Development of atopic sensitization in Finnish and Estonian children: A latent class analysis in a multicenter cohort

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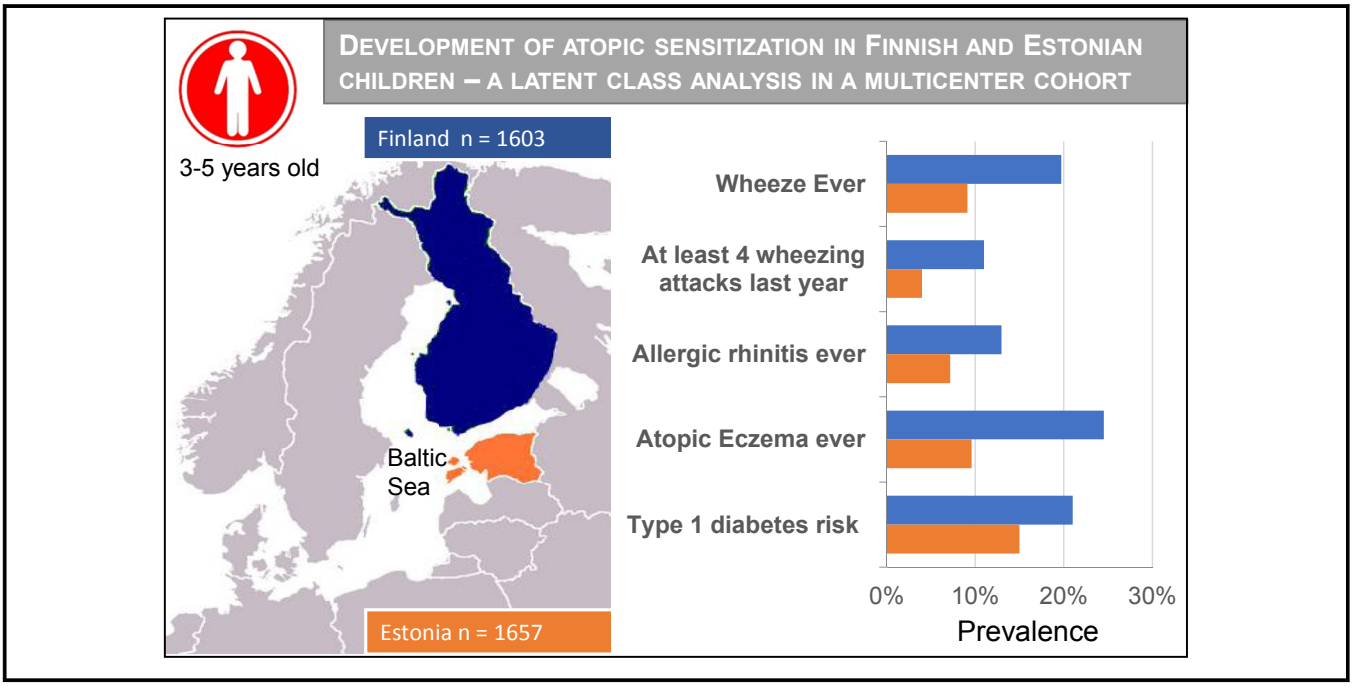
GRAPHICAL ABSTRACT



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Supported by the European Commission (grant no. HEALTH-F2-2008-202063) and the German Center for Lung Research (DZL).

Disclosure of potential conflict of interest: J. Weber reports grants from European Commission during the conduct of the study. E. von Mutius reports grants from the European Commission during the conduct of the study; personal fees from Pharma Ventures, Peptinnovate, OM Pharma SA, Boehringer Ingelheim, the European Commission/European Research Council Executive Agency, the Chinese University of Hong Kong, the University of Tampere/Tampereen Yliopisto, Utrecht Universiteit, the University of Turku/Turun Yliopisto, HAL Allergie GmbH, Ökosoziales Forum Oberösterreich, Mundipharma Deutschland GmbH & Co KG, and the University of Helsinki/Helsingin yliopisto outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication June 7, 2018; revised October 26, 2018; accepted for publication December 7, 2018.

Available online January 23, 2019.

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0091-6749/$36.00

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https://doi.org/10.1016/j.jaci.2018.12.1014

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

Objective: We sought to define phenotypes of atopic sensitization in early childhood and 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 years, respectively, and categorized into 3 CAP classes. Latent class analysis was performed with the statistical software package poLCA in R software.

Results: Both populations differed in terms of socioeconomic status and environmental determinants, such as pet ownership, farm-related exposure, time spent playing outdoors, and prevalence of allergic diseases (all $P < .001$). Nevertheless, we found similar latent classes in both populations: an unsensitized class, a food class, 2 inhalant classes differentiating between seasonal and perennial aeroallergens, and a severe atopy class. The latter was characterized by high total and specific IgE levels and strongly associated with wheeze (odds ratio [OR], 5.64 [95% CI, 3.07-10.52] and 4.56 [95% CI, 2.35-8.52]), allergic rhinitis (OR, 22.4 [95% CI, 11.67-44.54] and 13.97 [95% CI, 7.33-26.4]), and atopic eczema (OR, 9.39 [95% CI, 4.9-19.3] and 9.5 [95% CI, 5.2-17.5] for Finland and Estonia, respectively). Environmental differences were reflected in the larger seasonal inhalant atopy class in Finland, although composition of classes was comparable between countries.

Conclusion: Despite profound differences in environmental exposures, there might exist genuine patterns of atopic sensitization. The distribution of these patterns might determine the contribution of atopic sensitization to disease onset. (J Allergy Clin Immunol 2019;143:1904-13.)

Key words: *Latent class analysis, unsupervised clustering, IgE, atopy, allergy, diabetes type 1 risk, wheezing, Finland, Estonia, severe atopy*

Allergic diseases are more prevalent in industrialized countries compared with 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 40 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]

In addition to atopy, also autoimmune disorders, such as type 1 diabetes mellitus (T1D), are on the increase in Western countries.[6] For instance, a remarkably higher incidence of T1D was observed in Finnish schoolchildren less than 15 years of age compared with 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. Because 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, such as by concentrations of allergen-specific IgE (sIgE), monovalent 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 3 dimensions of allergen specificity, sIgE level, and time course in a data-driven approach, thereby being largely immune from investigator bias.[12] This methodology was used in Protection Against Allergy: Study in Rural Environments (PASTURE) and Multicenter Allergy Study (MAS) to cluster preschool children in 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 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 with 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 2 birth cohorts of 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, 2 countries separated by the Baltic Sea and with different socioeconomic and environmental developments over many decades and striking differences in atopy rates.

Abbreviations used

LC:	Latent class
LCA:	Latent class analysis
MAS:	Multicenter Allergy Study
OR:	Odds ratio
PARF:	Population-attributable risk fraction
PASTURE:	Protection Against Allergy: Study in Rural Environments
sIgE:	Specific IgE
T1D:	Type 1 diabetes mellitus
tIgE:	Total IgE

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, 1,574 children of 5,830 Finnish families and 1,681 children of 10,152 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 approved by the local institutional review boards of the participating hospitals.

