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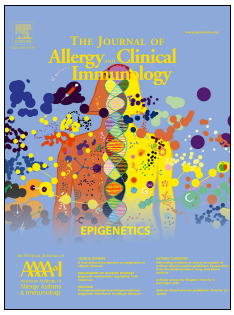

Development Of Atopic Sensitization In Finnish And Estonian Children – A Latent Class Analysis In A Multicenter Cohort

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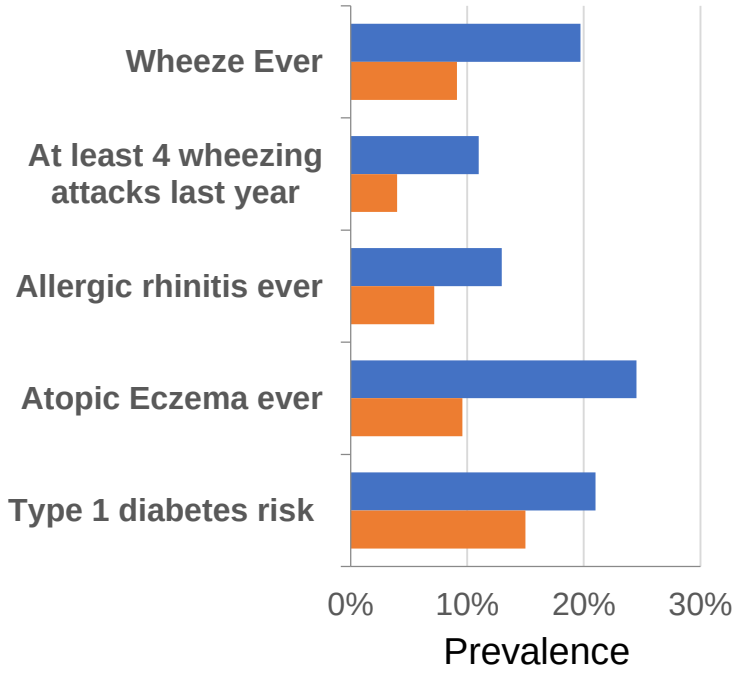
3-5 years old



DEVELOPMENT OF ATOPIC SENSITIZATION IN FINNISH AND ESTONIAN CHILDREN – A LATENT CLASS ANALYSIS IN A MULTICENTER COHORT

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Running Head: Atopic Sensitization in Finnish and Estonian children

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CONFLICT OF INTEREST

Dr. Weber reports grants from European Commission, during the conduct of the study. Dr. von Mutius reports grants from European Commission, during the conduct of the study; personal fees from Pharma Ventures, personal fees from Peptinnovate Ltd., personal fees from OM Pharma SA, personal fees from Boehringer Ingelheim, personal fees from European Commission/European Research Council Executive Agency, personal fees from The Chinese University of Hong Kong, personal fees from University of Tampere/Tampereen Yliopisto, personal fees from Utrecht Universiteit , personal fees from University of Turku/Turun Yliopisto, personal fees from HAL Allergie GmbH, personal fees from Ökosoziales Forum Oberösterreich, personal fees from Mundipharma Deutschland GmbH & Co. KG, personal fees from University of Helsinki/Helsingin yliopisto, outside the submitted work.

The remaining authors have nothing to disclose.

ABSTRACT

BACKGROUND

The prevalence of atopy is associated with a western lifestyle as illustrated by studies comparing neighboring regions with different socioeconomic backgrounds. Atopy might reflect various conditions differing in their susceptibility to environmental factors.

OBJECTIVE

To define phenotypes of atopic sensitization in early childhood and to examine their association with allergic diseases and hereditary background in Finland and Estonia

METHODS

The analysis included 1603 Finnish and 1657 Estonian children from the DIABIMMUNE multicenter young children cohort. Specific IgE levels were measured at age 3, 4 and 5 respectively, and categorized into three CAP classes. Latent Class Analysis (LCA) was performed with the statistic software package poLCA in R.

RESULTS

Both populations differed in terms of socioeconomic status and environmental determinants such as pet ownership, farm-related exposure, time of playing outdoors and prevalence of allergic diseases (all p-values <0.001). Nevertheless, we found similar latent classes (LC) in both populations: an unsensitized class, a food class, two inhalant classes differentiating between seasonal and perennial aero-allergens, and a severe atopy class. The latter was characterized by high total and specific IgE levels and strongly associated with wheeze (odds ratio 5.64 [3.07-10.52] and 4.56 [2.35-8.52]), allergic rhinitis (22.4 [11.67-44.54] and 13.97 [7.33-26.4]) and atopic eczema (9.39 [4.9-19.3] and 9.5 [5.2-17.5], for Finland and Estonia, respectively). Environmental differences were reflected in the larger seasonal inhalant atopy class in Finland though composition of classes was comparable between countries.

CONCLUSION

Despite profound differences in environmental exposures there may exist genuine patterns of atopic sensitization. The distribution of these patterns may determine the contribution of atopic sensitization to disease onset.

KEY MESSAGE

- Previously identified latent classes of atopy were replicated in Finland and Estonia, two countries with different environmental exposures.
- The inhalant atopy classes were related to atopic diseases with the strongest association for the highly sensitized severe atopy class.
- The differential associations of LC with allergic disease and genetic T1D susceptibility might point towards distinct immunologic mechanisms linking the various forms of atopy to allergy and autoimmunity.

CAPSULE SUMMARY

In two countries differing fundamentally in environmental and socioeconomic determinants and sensitization rates, similar atopy classes were found regarding composition and disease relevance.

KEY WORDS

Latent Class Analysis, unsupervised clustering, IgE, atopy, allergy, diabetes type 1 risk, wheezing, Finland, Estonia, severe atopy

WORD COUNT

3,364

ABBREVIATIONS

AIC	Akaike Information Criterion
BIC	Bayesian Information Criterion
CAP	Carrier polymer system
CL	Confidence Limit
DMT1	Diabetes mellitus type 1
IgE	Immunoglobulin E
sIgE	Specific Immunoglobulin E
tIgE	Total Immunoglobulin E
ISAAC	International Study of Asthma and Allergies in Childhood
kU/L	Kilo units per liter
LC	Latent Class
LCA	Latent Class Analysis
MAS	Multizentrische Allergiestudie
OR	Odds Ratio
PASTURE	Protection Against Allergy: Study in Rural Environments
SES	Socioeconomic status
T_{H1}	T helper 1 cells
T_{H2}	T helper 2 cells

INTRODUCTION

Allergic diseases are more prevalent in industrialized countries as compared to less affluent countries.[1] These differences are most prominent in neighboring areas,[2] which might be perceived as "living laboratories" in the analysis of atopic diseases.[3] A prominent example presents Karelia, an area covering a Finnish and a Russian part, which differ strongly in atopy prevalence and standard of living. Another example for such an experiment "by nature" might be seen in the forty years of separation between East and West Germany, where the inner-German border constituted a gradient in standard of living. The prevalence of atopy was significantly lower in the Eastern part, but caught up with West Germany within a decade after reunification.[4, 5] Besides atopy, also autoimmune disorders, such as type 1 diabetes mellitus (T1D), are on the rise in Western countries.[6] For instance, a remarkably higher incidence of T1D was observed in Finnish school children under the age of 15 years as compared to the incidence in Russian Karelia.[7] In addition, a genetic predisposition has been proposed for T1D, which was encoded predominantly by HLA loci.[8]

Atopy is known to be associated with allergic[9-12] and autoimmune[13-15] diseases. As these conditions emerge from different pathologies, the question arises whether they relate to different aspects of atopy and how these aspects can be disentangled. A variety of studies have sought to apply various classifications to atopy, e.g. by concentrations of allergen specific IgE, mono- versus polyvalent sensitization, or time course.[9, 11, 16-21] In our hands, latent class analysis (LCA) proved to be a suitable instrument for integrating the three dimensions allergen specificity, levels of specific IgE (sIgE), and time course in a data-driven approach, thereby being largely immune from investigator bias.[12] This methodology was used in PASTURE and MAS to cluster preschool children into 3 atopy phenotypes with respect to disease relevance. The severe atopy phenotype was strongly related to respiratory allergy and impaired lung function; the symptomatic phenotype included the inhalant classes and was associated with respiratory allergy to a lower extent and unrelated to lung function impairment. Finally, a benign atopy phenotype covered the food classes and was not associated to any allergic disease.[12] We hypothesized that with this classification we might be able to understand, which atopy types were most susceptible to environmental influences or family background of allergy and autoimmunity.

Therefore, the aim of this study was to replicate the previous LCA findings from two birth cohorts in the young children cohort of the DIABIMMUNE study (www.diabimmune.org) and to use the unique setting for a direct comparison of atopy phenotypes and their hereditary background between Finland and Estonia, two countries separated by the Baltic Sea and with different socioeconomic and environmental developments over many decades and striking differences in atopy rates.

METHODS

STUDY DESIGN

The DIABIMMUNE study was performed in urban or suburban areas of Tartu in Estonia and Espoo in Finland. Families with 3 year old children born between January 2006 and July 2008 were identified from population registers and invited for participation. Between September 2009 and July 2011, 1574 children out of 5830 Finnish families and 1681 children out of 10152 Estonian families were enrolled in the young children cohort and followed-up for 2 years (www.diabimmune.org). Blood was taken at the age of 3 and 5 years; for children with high risk of diabetes and high IgE levels at recruitment (high risk subpopulation) an additional follow-up visit with blood sampling was performed at the age of 4 years. The DIABIMMUNE study was conducted in accordance with the Declaration of Helsinki and was approved by the local institutional review boards of the participating hospitals.

QUESTIONNAIRES

Information about atopic eczema, allergic rhinitis and wheezing was obtained by the validated questions from the International Study of Asthma and Allergies in Childhood (ISAAC).[22] Furthermore, information about socioeconomic background, parental diseases, environmental factors, infections and medication was collected at age 3 and allergic disease status at age 5 by parental report.

