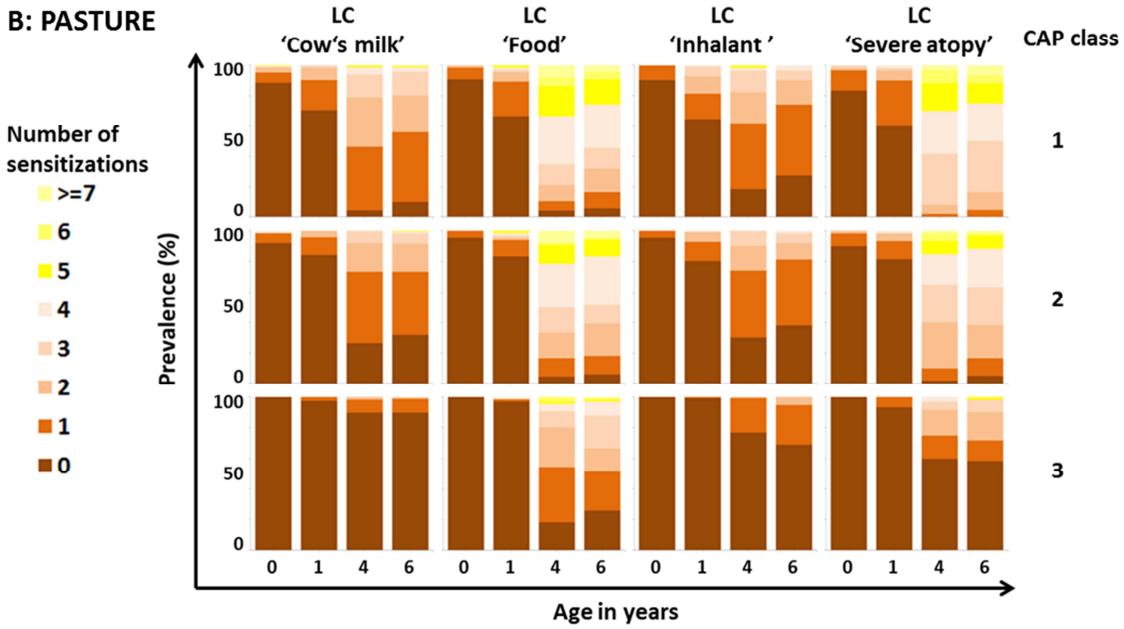

MAS: Late onset wheeze
LC 'Severe atopy'
LC 'Mite'
LC 'Seasonal'
Any inhalant: CAP 3
Any inhalant: CAP 1
LC 'Food'
Any food: CAP 3
Any food: CAP 1
0.1
1
5 10
50 150
PASTURE: Late onset wheeze
LC 'Severe atopy'
LC 'Inhalant'
Any inhalant: CAP 3
Any inhalant: CAP 1
LC 'Food'
LC 'Cow's milk'
Any food: CAP 3
Any food: CAP 1
MAS: Lifetime hayfever
PASTURE: Lifetime hayfever
MAS: Lifetime atop. dermatitis
PASTURE: Lifetime atop. dermatitis
OR and 95% CI
OR and 95% CI


MAS: Maternal atopy
LC 'Severe atopy'
LC 'Mite'
LC 'Seasonal'
LC 'Food'
0.1
1
5
10
20
PASTURE: Maternal atopy
LC 'Severe atopy'
LC 'Inhalant'
LC 'Food'
LC 'Cow's milk'
0.1
1
5
10
20
MAS: Paternal atopy
LC 'Severe atopy'
LC 'Mite'
LC 'Seasonal'
LC 'Food'
0.1
1
5
10
20
PASTURE: Paternal atopy
LC 'Severe atopy'
LC 'Inhalant'
LC 'Food'
LC 'Cow's milk'
0.1
1
5
10
20
MAS: Parental atopy
LC 'Severe atopy'
LC 'Mite'
LC 'Seasonal'
LC 'Food'
0.1
1
5
10
20
OR and 95% CI
PASTURE: Parental atopy
LC 'Severe atopy'
LC 'Inhalant'
LC 'Food'
LC 'Cow's milk'
0.1
1
5
10
20
OR and 95% CI


A: MAS
LC 'Unsensitized'
LC 'Food'
LC 'Seasonal'
LC 'Mite'
LC 'Severe atopy'
LCA
with full information
only year 6
only CAP class 1
only reduced specificities
Foods
Cow's milk
Hen's egg
Wheatflour
Seasonals
Grasspollen
Birchpollen
Perennials
Cat
Mite
Prevalence (%)
Age in years
N=485
N=74
N=62
N=33
N=26
N=564
N=18
N=19
N=48
N=31
N=463
N=72
N=72
N=40
N=33
N=458
N=104
N=57
N=43
N=18
B: PASTURE
LC 'Unsensitized'
LC 'Cow's milk'
LC 'Food'
LC 'Inhalant'
LC 'Severe atopy'
LCA
with full information
only year 6
only CAP class 1
only reduced specificities
Foods
Cow's milk
Hen's egg
Wheatflour
Nuts
Seasonals
Grasspollen
Treepollen
Perennials
Cat
Dog
Mite
Prevalence (%)
Age in years
N=411
N=142
N=50
N=121
N=42
N=518
N=100
N=32
N=75
N=41
N=382
N=141
N=49
N=140
N=54
N=409
N=190
N=48
N=74
N=45


MAS:
LC 'Severe atopy' on lifetime asthma
LCA with full information
LCA only year 6
LCA only CAP class 1
LCA only red. specificities
0.1
1
10
50
300
PASTURE:
LC 'Severe atopy' on lifetime asthma
LCA with full information
LCA only year 6
LCA only CAP class 1
LCA only red. specificities
0.1
1
10
50
300
MAS:
LC 'Food' on lifetime asthma
LCA with full information
LCA only year 6
LCA only CAP class 1
LCA only red. specificities
0.1
1
5
10
30
OR and 95% CI
PASTURE:
LC 'Food' on lifetime asthma
LCA with full information
LCA only year 6
LCA only CAP class 1
LCA only red. specificities
0.1
1
5
10
30
OR and 95% CI
MAS:
LC 'Severe atopy' on FEV1 z-score
LCA with full information
LCA only year 6
LCA only CAP class 1
LCA only red. specificities
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
PASTURE:
LC 'Severe atopy' on FEV1 z-score
LCA with full information
LCA only year 6
LCA only CAP class 1
LCA only red. specificities
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
MAS:
LC 'Food' on FEV1 z-score
LCA with full information
LCA only year 6
LCA only CAP class 1
LCA only red. specificities
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
ß-values and 95% CI
PASTURE:
LC 'Food' on FEV1 z-score
LCA with full information
LCA only year 6
LCA only CAP class 1
LCA only red. specificities
-1.5
-1.0
-0.5
0.0
0.5
1.0
1.5
ß-values and 95% CI


A: MAS
LC 'Food'
LC 'Seasonal'
LC 'Mite'
LC 'Severe atopy'
CAP class
Number of sensitizations
7
6
5
4
3
2
1
0
Prevalence (%)
100
50
0
1
2
3
1 2 3 5 6 1 2 3 5 6 1 2 3 5 6 1 2 3 5 6
Age in years


B: PASTURE
LC 'Cow's milk'
LC 'Food'
LC 'Inhalant '
LC 'Severe atopy'
CAP class
Number of sensitizations
>=7
6
5
4
3
2
1
0
Prevalence (%)
100
50
0
1
2
3
0 1 4 6 0 1 4 6 0 1 4 6 0 1 4 6
Age in years