Questionnaires

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

Outcome definitions

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

Wheezing was defined as a lifetime prevalence of wheeze up to the age of 5 years, whereas strong wheezing was characterized by 4 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), the DR4-DQ8 haplotype (DQB1*03:02/4 without the presence of DRB1*04:03/6), or both with no protective haplotypes were defined as children at risk for T1D.[23] Selected were those with positive test results for DQA1*05-DQB1*02, DQB1*032/4, or both but without DRB1*0403/6 (DR4-DQ8) and with negative results for protective haplotypes.[8] The risk was defined by the presence and combination of these HLA alleles.[23] The 3 highest risk classes, conferring susceptibility to T1D, were grouped together against the no or very low risk class.

Statistical analysis

sIgE values were categorized into CAP classes at 3 cutoff levels (0.35, 0.7, and 3.5 kU/L). For the LCA, the poLCA package in R 3.2 software was used.[24] We performed 4 longitudinal LCAs: 2 country-specific models and 2 for each country within a high-risk population a model with 3 time points of sIgE measurements (at ages 3, 4, and 5 years). Each subject was unambiguously assigned to the class for which the posterior probability was greatest. Each latent class (LC) 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 the reference group. Associations were reported as odds ratios (ORs) with 95% CIs. Characteristics between countries were compared by using the Fisher exact test, Kruskal-Wallis test, and logistic regression. *P* values of less than .05 were considered statistically significant. Population-attributable risk fractions were based on the ORs for the respective disease and the prevalence of each LC.

RESULTS

The 2 study populations were equal in size (Table I) but differed significantly with respect to socioeconomic status, environmental exposures, and parental diseases (Fig 1 and see Table E1 in this article's Online Repository at www.jacionline.org). The risks of allergic disease and sensitization to major allergens, such as hen's egg, cow's milk, and birch, were increased in Finland, whereas only dust mite levels were substantially greater in Estonia ($P < .001$, Fig 2).

Children with available sIgE data at both time points did not differ from the entire population with respect to parents' and children's disease prevalence, exposure to farming, and socioeconomic patterns, except for maternal education in Finland (see Table E2 in this article's Online Repository at www.jacionline.org). The 4-class LCA models yielded consistently the lowest Bayesian information criterion and the 6-class solution yielded consistently the lowest Akaike information criterion, whereas entropy was maximized in the 4- and 5-class solutions (see Table E3 in this article's Online Repository at www.jacionline.org). For interpretability and comparability with earlier studies, we performed main analyses with the 5-class solutions and sensitivity analyses with the other solutions.

TABLE I. Study population with complete data for questionnaires and IgE measurement at recruitment and follow-up

	Finnish study population		Estonian study population	
	No.	Percent	No.	Percent
Age 3 y				
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 y				
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 y				
Complete IgE data	1124	70.1	1165	70.3

LCs of the 2 countries were similar with respect to lead allergens (Fig 3, *A* and *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 labeled severe atopy for the features listed below.

Although the high-risk subpopulation was smaller in Estonia (17.0% vs 31.8% in Finland), LCA revealed similar classes and interpretations (see Fig E1, *A* and *B*, in this article's Online Repository at www.jacionline.org). The 3 measurement time points of the high-risk subpopulation demonstrated that the steepest increase in inhalant sIgE levels occurred before age 4 years (see Fig E1, *A* and *B*).

Distribution of LCs in the full sample reflected the greater sensitization rate in Finland with the most striking difference in LC3, the seasonal class (Fig 4). In contrast, countries did not differ in overall sensitization in the high-risk subpopulations (see Table E4 in this article's Online Repository at www.jacionline.org). Across all class solutions in both countries, the severe atopy class LC5 harbored children with the greatest concentrations of total IgE (tIgE; Fig 5 and see Fig E2, *A*, in this article's Online Repository at www.jacionline.org).

The upper panel of Fig 6 demonstrates a gradient in associations of individual LCs with wheeze, allergic rhinitis, and eczema in both countries. In addition to a weak inverse

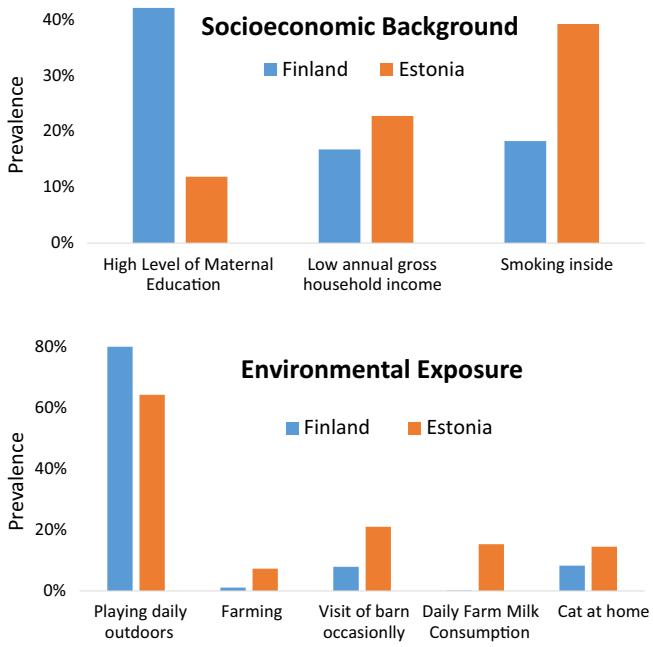

FIG 1. Differences in population characteristics between Finland and Estonia. All $P < .001$.