OUTCOME DEFINITIONS

Concentrations of specific IgE antibodies (sIgE) to common food (hen´s egg, cow's milk, peanut) and aero-allergens (cat, dog, house dust mite, timothy grass and birch pollen) was measured in a SFS-EN ISO/IEC 17025:2005- and SFS-EN ISO 15189:2007- accredited centralized laboratory at the Unit of Medical Research Unit, Seinäjoki Central Hospital, in Finland by using automated Phadia 250 ImmunoCAP fluoroenzyme immunoassay analyzer (Phadia Diagnostics, Thermo Fisher Scientific). The analyses were carried out blind to the knowledge of the clinical and demographic data. Concentrations of at least 0.35 kU/L (corresponding to CAP class 1) were considered positive. We also assessed cut-off levels for CAP class 2 and 3 corresponding to 0.7 kU/L and 3.5 kU/L, respectively.

Wheezing was defined as lifetime prevalence of wheeze up to the age of 5 years, while strong wheezing was characterized by four or more attacks within the year before the follow-up visit.

Risk for T1D was assessed by HLA-DQ genotypes. Children with the DR3-DQ2 haplotype (the DQA1*05-DQB1*02 combination) and/or the DR4-DQ8 haplotype (DQB1*03:02/4 without the presence of DRB1*04:03/6) with no protective haplotypes, were defined as children at risk for T1D.[23]

Selected were those tested positive for DQA1*05-DQB1*02 and/or DQB1*032/4, but without DRB1*0403/6 (DR4-DQ8), and negative for protective haplotypes.[8] The risk was defined by the presence and combination of these HLA alleles.[23] The three highest risk classes, conferring susceptibility to T1D, were grouped together against the no or very low risk class.

STATISTICAL ANALYSIS

The sIgE values were categorized into CAP classes at three cut-off levels (0.35 kU/L, 0.7 kU/L and 3.5kU/L). For the LCA, the poLCA package in R 3.2. was used.[24] We performed four longitudinal LCAs: two country specific models and two for each country within a high risk population a model with three time points of sIgE measurements (at ages 3, 4, and 5 years). Each individual was unambiguously assigned to the class for which the posterior probability was highest. Each latent class was deliberately labeled according to the most prevalent allergen specificities. Logistic regression models were calculated to assess the association of allergic diseases and risk for T1D with class membership. The unsensitized class served as reference group. Associations were reported as odds ratios (OR) with 95% confidence intervals (CI). Characteristics between countries were compared by Fisher´s exact test, Kruskal-Wallis test and logistic regression. *P* values less than 0.05 were considered statistically significant. Populations attributable risk fractions were based on the odds ratios for the respective disease and the prevalence of each LC.

RESULTS

The two study populations were equal in size (Table 1), but differed significantly with respect to socioeconomic status, environmental exposures, and parental diseases (Figure 1, Table E1). The risk of allergic diseases and sensitization to major allergens such as hen's egg, cow's milk, and birch were increased in Finland whereas only dust mite was substantially higher in Estonia (p-values <0.001, Figure 2).

Children with available sIgE data at both time points did not differ from the entire population with respect to parental and children´s disease prevalence, exposure to farming, and socioeconomic patterns except for maternal education in Finland (Table E 2). The 4-class LCA models yielded consistently the lowest Bayesian information criterion (BIC) and the 6-class solution the lowest Akaike criterion (AIC), whereas entropy was maximized in the 4- and 5-class solutions (Table E 3). For interpretability and comparability with earlier studies, we performed main analyses with the 5-class solutions and sensitivity analyses with the other solutions.

The LCs of the two countries were similar with respect to lead allergens (Figure 3 A/B): LC1 was an unsensitized class with an extremely low prevalence of sensitization. The food class LC2 was characterized by sensitization to egg and milk allergens with decreasing sIgE levels over time. The seasonal inhalant class LC3 was driven by sIgE to birch and the perennial inhalant class LC4 by cat sIgE combined with dog (Finland) or dust mite sIgE (Estonia). The smallest class, LC5, was characterized by sIgE to inhalant (both seasonal and perennial) allergens combined with food sIgE and was labelled severe atopy for the features listed below.

Though the high-risk subpopulation was smaller in Estonia (17.0% versus 31.8% in Finland), LCA revealed similar classes and interpretations (Figure 3 C/D). The three measurement time points of the high-risk subpopulation demonstrated that the steepest increase in inhalant sIgE occurred before age 4 (Figure 3C/D).

The distribution of LCs in the full sample reflected the higher sensitization rate in Finland with the most striking difference in LC3, the seasonal class (Figure 4). In contrast, the countries did not differ in overall sensitization in the high-risk subpopulations (Table E4). Across all class solutions in both countries, the severe atopy class LC5 harbored children with the highest concentrations of total IgE (tIgE) (Figure 5 and Figure E 1A).

The upper panel of Figure 6 demonstrates a gradient in the associations of the individual LCs with wheeze, allergic rhinitis, and eczema in both countries. Besides a weak inverse association with allergic rhinitis in Finland, the food class was not associated with allergic disease thereby

corresponding to the benign atopy phenotype. However, the seasonal and the perennial classes were substantially related to all allergic diseases as a sign of symptomatic atopy. The strongest associations with disease were found for the severe atopy phenotype (LC 5). It was strongly associated with wheeze (OR=5.64 [3.07-10.52] and 4.56 [2.35-8.52]), allergic rhinitis (22.4 [11.67-44.54] and 13.97 [7.33-26.4]) and atopic eczema (9.39 [4.9-19.3] and 9.5 [5.2-17.5], for Finland and Estonia, respectively). The lower panel of Figure 6 reveals an association of severe atopy (LC5) with family history of atopy, whereas in Estonia the T1D risk genotype predisposed to symptomatic atopy (LC3 and LC4) but not severe atopy (LC5).

Despite its small size, the severe atopy class contributed prominently to wheeze, allergic rhinitis, and eczema as illustrated by population attributable risk fractions (PARFs, Figure 7). In Finland, however, the seasonal class (LC3) contributed most importantly to all disease.

Similar disease associations were found for the 6-class solution of both countries (Figure E 1B) and the high-risk subpopulation (Figure E 2).

DISCUSSION

Regardless of substantial environmental and socioeconomic differences and different atopy rates between Finland and Estonia, the postulated latent classes of food, seasonal, perennial, and severe atopy were found in both countries with high consistency. These four latent classes corresponded to the previously established trichotomy of benign, symptomatic, and severe atopy as illustrated by various degrees of disease relevance, tIgE levels, and family history of atopy. The higher sensitization prevalence in Finland was mainly attributable to the seasonal inhalant class, which also explained the higher proportion of allergic disease in Finland as shown by the corresponding PARF. The severe atopy class was equal in size and explained equal shares of allergic rhinitis and wheeze in both countries. Seasonal atopy was associated with T1D risk in Estonia and by trend in Finland.

Despite their geographical proximity and similar climate, the studied regions of Finland and Estonia differ in many environmental exposures. During the 20th century, the two countries experienced different social and economic developments.[25] Combined with faster urbanization and a Western lifestyle the Finnish economy prospered rapidly after World War II. Estonia's economic growth matured predominantly after the Fall of the Soviet Union.[26] As proxy variables of socioeconomic disparity, we assessed data on annual household income and maternal education in the current analysis. Environmental exposures were represented by animal and pet exposure, environmental tobacco smoke, farm milk consumption and playing outdoors (Figure 1). This list is obviously incomplete; previous comparisons of East and West European countries suggest additional differences in indoor climate,[27] pollution,[28] pollen trends,[29] and family size.[30]

A striking finding of this analysis was the difference in disease prevalence between both countries, with wheeze and allergic rhinitis being more common in Finland. These dissimilarities haven been often observed between East and West Europe. For example, the incidence of allergic disease was found to be elevated in urban areas and in industrialized countries in comparison to the post socialist countries of Eastern Europe.[31] An asthma diagnosis was more often observed in Swedish schoolchildren as compared to Estonian children.[32] Similarly, the prevalence of atopic diseases was increased in West Germany in comparison to East Germany[4] though levelling out within a decade after reunification.[5]

In Karelia, another interface between East and West, a gradient in asthma prevalence and atopic sensitization persists.[33] While remaining stable in Russian Karelia, sensitization rates to pollen and to cat increased from 1997 to 2007 in the Finnish part of Karelia. Similarly, sensitization rates differed between Swedish and Polish schoolchildren.[34] Even within Poland, differences were noted: In urban environments, children were increasingly sensitized to tree pollen, grass, corn, weeds and animals.[35]

A similar gradient was observed between the city of Montreal and Prince Edward Island as a rural region of Canada.[36]

The overall gradient in sensitization between Finland and Estonia observed in this study fits well into this picture. However, the forms of atopic sensitization follow the same pattern irrespectively of the environmental discrepancies. Only in the class of perennial sensitization the lead allergen differed. Whereas in Finnish children the perennial class was dominated by sIgE to cat, in Estonia sIgE to mite was most prevalent in this class. House dust mite sIgE might be more a marker for exposure,[37] which might be limited in Finland due to its higher latitude. At least for Swedish regions a lack of dust mites has been described.[38]

The described gradient in atopic sensitization can, however, resolve in response to environmental changes within a few years as illustrated by the rapid assimilation of atopy prevalence within Germany[5] or between rural and urban Poland.[39] Thus, atopy may virtually mirror environmental influences and illustrate the plasticity of the immune system also in adulthood.[40] The question now arises what atopy actually means.

We have previously classified atopy forms by latent class analysis in two birth cohorts, i.e. the Multicenter Allergy Study (MAS), conducted in five major cities in Germany, and the Protection Against Allergy: Study in Rural Environments (PASTURE), comprising children living in rural areas of five European countries.[12] Like in MAS and PASTURE, levels of sIgE in this study were found to be generally increasing from 3 to 5 years of age except for food sIgE.