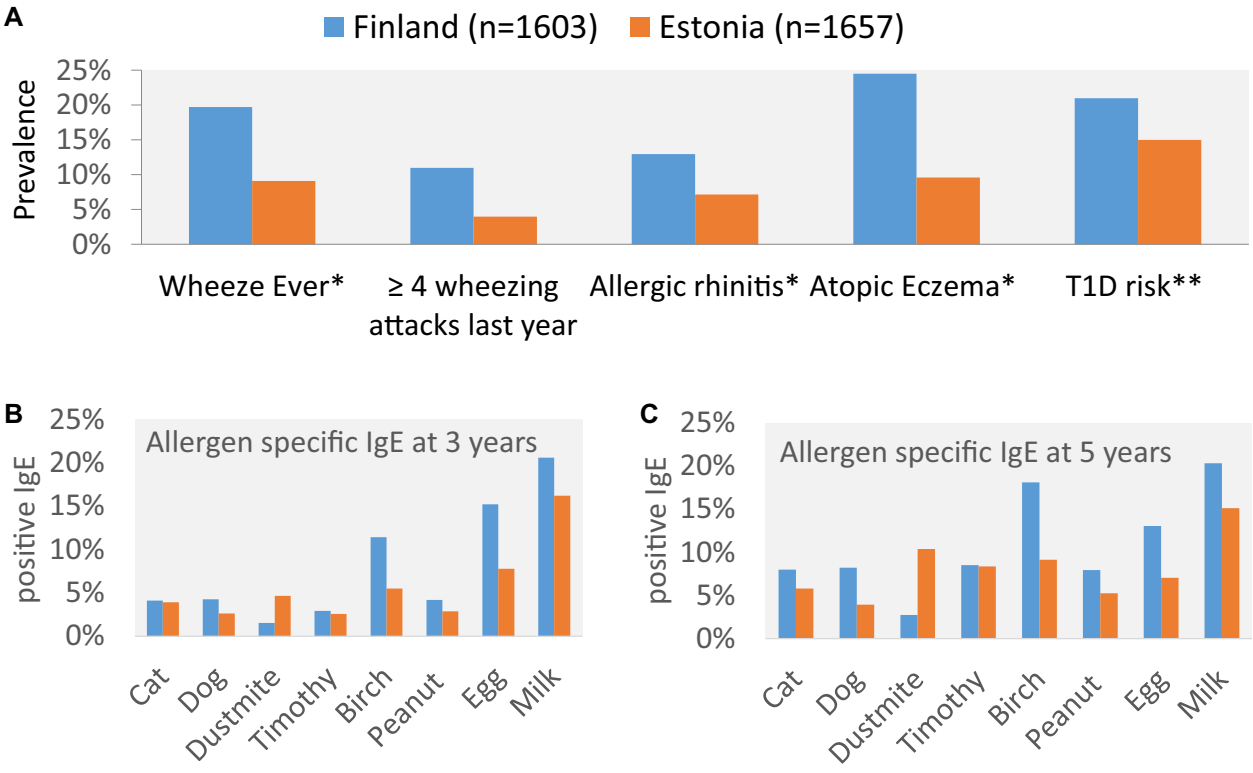

FIG 2. A-C, Prevalence of allergic disease and atopic sensitization (sIgE ≥ 0.35 kU/L) in Finland and Estonia. *International Study of Asthma and Allergies in Childhood questions (see Table E6 in this article's Online Repository at www.jacionline.org). **T1D risk = genetic risk of type 1 diabetes mellitus.

association with allergic rhinitis in Finland, the food class was not associated with allergic disease, thereby corresponding to the benign atopy phenotype. However, seasonal and 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 (LC5), which was strongly associated with wheeze (OR, 5.64 [95% CI, 3.07-10.52] and 4.56 [95% CI, 2.35-8.52]), allergic rhinitis (OR, 22.4 [95% CI, 11.67-44.54] and 13.97 [95% CI, 7.33-26.4]), and atopic eczema

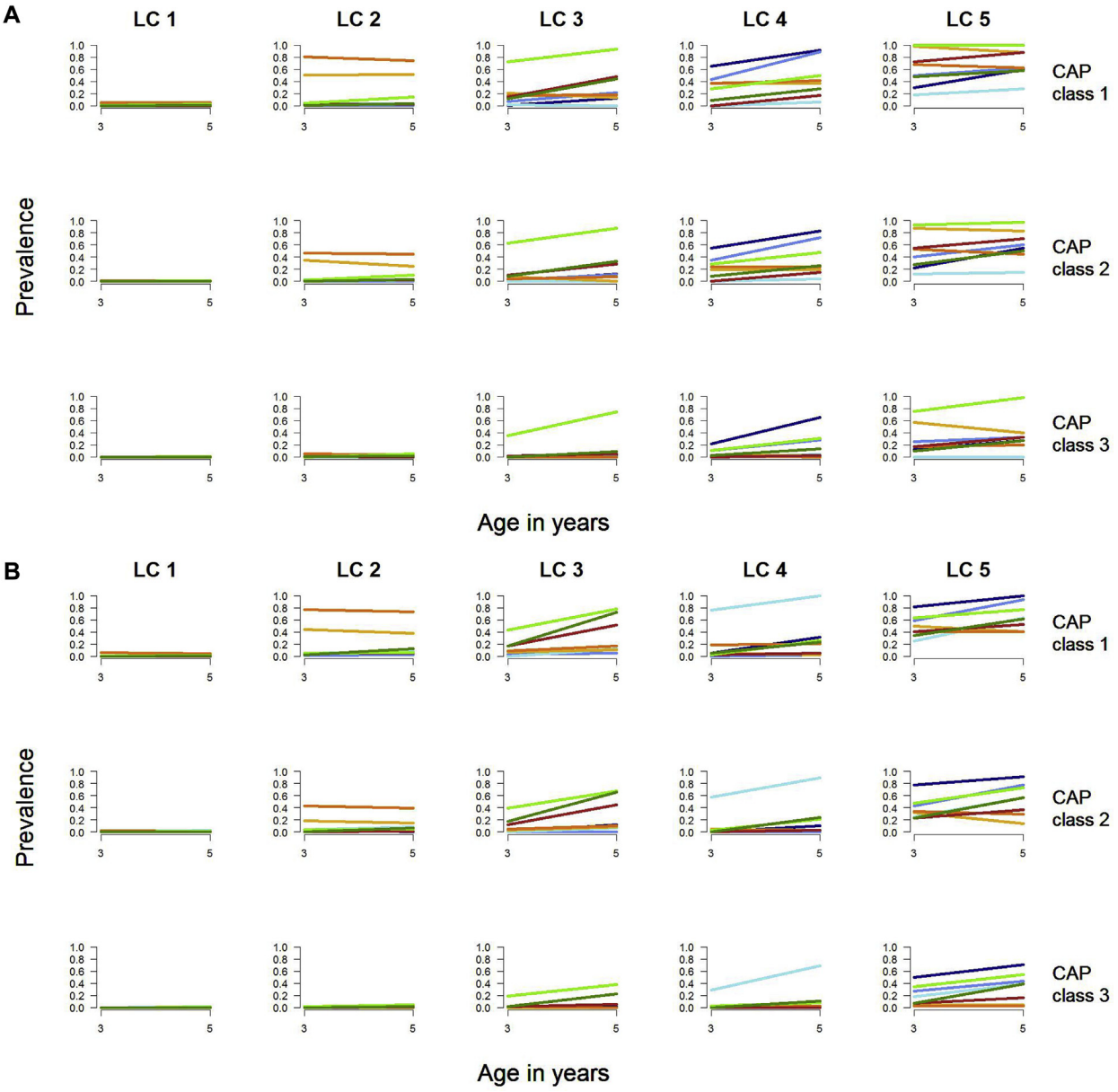

FIG 3. **A** and **B**, Prevalence of positive IgE levels for the 5-class solutions. Perennial inhalant allergen specificities: dark blue, cat; light blue, dog; very light blue, dust mite. Seasonal inhalant allergen specificities: light green, birch; dark green, timothy. Food allergen specificities: red, peanut; orange, milk; yellow, egg. Full samples included 1124 children in Finland and 1165 children in Estonia.

(OR, 9.39 [95% CI, 4.9-19.3] and 9.5 [95% CI, 5.2-17.5] for Finland and Estonia, respectively). The lower panel of Fig 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) (see Table E5 in this article's Online Repository at www.jacionline.org).

Despite its small size, the severe atopy class contributed prominently to wheeze, allergic rhinitis, and eczema, as shown by population-attributable risk fractions (PARFs; Fig 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 (see Fig E2, *B*) and the high-risk subpopulation (see Fig E3 in this article's Online Repository at www.jacionline.org).

DISCUSSION

Regardless of substantial environmental and socioeconomic differences and different atopy rates between Finland and Estonia, the postulated LCs of food-induced, seasonal, perennial, and severe atopy were found in both countries with high consistency. These 4 LCs corresponded to the previously established trichotomy of benign, symptomatic, and severe atopy,

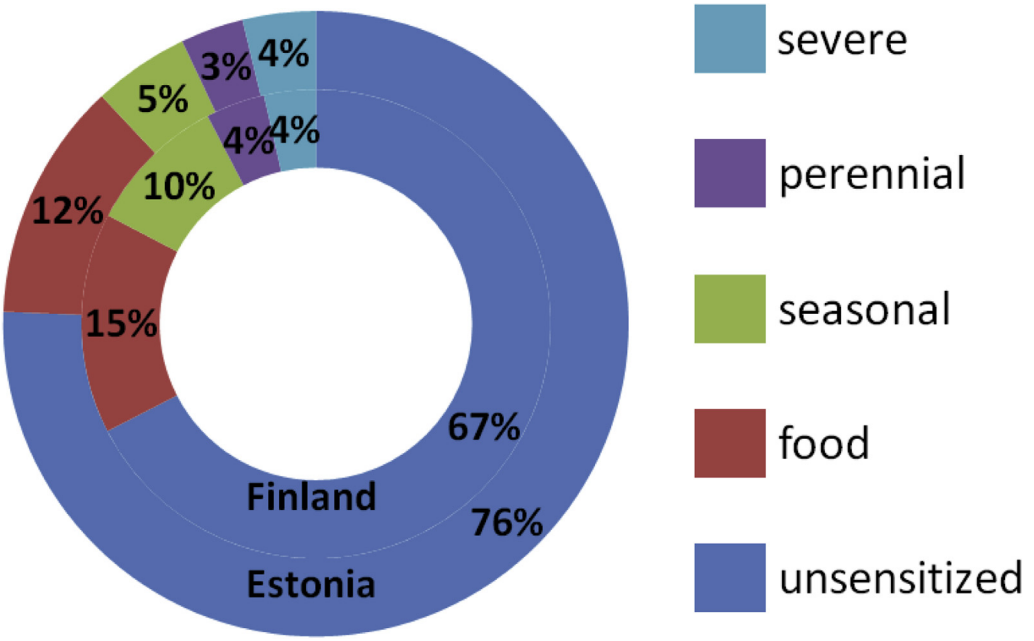

FIG 4. Distribution of LCs by country. Distribution of class sizes differs significantly between countries ($P < .001$).

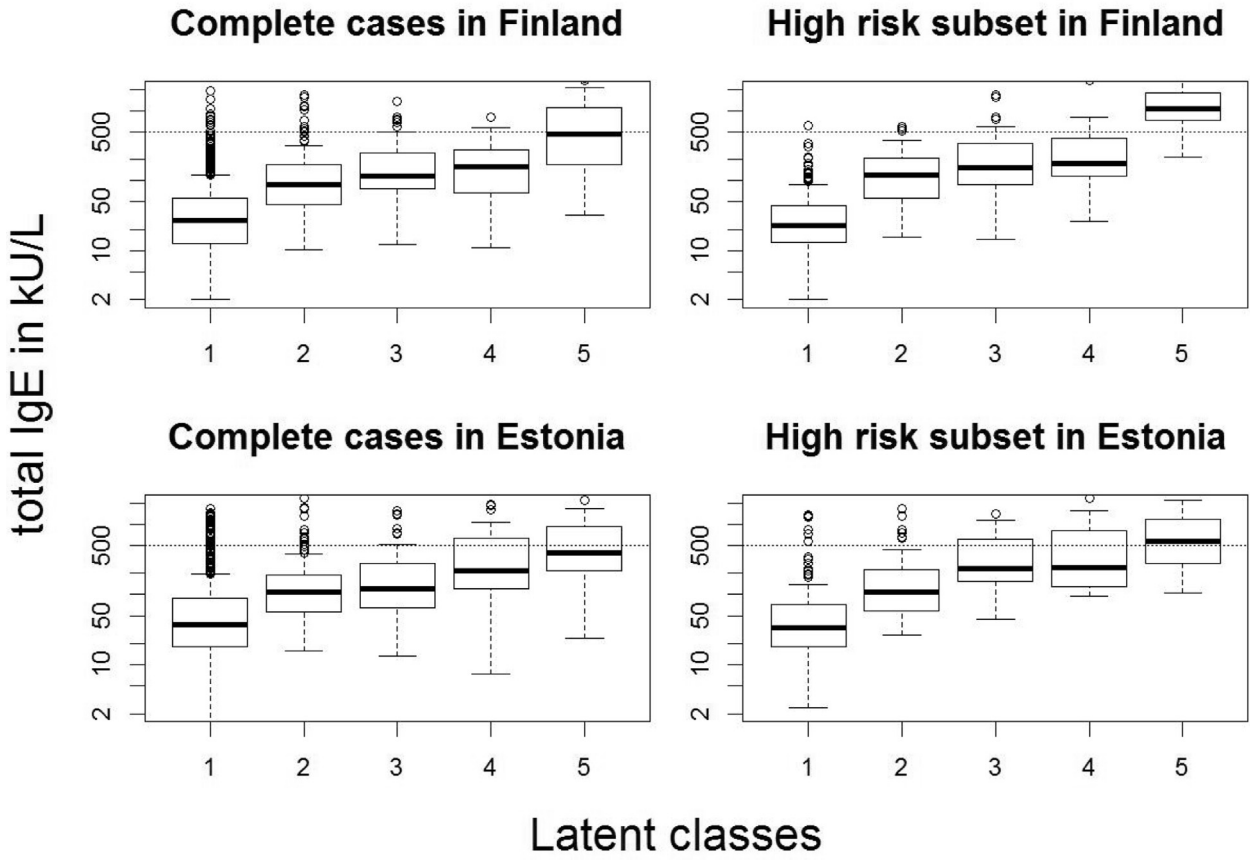

FIG 5. tIgE levels across LCA at 5 years. tIgE levels increase significantly over the LCs ($P < .001$ for all panels).

as shown by various degrees of disease relevance, tIgE levels, and family history of atopy. The greater sensitization prevalence in Finland was mainly attributable to the seasonal inhalant class, which also explained the greater proportion of allergic disease in Finland, as shown by the corresponding PARF. The severe atopy class was equal in size and explained equal