The gradient of disease relevance previously detected in MAS and PASTURE was replicated in the two arms of the DIABIMMUNE study under investigation: Benign atopy included classes with sIgE only to food and without any disease association, though MAS revealed one food class and PASTURE two food classes. Symptomatic atopy reflected the inhalant classes (a seasonal and a perennial class in MAS and a single inhalant class in PASTURE) with their moderate associations with chronic inflammatory conditions such as allergic rhinitis, atopic eczema, and wheezing. Consistently with findings from MAS and PASTURE, severe atopy was characterized by the highest risk for all the above diseases and high sIgE levels to various allergens ("polysensitization"). In MAS and PASTURE, the severe atopy classes were strongly correlated with impaired lung function thereby contrasting with the other inhalant classes,[41] which is of specific clinical interest. Despite a slightly different approach, also the Manchester and Isle of Wight studies revealed a similar association of a highly sensitized class with reduced lung functioning at the age of 10 years.[42] In the current analysis, pulmonary function testing was not feasible due to the young age of the children. Nevertheless, the strong

relation to total IgE supports the specific severity of this atopy from. Total IgE has well been described as a predictor for allergic diseases.[10, 11, 43-45]

Furthermore, our findings are in line with a study observing a relation of asthma to an inhalant sensitization class with food co-sensitization.[46] In another study highly sensitized children at the age of 2 were found to be five times more likely to develop asthma within the following 2 years.[20] Likewise, the additional follow up at age 4 of the present study allowed an analysis of the dynamics of sIgE between ages 3 and 5, a time window not well covered by previous analyses.[12] Here we found sIgE levels to increase particularly before age 4 years, suggesting this period to be critical for the development of (severe) atopic sensitization. This may have clinical implications, because atopy-related asthma forms ("late onset wheeze") often manifest after age 4.[47] This asthma form is characterized by a loss of lung function,[47] which might be prevented if these children were identified early in life.[21] At least there is evidence for improvement of asthma by reducing IgE level by monoclonal antibodies.[48]

The rise of both atopic and autoimmune diseases challenged the T_{H1}/T_{H2} paradigm, which originally suggested an antagonism of T_{H1} and T_{H2} dominated immune conditions and diseases.[6] However, the concomitant occurrence of T_{H1} and T_{H2} prone diseases like atopy and diabetes suggests common genetic traits.[49] Interestingly we found the hereditary predisposition to T1D to be associated with the inhalant LCs but not with severe atopy. This finding was robust over the four- to six-class solutions of LCA in Estonia and seen by trend in Finland. In contrast to the hereditary background of T1D, family history of atopy was most strongly associated with LC severe atopy. This class also exhibited the strongest associations with atopic diseases in the children as illustrated by allergic rhinitis and asthma, which in the present analysis was represented by wheeze at age 5 years. The rather low prevalence of autoimmune diseases in this young study population precluded a further investigation into the associations of atopy and autoimmune diseases.[50, 51] Nevertheless the described findings support the concept that atopy consists of various different entities, some being associated with autoimmunity and susceptibility to environmental exposures, whereas others reflecting more genetically determined forms predisposing to T_{H2} diseases.

The DIABIMMUNE study was mainly set up to study the development of T1D and the immunologic pathways involved thereby explaining the relatively sparse data on asthma and atopic diseases. In particular, the definition of eczema was not sufficiently precise thereby leaving room for interpretation between country-specific medical cultures. This may explain the different PARFs of severe atopy for eczema between countries.

On the other hand, the DIABIMMUNE study provided the unique opportunity to address the relationship between atopy and T1D. In fact, the hygiene hypothesis of asthma and allergies has stimulated a vivid debate whether infections in early childhood could foster or protect from ß-cell autoimmunity.[52-54] An argument for the protective role of infections was the inverse relationship of T1D risk and sib ship size observed by Cardwell and colleagues and replicated in the present study (data not shown).[55] Furthermore, changes in early microbial exposure altering the maturation of the immune system are currently a matter of debate. Vatanen et al. (2016) found that lipopolysaccharide from Bacteroides, which impede immune signaling and reduce endotoxin tolerance, are more common in Finland than in Russian Karelia, providing a possible explanation for the persistent gradient in the burden of disease.[56]

To evaluate the robustness of the associations found in this study, we performed sensitivity analyses with four- and six-class models. In the four-class model, the seasonal and perennial inhalant classes were grouped together, and this group associated significantly with the HLA-defined T1D risk alleles. Moreover, the severe atopy class maintained the highest odds for the development of atopic diseases. The large sample size also allowed for a robust model with six classes, which separated LC severe atopy into a class with higher and a smaller class with relatively lower sIgE levels to seasonal allergens and moderate disease relevance. The latter dichotomy may again refine the characterization of severe atopy. Solutions with 7 or more classes were not explored due to the sample size, which may lead to insufficiently small class sizes.[42]

CONCLUSION

Taken together, we found very similar patterns of latent classes in both countries despite substantial differences in socioeconomic and environmental factors and distribution of single allergen specificities. The seasonal inhalant class seems to be most susceptible to environmental influences as reflected by substantially differing PARFs between two different countries. The phenomenon of severe atopy was mainly determined by elevated levels of sIgE and tIgE. The differential associations of LCs with allergic diseases and genetic T1D susceptibility might point towards distinct immunologic mechanisms linking the various forms of atopy to allergy and autoimmunity, which may be driven by the interaction of environment and genetic background.

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TABLES

TABLE 1: STUDY POPULATION WITH COMPLETE DATA FOR QUESTIONNAIRES AND IGE MEASUREMENT AT RECRUITMENT AND FOLLOW-UP

	Finnish study population		Estonian study population	
	N	%	N	%
Age 3				
Recruitment	1603	100	1657	100
Main questionnaire	1517	94.6	1236	74.6
Environmental exposure	1525	95.1	1636	98.7
IgE Measurement	1506	93.9	1635	98.7
Age 5				
Main questionnaire	1338	83.3	828	50.0
Allergy questionnaire	1328	82.8	1320	79.7
Autoimmune disease form	1273	79.4	607	36.6
IgE Measurement	1349	84.1	1291	78.0
Age 3 and 5				
Complete IgE data	1124	70.1	1165	70.3

LEGEND TO FIGURES

FIGURE 1: DIFFERENCES IN POPULATION CHARACTERISTICS BETWEEN FINLAND AND ESTONIA

All p-values < 0.001.

FIGURE 2: PREVALENCE OF ALLERGIC DISEASES AND ATOPIC SENSITIZATION (SIGE ≥ 0.35 KU/L) IN FINLAND AND ESTONIA

* ISAAC questions

FIGURE 3: PREVALENCE OF POSITIVE IGE FOR THE 5-CLASS SOLUTIONS

Perennial inhalant allergen specificities: dark blue = cat, light blue = dog, very light blue = dust mite

Seasonal inhalant allergen specificities: light green = birch, dark green = timothy

Food allergen specificities: red = peanut, orange = milk, yellow = egg

The full samples included 1124 children in Finland and 1165 children in Estonia, whereas the high-risk population comprised 357 and 198 children, respectively.

FIGURE 4: DISTRIBUTION OF THE LATENT CLASSES BY COUNTRY

The distribution of class sizes differs significantly between countries ($p<0.001$).

FIGURE 5: LEVELS OF TOTAL IGE ACROSS LCA AT 5 YEARS

Levels of total IgE increase significantly over the latent classes ($p<0.001$ for all panels).

FIGURE 6: ASSOCIATIONS OF LATENT CLASSES WITH DISEASES AND HEREDITARY BACKGROUND

FI = Finland, EE = Estonia. Odds ratios are given with 95%-confidence intervals. There were many missing values for maternal and paternal history of atopy in the Estonian population as indicated by smaller symbols for the effect estimate. T1D = Type 1 diabetes mellitus risk represented by HLA-DQ risk alleles

FIGURE 7: POPULATION ATTRIBUTABLE RISK FRACTIONS BY LATENT CLASSES

PARF = population attributable risk fraction

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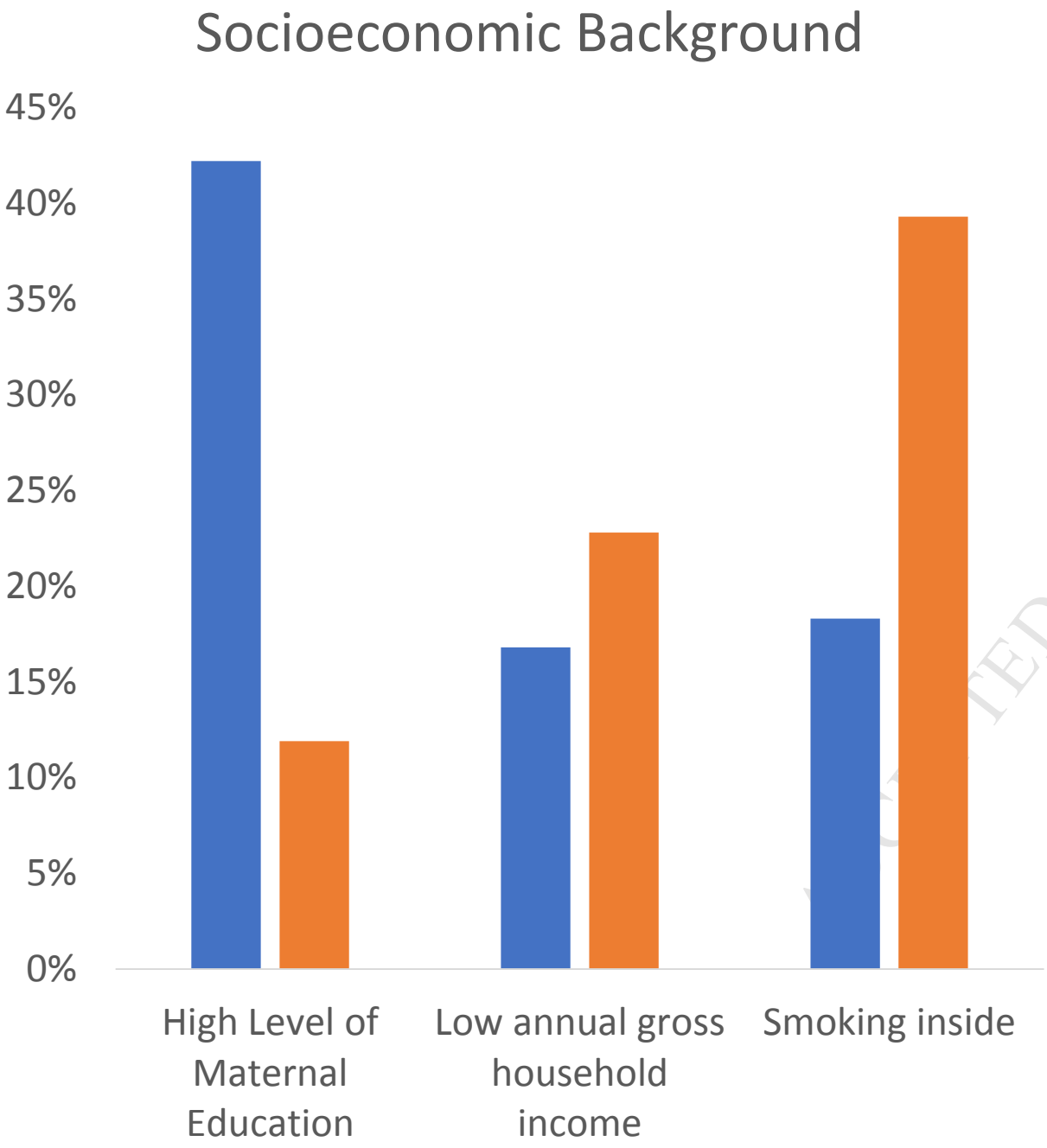