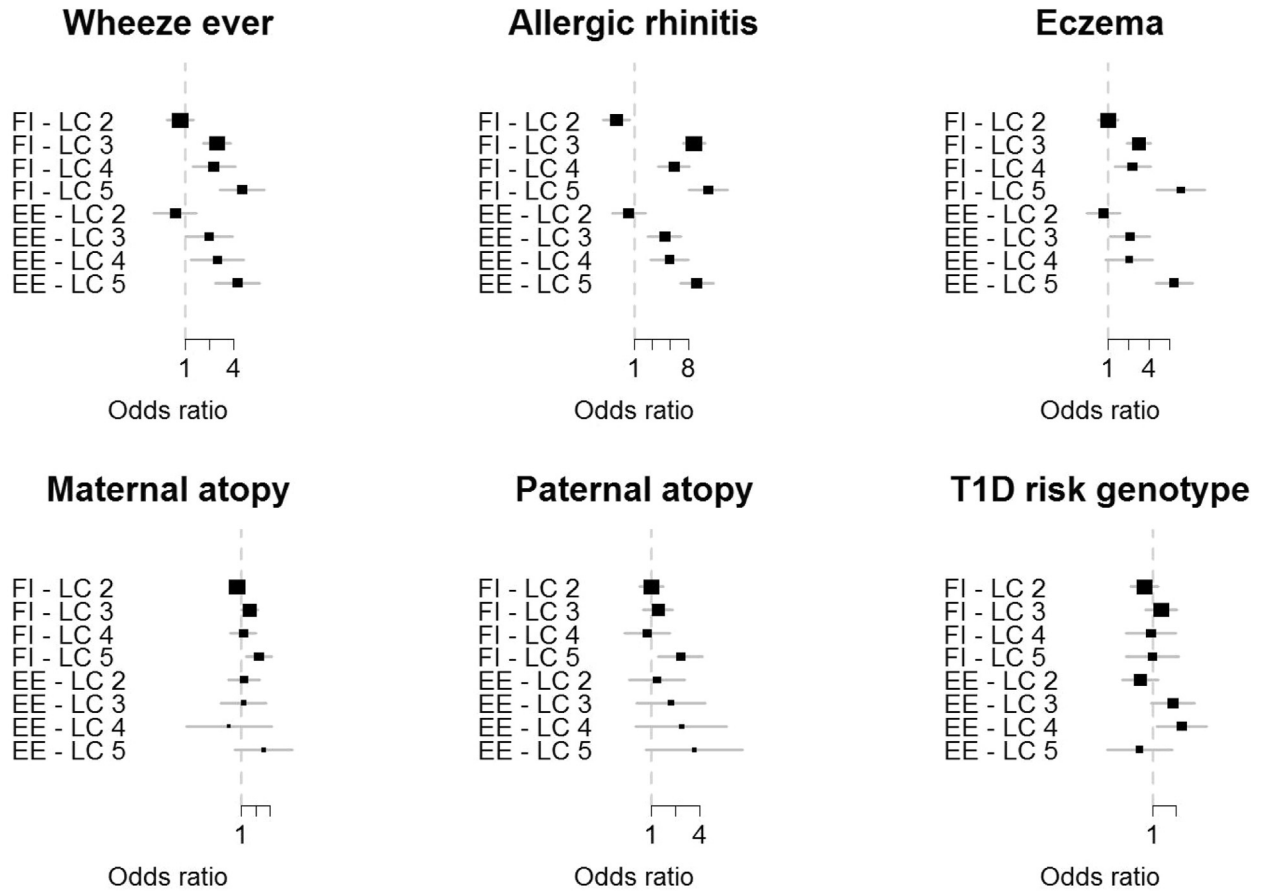

FIG 6. Associations of LCs with diseases and hereditary background. *EE*, Estonia; *FI*, Finland. ORs are presented with 95% CIs. There were many missing values for maternal and paternal history of atopy in the Estonian population, as indicated by smaller symbols for the effect estimate. T1D risk is represented by HLA-DQ risk alleles.

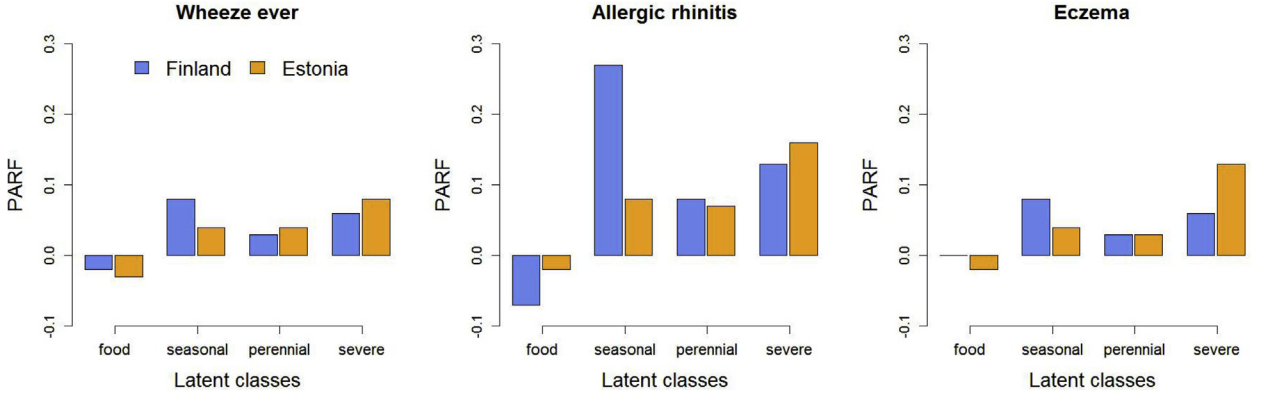

FIG 7. PARFs by LCs.

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 geographic proximity and similar climate, the studied regions of Finland and Estonia differ in many environmental exposures. During the 20th century, the 2 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 (Fig 1). This list is obviously incomplete; previous comparisons

of Eastern and Western 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 have been often observed between Eastern and Western Europe. For example, the incidence of allergic disease was found to be increased in urban areas and industrialized countries in comparison with the postsocialist countries of Eastern Europe.[31] An asthma diagnosis was more often observed in Swedish schoolchildren compared with Estonian children.[32] Similarly, the prevalence of atopic diseases was increased in West Germany in comparison with East Germany,[4] although it leveled out within a decade after reunification.[5]

In Karelia, another interface between Eastern and Western Europe, a gradient in asthma prevalence and atopic sensitization persists.[33] Although remaining stable in Russian Karelia, sensitization rates to pollen and 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 irrespective of environmental discrepancies. Only in the class of perennial sensitization did the lead allergen differ. Although 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 level might be more a marker for exposure,[37] which might be limited in Finland because of its higher latitude. At least for Swedish regions, a lack of dust mites has been described.[38]

However, the described gradient in atopic sensitization can resolve in response to environmental changes within a few years, as shown by the rapid assimilation of atopy prevalence within Germany[5] or between rural and urban Poland.[39] Thus atopy can virtually mirror environmental influences and illustrate the plasticity of the immune system also in adulthood.[40]

The question now arises of what atopy actually means. We have previously classified atopy forms using LCA in 2 birth cohorts: MAS, which was conducted in 5 major cities in Germany, and PASTURE, comprising children living in rural areas of 5 European countries.[12] Like in MAS and PASTURE, sIgE levels in this study were found to generally increase from 3 to 5 years of age, except for those for food sIgE.

The gradient of disease relevance previously detected in MAS and PASTURE was replicated in the 2 arms of the DIABIMMUNE study under investigation: Benign atopy included classes with sIgE only to food and without any disease association, although MAS revealed 1 food class and PASTURE revealed 2 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. Consistent with findings from MAS and PASTURE, severe atopy was characterized by the greatest 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, the Manchester and Isle of Wight studies also revealed a similar association of a highly sensitized class with reduced lung function at the age of 10 years.[42] In the current analysis pulmonary function testing was not feasible because of the young age of the children. Nevertheless, the strong relation to tIgE levels supports the specific severity of this atopy from. tIgE levels have been well 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 cosensitization.[46] In another study highly sensitized children at age 2 years were found to be 5 times more likely to have asthma within the following 2 years.[20] Likewise, the additional follow-up at age 4 years of the present study allowed an analysis of the dynamics of sIgE between ages 3 and 5 years, 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 might have clinical implications because atopy-related asthma forms ("late-onset wheeze") often manifest after age 4 years.[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 levels with mAbs.[48]

The increase in 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, such as atopy and diabetes, suggests common genetic traits.[49] Interestingly, we found the hereditary predisposition to T1D to be associated with inhalant LCs but not with severe atopy. This finding was robust over the 4- to 6-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 shown by allergic rhinitis and asthma, which was represented by wheeze at age 5 years. The rather low prevalence of autoimmune disease 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 and 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 might explain the different PARFs of severe atopy for eczema between countries.

On the other hand, the DIABIMMUNE study provided a unique opportunity to address the relationship between atopy and T1D. In fact, the hygiene hypothesis of asthma and allergies has

stimulated a vivid debate about whether infections in early childhood could foster or protect against β-cell autoimmunity.[52-54] An argument for the protective role of infections was the inverse relationship of T1D risk and sibship size observed by Cardwell et al[55] and replicated in the present study (data not shown). Furthermore, changes in early microbial exposure altering the maturation of the immune system are currently a matter of debate. Vatanen et al[56] found that LPSs 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.

To evaluate the robustness of the associations found in this study, we performed sensitivity analyses with 4- and 6-class models. In the 4-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 development of atopic diseases. The large sample size also allowed for a robust model with 6 classes, which separated the LC of severe atopy into a class with higher and lower classes, with relatively lower sIgE levels to seasonal allergens and moderate disease relevance. The latter dichotomy might again refine the characterization of severe atopy. Solutions with 7 or more classes were not explored because of the sample size, which could lead to insufficiently small class sizes.[42]

In conclusion, we found very similar patterns of LCs 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 2 different countries. The phenomenon of severe atopy was mainly determined by increased levels of sIgE and tIgE. The differential associations of LCs with allergic diseases and genetic T1D susceptibility might point toward distinct immunologic mechanisms linking the various forms of atopy to allergy and autoimmunity, which might be driven by the interaction of environmental and genetic background.

We thank Katriina Koski and Matti Koski (University of Helsinki) for the coordination and database work in the DIABIMMUNE study.

Key messages

- **Previously identified LCs of atopy were replicated in Finland and Estonia, 2 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.**
- **Differential associations of LCs with allergic disease and genetic T1D susceptibility might point toward distinct immunologic mechanisms linking the various forms of atopy to allergy and autoimmunity.**

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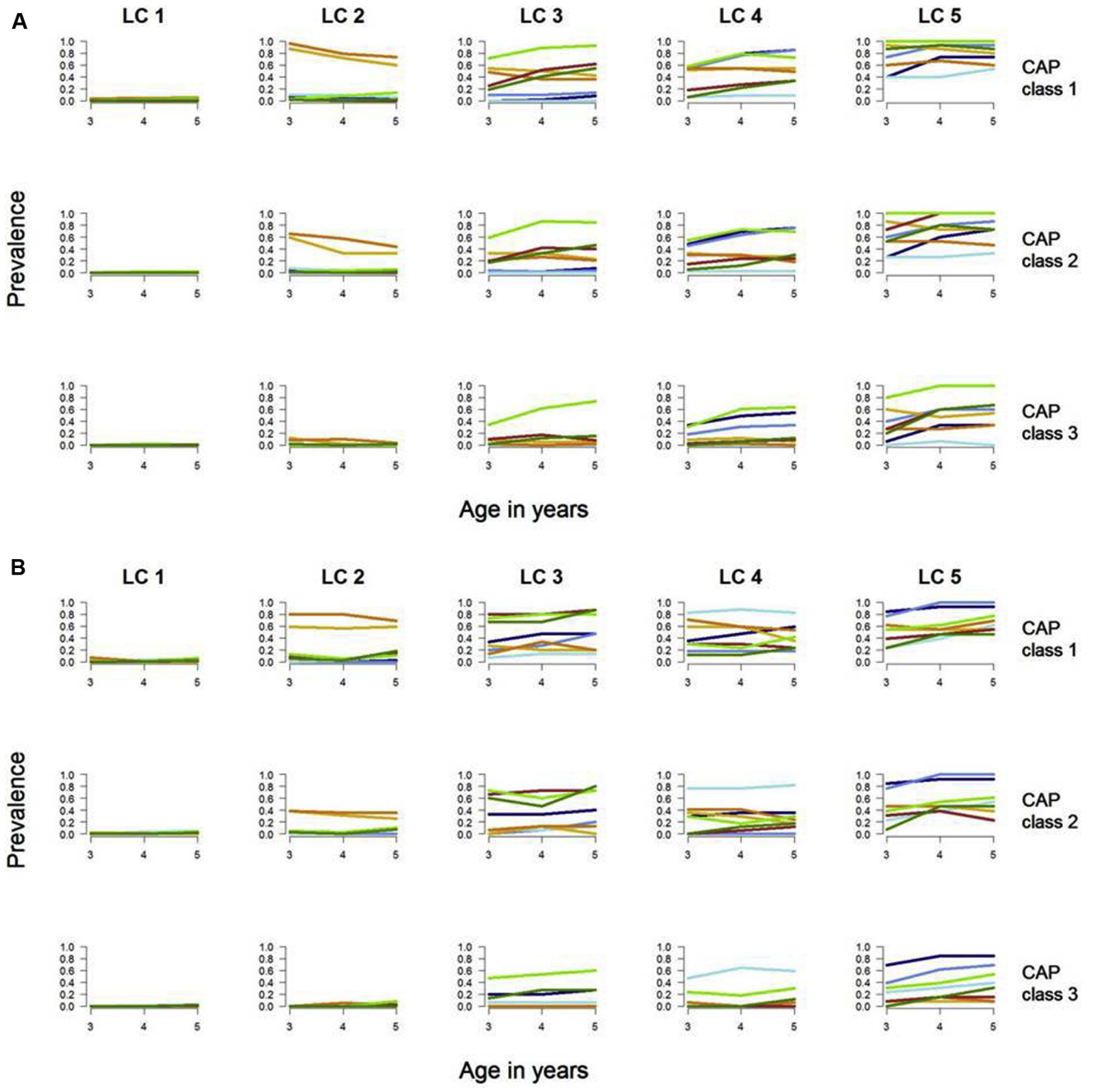

FIG E1. **A** and **B**, Prevalence of positive IgE levels for the 5-class solutions in the high-risk populations. Perennial inhalant allergen specificities: dark blue, cat; light blue, dog; very light blue, dust mite. Seasonal inhalant allergen specificities: light green, birch; dark green, timothy. Food allergen specificities: red, peanut; orange, milk; yellow, egg. High-risk populations included 357 children in Finland (Fig E1, *A*) and 198 children in Estonia (Fig E1, *B*).