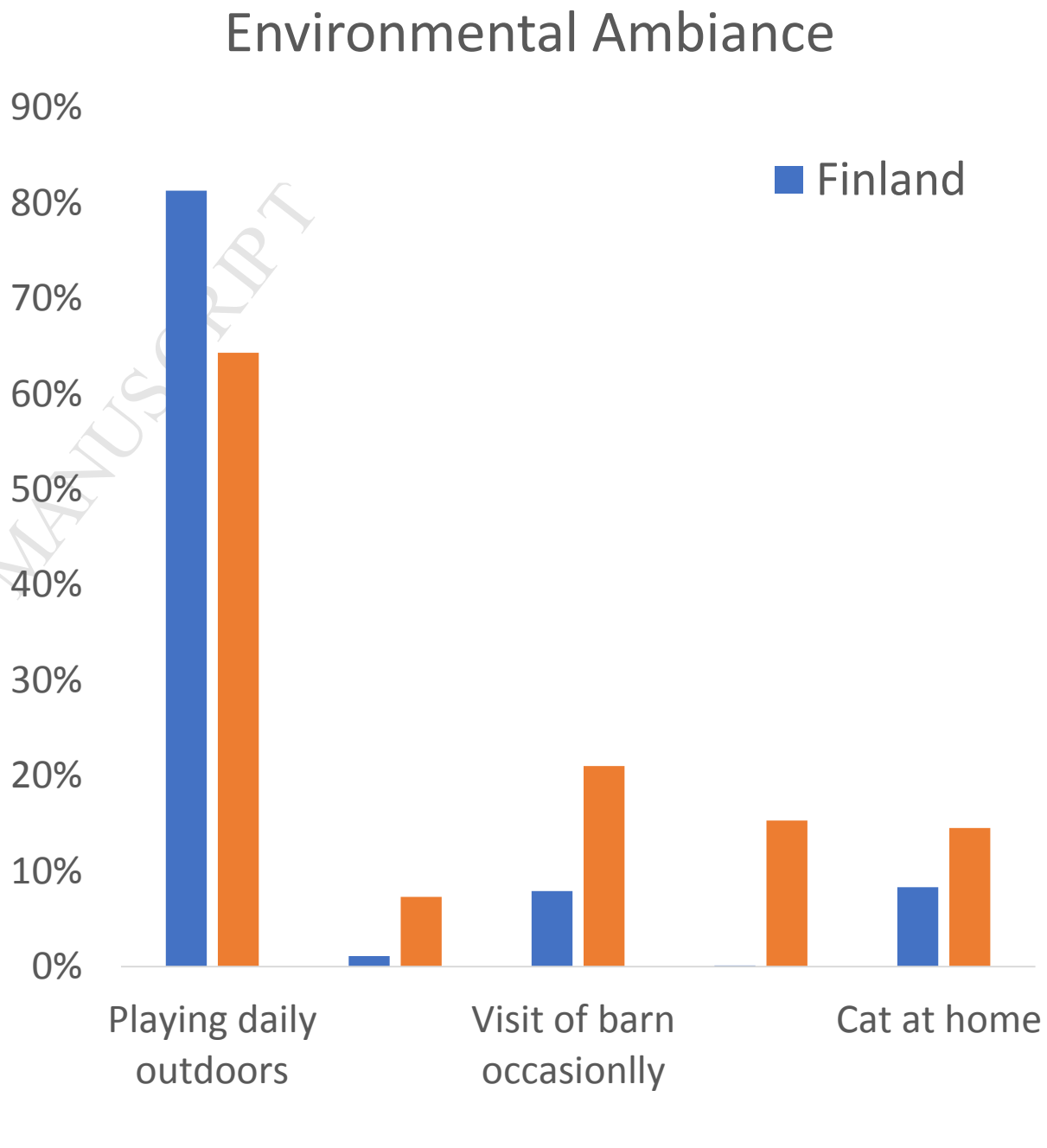
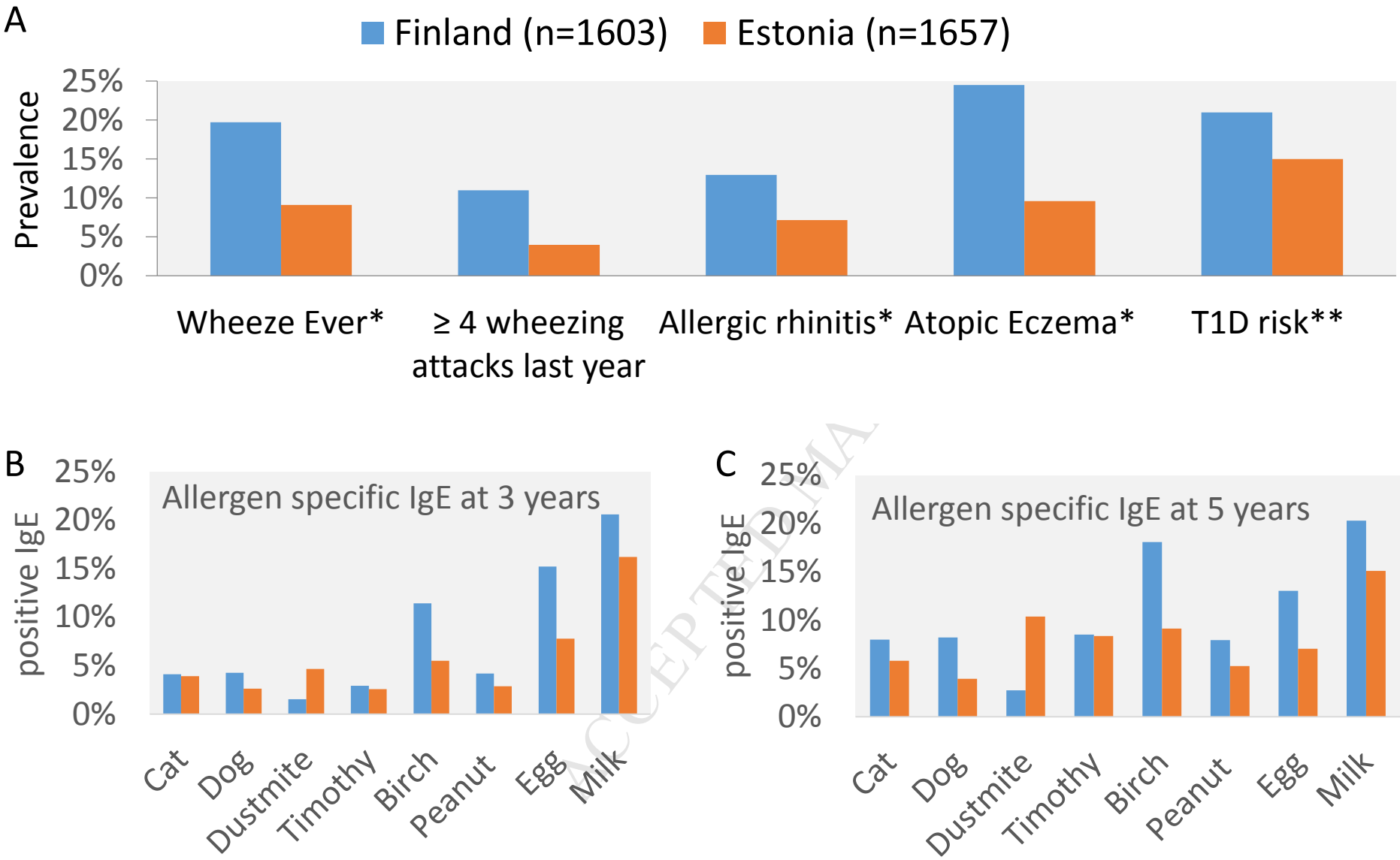
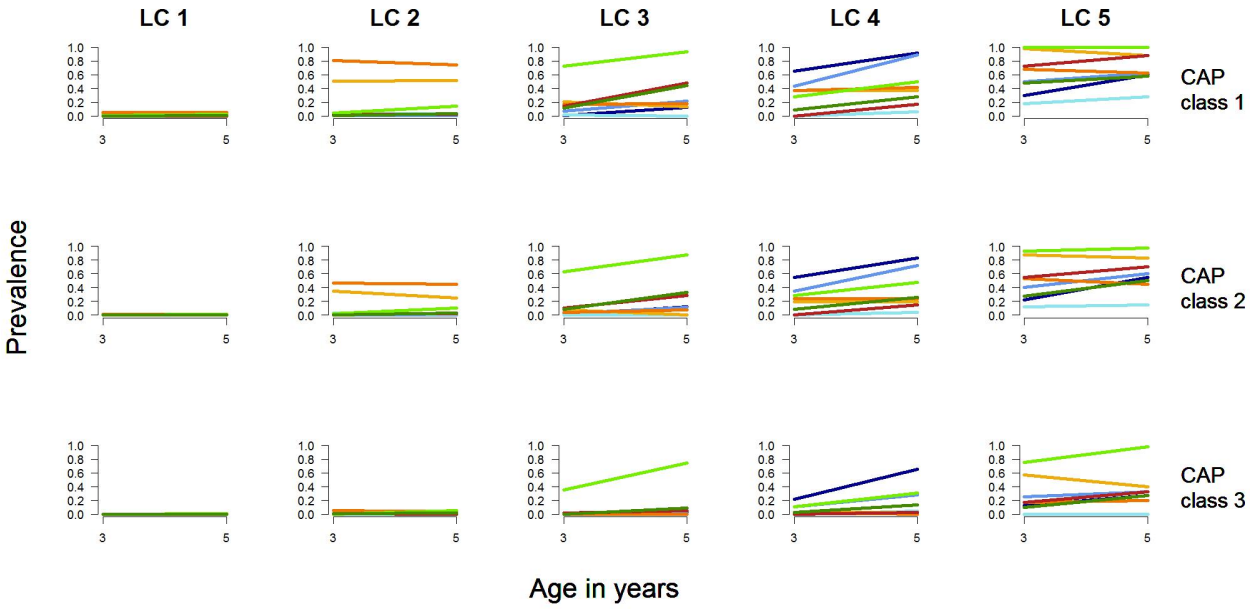
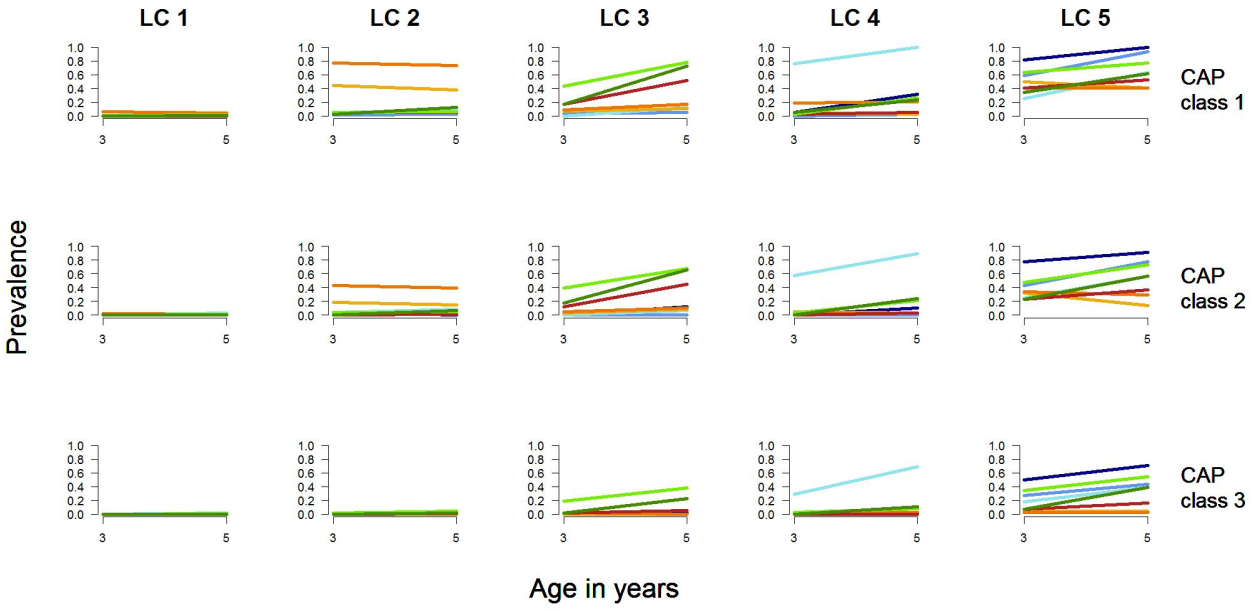
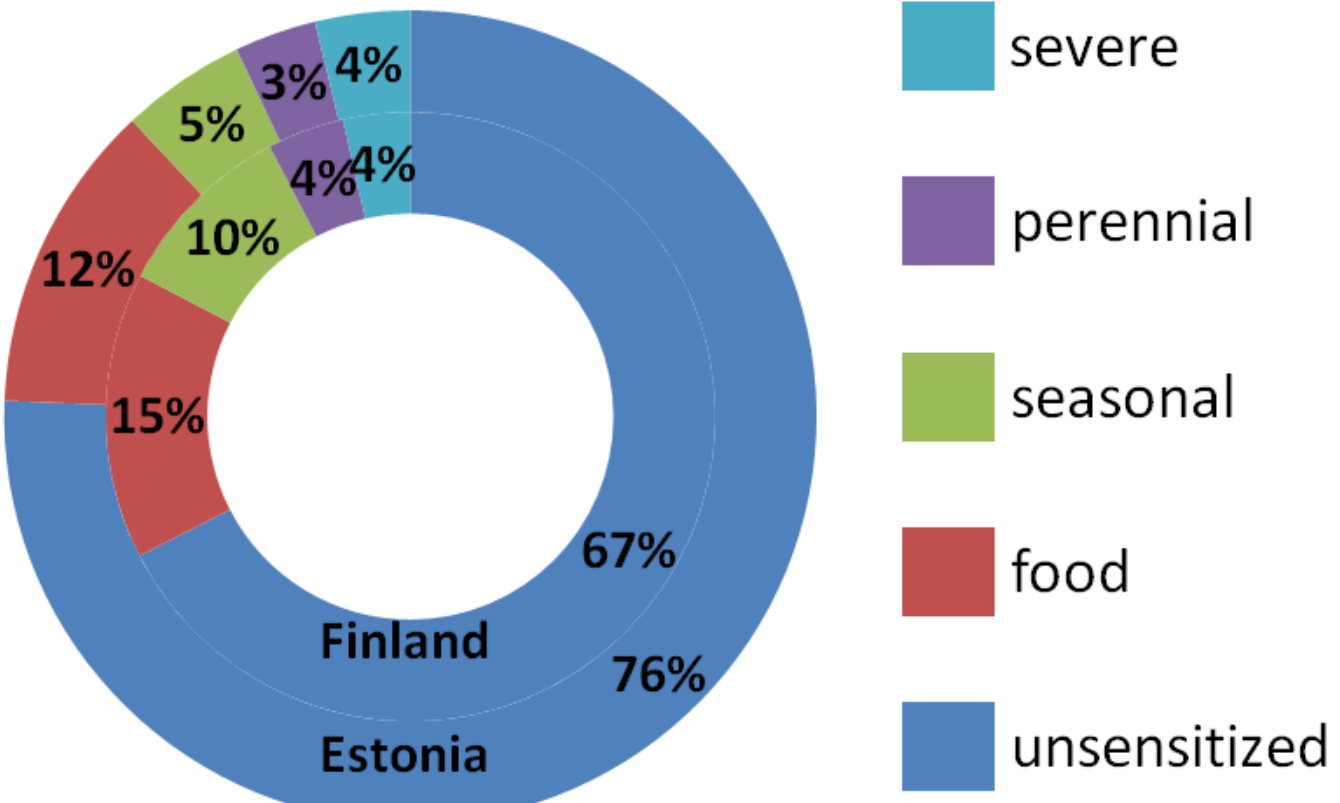