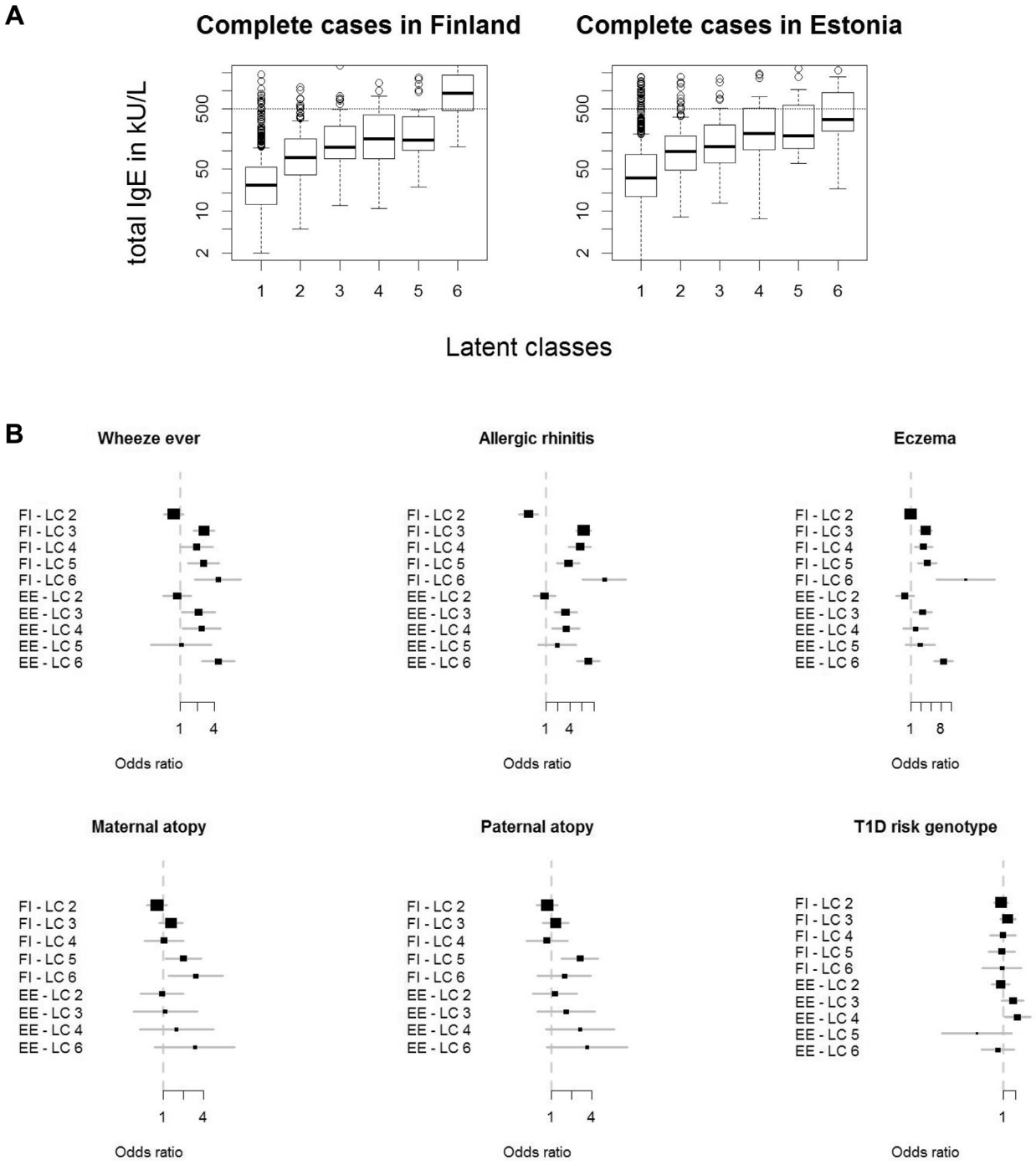

FIG E2. The 6-class solution. **A,** tIgE levels across LCs. **B,** Associations of LCs with diseases and hereditary background. *EE*, Estonia; *FI*, Finland.

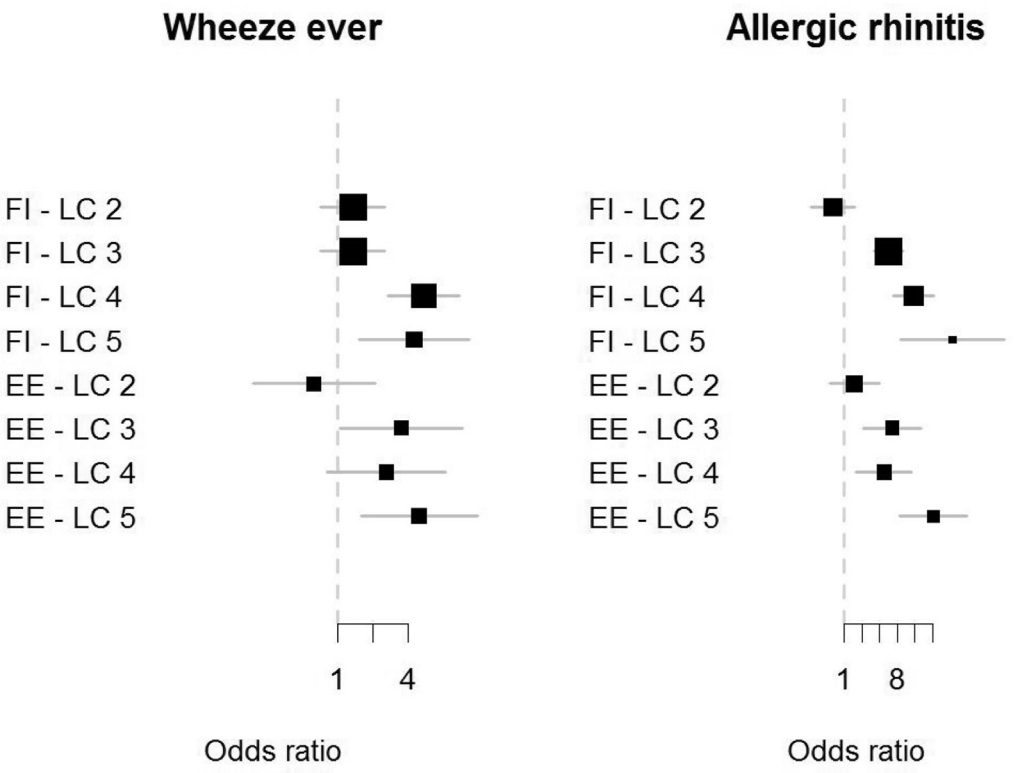

FIG E3. Disease associations in the high-risk population. Because of a lower sample size and empty cells, associations could not be calculated for eczema. *EE*, Estonia; *FI*, Finland.