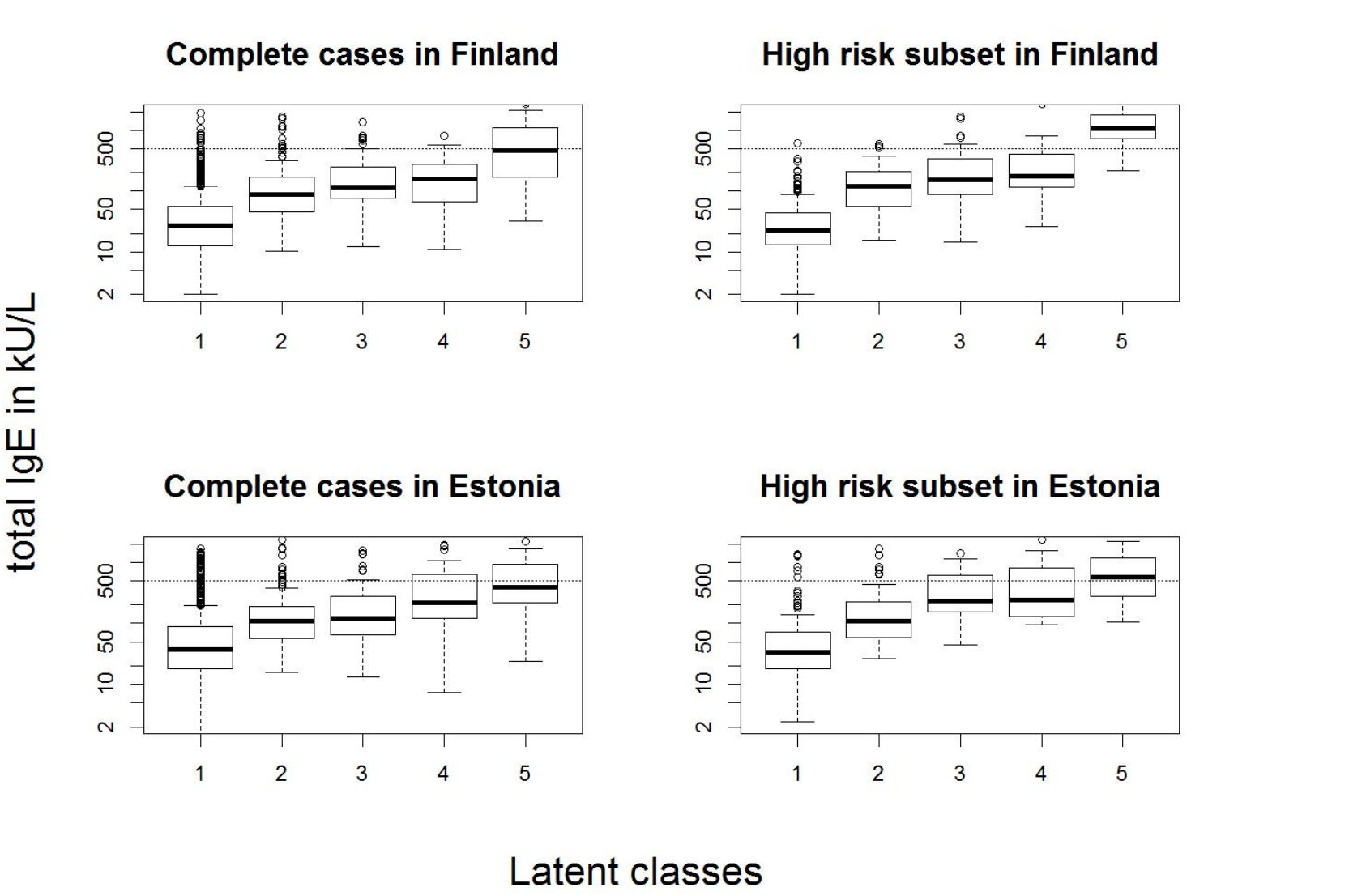


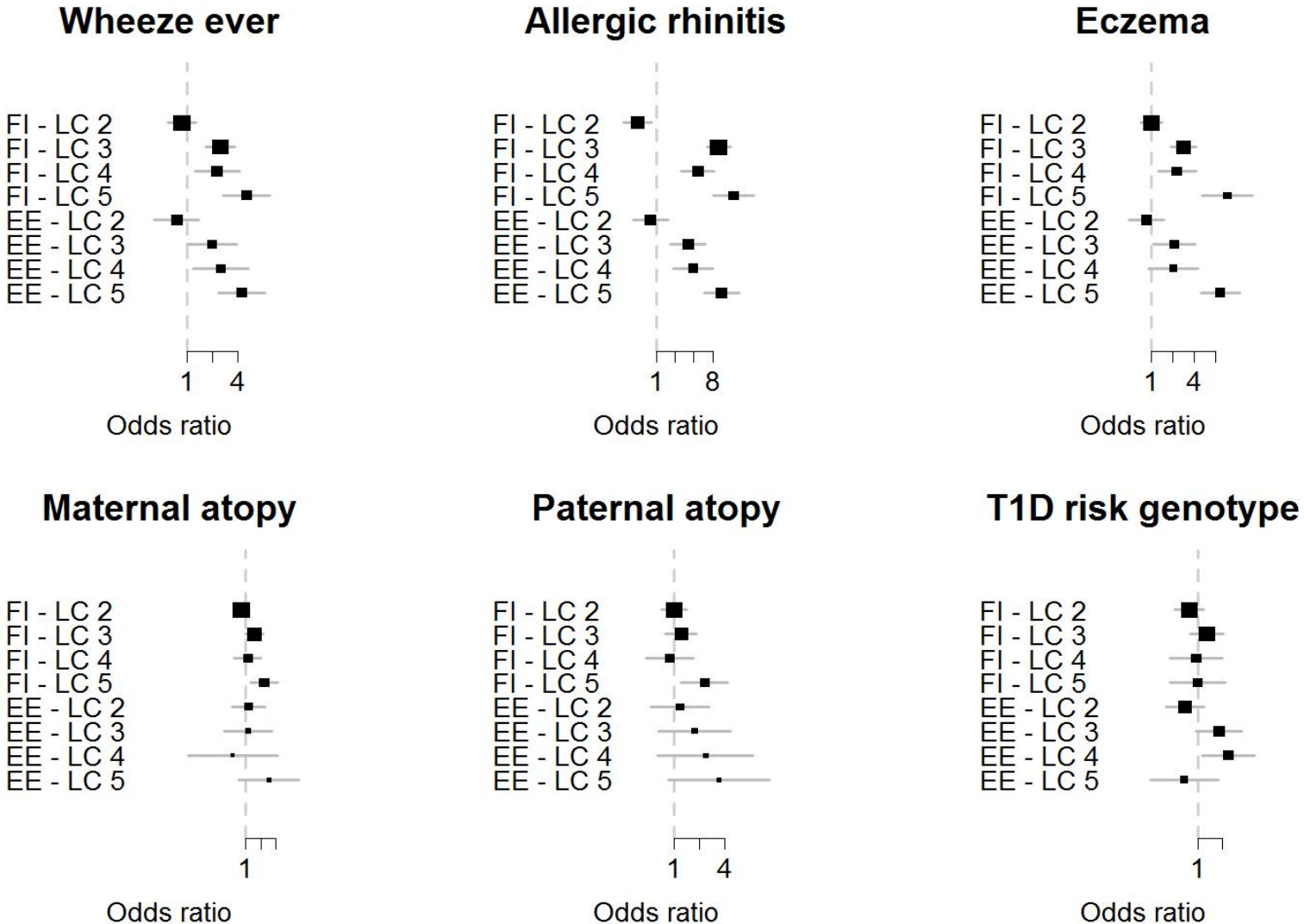


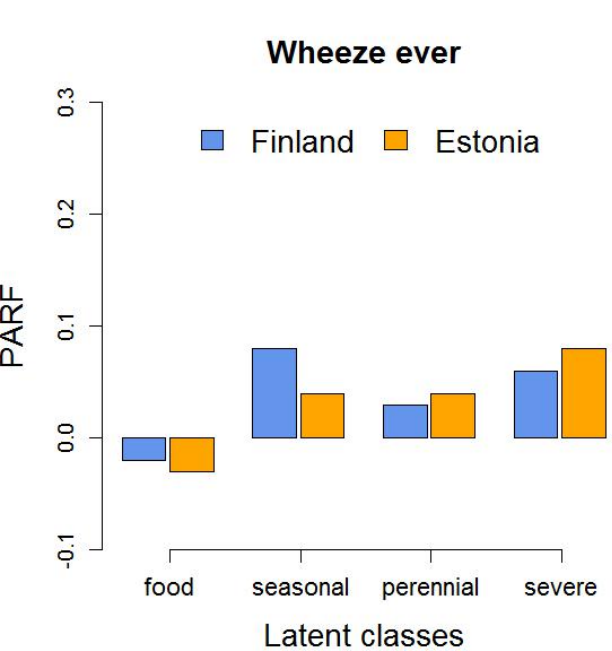


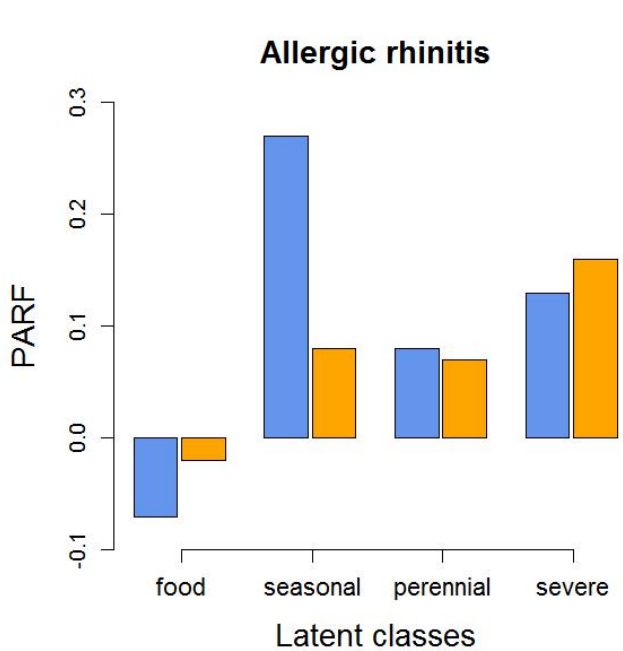


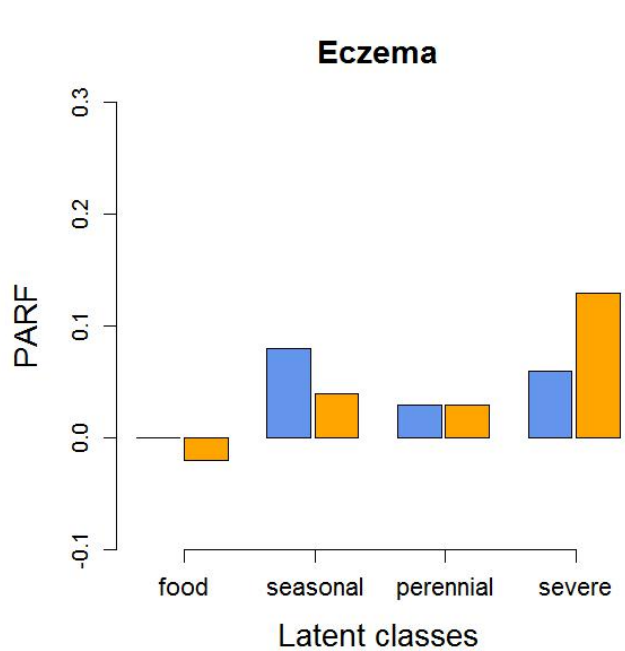

4%
3%
5%
4%
4%
10%
12%
15%
67%
Finland
76%
Estonia
severe
perennial
seasonal
food
unsensitized

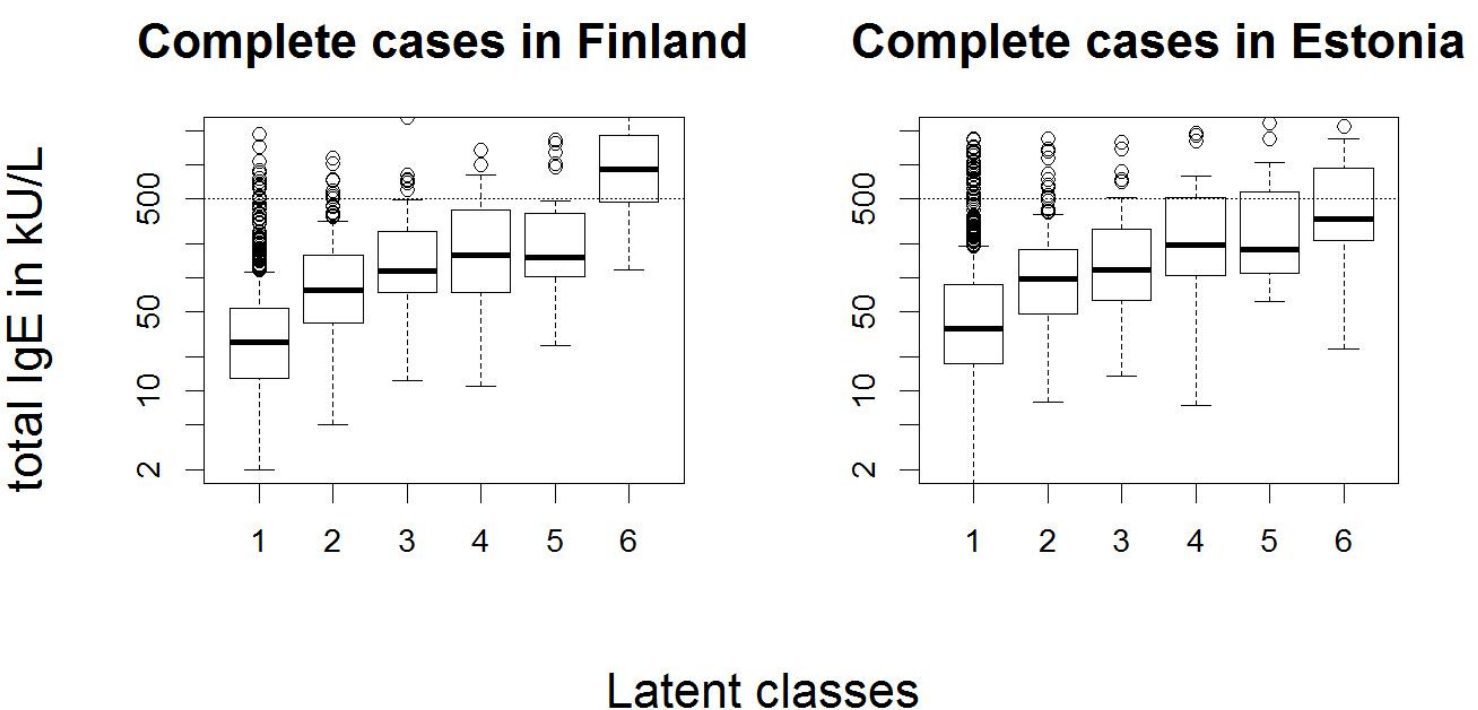

Complete cases in Finland
High risk subset in Finland
Complete cases in Estonia
High risk subset in Estonia
total IgE in kU/L
2
10
50
500
1
2
3
4
5
Latent classes

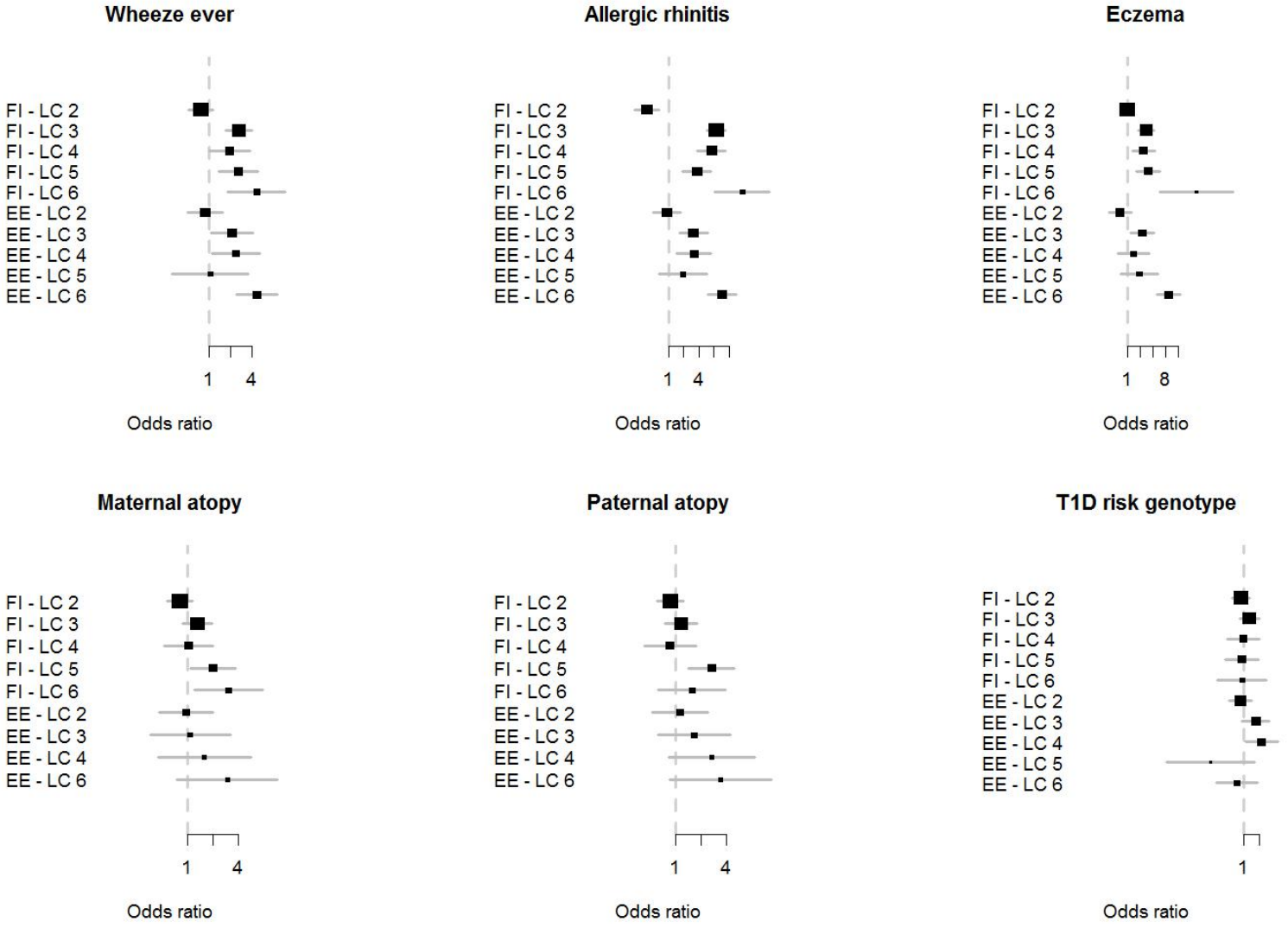

Wheeze ever
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
1
4
Odds ratio
Allergic rhinitis
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
1
8
Odds ratio
Eczema
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
1
4
Odds ratio
Maternal atopy
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
1
Odds ratio
Paternal atopy
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
1
4
Odds ratio
T1D risk genotype
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
1
Odds ratio

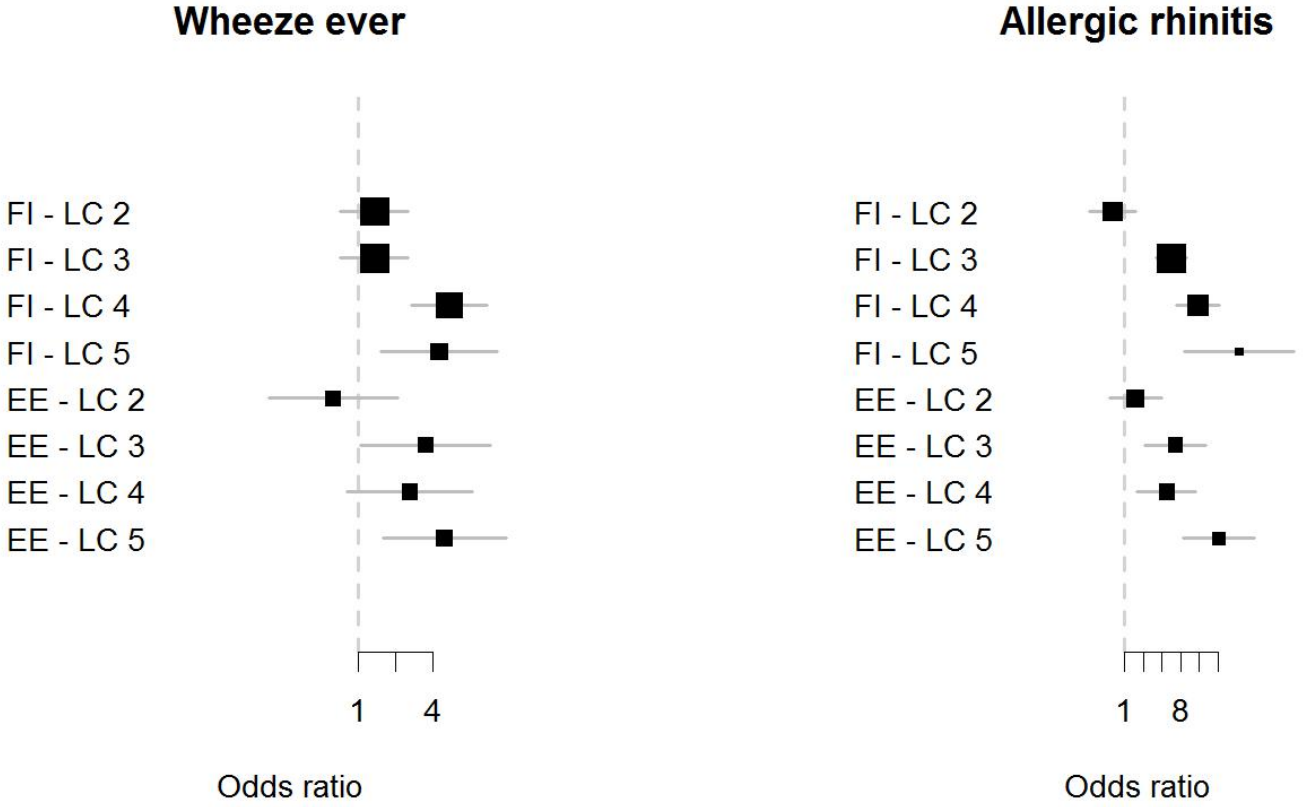

Wheeze ever
Finland
Estonia
PARF
0.3
0.2
0.1
0.0
-0.1
food
seasonal
perennial
severe
Latent classes


Allergic rhinitis
PARF
0.3
0.2
0.1
0.0
-0.1
food
seasonal
perennial
severe
Latent classes


Eczema
PARF
0.3
0.2
0.1
0.0
-0.1
food
seasonal
perennial
severe
Latent classes


Complete cases in Finland
Complete cases in Estonia
total IgE in kU/L
2
10
50
500
1
2
3
4
5
6
Latent classes


Wheeze ever
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
FI - LC 6
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
EE - LC 6
1
4
Odds ratio
Allergic rhinitis
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
FI - LC 6
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
EE - LC 6
1
4
Odds ratio
Eczema
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
FI - LC 6
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
EE - LC 6
1
8
Odds ratio
Maternal atopy
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
FI - LC 6
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 6
1
4
Odds ratio
Paternal atopy
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
FI - LC 6
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 6
1
4
Odds ratio
T1D risk genotype
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
FI - LC 6
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
EE - LC 6
1
Odds ratio


Wheeze ever
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
1
4
Odds ratio
Allergic rhinitis
FI - LC 2
FI - LC 3
FI - LC 4
FI - LC 5
EE - LC 2
EE - LC 3
EE - LC 4
EE - LC 5
1
8
Odds ratio

ONLINE SUPPLEMENT TO:

DEVELOPMENT OF ATOPIC SENSITIZATION IN FINNISH AND ESTONIAN CHILDREN – A LATENT CLASS ANALYSIS IN A MULTICENTER COHORT

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Running Head: Atopic Sensitization in Finnish and Estonian children

FIGURES

FIGURE E 1: THE 6-CLASS SOLUTION

A: Levels of total IgE across latent classes

B: Associations of latent classes with diseases and hereditary background

FIGURE E 2: DISEASE ASSOCIATIONS IN THE HIGH-RISK POPULATION

Due to a lower sample size and empty cells associations could not be calculated for eczema.