TABLE E1. Description of study population by center

	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	.660
Female	735	45.8		787	47.5		
Socioeconomic background							
Level of education (mother)							
Low	243	15.2	192	511	30.8	104	<.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	<.001
High	1149	71.7		1246	75.2		
Smoking inside							
3 y	277	18.3		480	39.0		<.001
Environmental ambience							
Play outdoors							
Daily	1378	81.3		1132	64.8		<.001
Weekly	115	6.8	84	300	17.2	21	
seldom	26	1.5		204	11.7		
Farming							
Visit of barn	17	1.1	57	90	7.3	43	<.001
Daily	5	0.3	45	39	3.2	71	<.001
Occasionally	119	7.9	44	259	21.0	62	<.001
Farm milk consumption							
Daily/weekly	2	0.1	173	254	15.3	113	<.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	<.001
Parental diseases							
Parental diabetes	161	10.0	76	56	3.4	12	<.001
Paternal asthma	99	6.2	76	37	2.3	12	<.001
Maternal asthma	120	7.5	76	48	2.9	12	<.001

For comparison of study centers by characteristics in Table E1, *P* values were derived by using the Fisher exact test and Kruskal-Wallis test.
NA, Not available.

TABLE E2. 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
Sex						
Male	566	102	.227	284	30	.199
female	497	108		255	38	
High annual income						
Low	693	86	.501	241	33	.278
High	521	114		291	33	
Maternal education						
Low	166	23	.027	150	23	.411
Intermediate	411	74		309	38	
High	469	111		80	6	
Farming*	14	3	.583	62	28	.631
Allergic rhinitis*	184	24	.051	104	15	.884
Atopic eczema at age 3 y*	332	61	.626	136	23	.363
Wheezing*	257	59	.189	133	18	.999
Parental asthma*	139	26	.911	27	2	.761
Paternal diabetes*	120	15	.085	13	2	.680

P values were derived by using the Fisher exact test or Kruskal-Wallis test.
*Binary variables: only positive (= yes) data are shown.

TABLE E3. Model fit criteria

	Finland			Estonia		
Classes	**AIC**	**BIC**	**Entropy**	**AIC**	**BIC**	**Entropy**
All children (2 time points)						
4	11,153	12,113	95.99%	9,476	10,463	95.93%
5	11,010	12,211	96.45%	9,385	10,619	96.28%
6	10,930	12,372	95.8%	9,330	10,813	94.42%
High risk (3 time points)						
4	7,476	8,573	99.45%	4,381	5,338	99.37%
5	7,383	8,756	99.49%	4,368	5,565	99.49%
6	7,344	8,992	99.31%	4,410	5,847	99.53%

AIC, Akaike information criterion; *BIC*, Bayesian information criterion.

TABLE E4. 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%

Distribution of classes of the high-risk subpopulation varies in size between countries with borderline significance ($P = .070$).

TABLE E5. ORs to Fig 6 in the main body

Country	LC	Condition	OR (95% CI)	*P* value
EE	LC2	Wheeze ever	0.74 (0.39-1.31)	.33
EE	LC3	Wheeze ever	1.95 (0.94-3.74)	.056
EE	LC4	Wheeze ever	2.48 (1.08-5.16)	.021
EE	LC5	Wheeze ever	4.48 (2.29-8.58)	<.001
EE	LC2	Allergic rhinitis	0.81 (0.4-1.51)	.537
EE	LC3	Allergic rhinitis	3.22 (1.62-6.05)	<.001
EE	LC4	Allergic rhinitis	3.86 (1.73-7.92)	<.001
EE	LC5	Allergic rhinitis	11.02 (5.75-21.12)	<.001
EE	LC2	Eczema	0.86 (0.47-1.48)	.608
EE	LC3	Eczema	2.1 (1.03-3.94)	.03
EE	LC4	Eczema	2.05 (0.86-4.39)	.078
EE	LC5	Eczema	9.21 (4.85-17.29)	<.001
EE	LC2	Maternal atopy	1.16 (0.51-2.36)	.704
EE	LC3	Maternal atopy	1.13 (0.32-3.03)	.834
EE	LC4	Maternal atopy	0.55 (0.03-2.89)	.572
EE	LC5	Maternal atopy	2.94 (0.63-10.91)	.124
EE	LC2	Paternal atopy	1.16 (0.49-2.46)	.714
EE	LC3	Paternal atopy	1.73 (0.57-4.39)	.286
EE	LC4	Paternal atopy	2.36 (0.52-8)	.2
EE	LC5	Paternal atopy	3.42 (0.72-12.68)	.082
EE	LC2	T1D risk genotype	0.7 (0.4-1.17)	.199
EE	LC3	T1D risk genotype	1.8 (0.93-3.29)	.064
EE	LC4	T1D risk genotype	2.32 (1.08-4.62)	.023
EE	LC5	T1D risk genotype	0.69 (0.24-1.63)	.447
FI	LC2	Wheeze ever	0.85 (0.57-1.26)	.442
FI	LC3	Wheeze ever	2.46 (1.62-3.71)	<.001
FI	LC4	Wheeze ever	2.27 (1.21-4.18)	.009
FI	LC5	Wheeze ever	5.1 (2.66-9.97)	<.001
FI	LC2	Allergic rhinitis	0.51 (0.29-0.84)	.011
FI	LC3	Allergic rhinitis	9.87 (6.42-15.18)	<.001
FI	LC4	Allergic rhinitis	4.62 (2.48-8.41)	<.001
FI	LC5	Allergic rhinitis	16.95 (8.33-37.34)	<.001
FI	LC2	Eczema	1.01 (0.7-1.45)	.94
FI	LC3	Eczema	2.83 (1.9-4.26)	<.001
FI	LC4	Eczema	2.29 (1.25-4.18)	.007
FI	LC5	Eczema	11.59 (5.31-28.79)	<.001
FI	LC2	Maternal atopy	0.82 (0.57-1.16)	.274
FI	LC3	Maternal atopy	1.48 (0.97-2.23)	.066
FI	LC4	Maternal atopy	1.11 (0.58-2.05)	.744
FI	LC5	Maternal atopy	2.34 (1.23-4.53)	.01
FI	LC2	Paternal atopy	0.99 (0.68-1.4)	.936
FI	LC3	Paternal atopy	1.21 (0.78-1.84)	.393
FI	LC4	Paternal atopy	0.88 (0.44-1.67)	.704
FI	LC5	Paternal atopy	2.29 (1.21-4.39)	.011
FI	LC2	T1D risk genotype	0.79 (0.52-1.19)	.276
FI	LC3	T1D risk genotype	1.28 (0.8-1.99)	.283
FI	LC4	T1D risk genotype	0.95 (0.44-1.88)	.899
FI	LC5	T1D risk genotype	1 (0.44-2.05)	.999

EE, Estonia; *FI*, Finland.

TABLE E6. ISAAC questions

Variable	ISAAC question	Response options	Operationalized
Atopic Eczema	Has your child ever had an itchy rash that was coming and going for at least 6 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 1-3 4-12 >12	>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 nose when he/she did not have a cold or the flu?	Yes No	

ISAAC, International Study of Asthma and Allergies in Childhood.