T1D = Type 1 diabetes mellitus risk represented by HLA-DQ risk alleles

TABLES

TABLE E 1: DESCRIPTION OF STUDY POPULATION BY CENTERS

		Finnish study population			Estonian study population			
		Total no.	No. (%)	NA	Total no.	No. (%)	NA	p-value
Participants characteristics								
Male		792	49.4	76	858	51.8	12	0.660
Female		735	45.8		787	47.5		
Socioeconomic background								
Level of education (Mother)	Low	243	15.2	192	511	30.8	104	<0.001
	Middle	581	36.2		934	56.4		
	High	676	42.2		197	11.9		
Annual gross household income	Low	270	16.8	273	377	22.8	123	<0.001
	High	1149	71.7		1246	75.2		
Smoking Inside	3 years	277	18.3		480	39.0		<0.001
Environmental ambience								
Play outdoors	Daily	1378	81.3	84	1132	64.8	21	<0.001
	Weekly	115	6.8		300	17.2		
	seldom	26	1.5		204	11.7		
Farming		17	1.1	57	90	7.3	43	<0.001
Visit of barn	Daily	5	0.3	45	39	3.2	71	<0.001
	Occasionally	119	7.9	44	259	21.0	62	<0.001
Farm Milk Consumption	Daily/Weekly	2	0.1	173	254	15.3	113	<0.001
	Occasionally	5	0.3		115	6.9		
	Never	1514	94.5		1266	76.4		
Cat at home		133	8.3	92	241	14.5	427	<0.001
Parental Diseases								
Parental Diabetes		161	10.0	76	56	3.4	12	<0.001
Paternal Asthma		99	6.2	76	37	2.3	12	<0.001
Maternal Asthma		120	7.5	76	48	2.9	12	<0.001

For the comparison of study centers by characteristics in Table E1, p-values were derived by Fisher´s Exact Test and Kruskall Wallis Test.

TABLE E 2: CHILDREN WITH AVAILABLE IGE VALUES AT BOTH TIME POINTS

		Finland (Included=1124; excluded=210)			Estonia (Included=1165; excluded=68)		
		included	excluded	p-value	included	excluded	p-value
Gender	Male	566	102	0.227	284	30	0.199
	female	497	108		255	38	
High Annual Income	Low	693	86	0.501	241	33	0.278
	High	521	114		291	33	
Maternal education	Low	166	23	0.027	150	23	0.411
	Intermediate	411	74		309	38	
	High	469	111		80	6	
Farming*		14	3	0.583	62	28	0.631
Allergic Rhinitis*		184	24	0.051	104	15	0.884
Atopic eczema at age 3*		332	61	0.626	136	23	0.363
Wheezing*		257	59	0.189	133	18	0.999
Parental Asthma*		139	26	0.911	27	2	0.761
Paternal Diabetes*		120	15	0.085	13	2	0.680

*Binary variables, only positive (=yes) data shown

P-values were derived by Fisher´s Exact Test or Kruskall-Wallis Test.

TABLE E 3: MODEL FIT CRITERIA

		Finland			Estonia		
	classes	AIC	BIC	Entropy	AIC	BIC	Entropy
All children (2 time points)	4	11153	12113	95.99 %	9476	10463	95.93 %
	5	11010	12211	96.45 %	9385	10619	96.28 %
	6	10930	12372	95.8 %	9330	10813	94.42 %
High risk (3 time points)	4	7476	8573	99.45 %	4381	5338	99.37 %
	5	7383	8756	99.49 %	4368	5565	99.49 %
	6	7344	8992	99.31 %	4410	5847	99.53 %

TABLE E 4: DISTRIBUTION OF CLASSES IN THE HIGH-RISK POPULATION

Center	LC1	LC2	LC3	LC4	LC5
Finland	57%	15%	15%	9%	4%
Estonia	58%	20%	8%	9%	7%

The distribution of classes of the high risk subpopulation varies in size between the countries with borderline significance (p=0.070)."

TABLE E5: ODDS RATIOS TO FIGURE 6 IN THE MAIN BODY

Country	Latent class	Condition	Odds ratio [95%-CI]	p-value
EE	LC 2	Wheeze ever	0.74 [0.39-1.31]	0.33
EE	LC 3	Wheeze ever	1.95 [0.94-3.74]	0.056
EE	LC 4	Wheeze ever	2.48 [1.08-5.16]	0.021
EE	LC 5	Wheeze ever	4.48 [2.29-8.58]	< 0.001
EE	LC 2	Allergic rhinitis	0.81 [0.4-1.51]	0.537
EE	LC 3	Allergic rhinitis	3.22 [1.62-6.05]	< 0.001
EE	LC 4	Allergic rhinitis	3.86 [1.73-7.92]	< 0.001
EE	LC 5	Allergic rhinitis	11.02 [5.75-21.12]	< 0.001
EE	LC 2	Eczema	0.86 [0.47-1.48]	0.608
EE	LC 3	Eczema	2.1 [1.03-3.94]	0.03
EE	LC 4	Eczema	2.05 [0.86-4.39]	0.078
EE	LC 5	Eczema	9.21 [4.85-17.29]	< 0.001
EE	LC 2	Maternal atopy	1.16 [0.51-2.36]	0.704
EE	LC 3	Maternal atopy	1.13 [0.32-3.03]	0.834
EE	LC 4	Maternal atopy	0.55 [0.03-2.89]	0.572
EE	LC 5	Maternal atopy	2.94 [0.63-10.91]	0.124
EE	LC 2	Paternal atopy	1.16 [0.49-2.46]	0.714
EE	LC 3	Paternal atopy	1.73 [0.57-4.39]	0.286
EE	LC 4	Paternal atopy	2.36 [0.52-8]	0.2
EE	LC 5	Paternal atopy	3.42 [0.72-12.68]	0.082
EE	LC 2	T1D risk genotype	0.7 [0.4-1.17]	0.199
EE	LC 3	T1D risk genotype	1.8 [0.93-3.29]	0.064
EE	LC 4	T1D risk genotype	2.32 [1.08-4.62]	0.023

EE	LC 5	T1D risk genotype	0.69 [0.24-1.63]	0.447
FI	LC 2	Wheeze ever	0.85 [0.57-1.26]	0.442
FI	LC 3	Wheeze ever	2.46 [1.62-3.71]	< 0.001
FI	LC 4	Wheeze ever	2.27 [1.21-4.18]	0.009
FI	LC 5	Wheeze ever	5.1 [2.66-9.97]	< 0.001
FI	LC 2	Allergic rhinitis	0.51 [0.29-0.84]	0.011
FI	LC 3	Allergic rhinitis	9.87 [6.42-15.18]	< 0.001
FI	LC 4	Allergic rhinitis	4.62 [2.48-8.41]	< 0.001
FI	LC 5	Allergic rhinitis	16.95 [8.33-37.34]	< 0.001
FI	LC 2	Eczema	1.01 [0.7-1.45]	0.94
FI	LC 3	Eczema	2.83 [1.9-4.26]	< 0.001
FI	LC 4	Eczema	2.29 [1.25-4.18]	0.007
FI	LC 5	Eczema	11.59 [5.31-28.79]	< 0.001
FI	LC 2	Maternal atopy	0.82 [0.57-1.16]	0.274
FI	LC 3	Maternal atopy	1.48 [0.97-2.23]	0.066
FI	LC 4	Maternal atopy	1.11 [0.58-2.05]	0.744
FI	LC 5	Maternal atopy	2.34 [1.23-4.53]	0.01
FI	LC 2	Paternal atopy	0.99 [0.68-1.4]	0.936
FI	LC 3	Paternal atopy	1.21 [0.78-1.84]	0.393
FI	LC 4	Paternal atopy	0.88 [0.44-1.67]	0.704
FI	LC 5	Paternal atopy	2.29 [1.21-4.39]	0.011
FI	LC 2	T1D risk genotype	0.79 [0.52-1.19]	0.276
FI	LC 3	T1D risk genotype	1.28 [0.8-1.99]	0.283
FI	LC 4	T1D risk genotype	0.95 [0.44-1.88]	0.899
FI	LC 5	T1D risk genotype	1 [0.44-2.05]	0.999

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60 TABLE E6: ISAAC QUESTIONS

Variable	ISAAC Question	Response options	Operationalized
Atopic Eczema	Has your child ever had an itchy rash which was coming and going for at least six months?	Yes No	
Wheeze ever	Has your child ever had wheezing or whistling in the chest at any time in the past?	Yes No	
Strong Wheeze	How many attacks of wheezing has your child had in the past 12 months	None 1 to 3 4 to 12 More than 12	More than 4 attacks in the last 12 months
Allergic Rhinitis	In the past 12 months, has your child had a problem with sneezing or a runny or blocked	Yes No	

	nose when he/she DID NOT have a cold or the flu?		

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