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Significant disparities in allergy prevalence and microbiota between the young people in Finnish and Russian Karelia

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Short title: Allergy gap between young people in Finnish and Russian Karelia

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Key words: allergy, Finland, microbiota, Russia, specific IgE.

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Abbreviations: ISAAC: International Study of Asthma and Allergies in Childhood; sIgE: allergen-specific serum immunoglobulin E; OTU: operational taxonomic unit

Abstract:

Background: Atopic allergy has been more common among schoolchildren in Finland, as compared to Russian Karelia. These adjacent regions show one of the most contrasting socio-economically differences in the world.

Objective: We explored changes in allergy from school age to young adulthood from 2003 to 2010/2012 in these two areas. The skin and nasal microbiota were also compared.

Methods: Randomly selected children from Finnish ($n = 98$) and Russian Karelia ($n = 82$) were examined in 2003, when the children were 7–11 years of age, and again in 2010 (Finnish Karelia) and 2012 (Russian Karelia). We analysed self-reported allergy symptoms and sensitization to common allergens by serum sIgE values. The skin (volar forearm) and nasal mucosa microbiota, collected in 2012 (age 15–20 yrs), identified from DNA samples, were compared with multivariate methods.

Results: Asthma, hay-fever, atopic eczema, self-reported rhinitis, as well as atopic sensitization, were 3–10 fold more common in Finland, as compared to Russian Karelia. Hay-fever and peanut sensitization were almost non-existent in Russia. These patterns remained throughout the 10-year follow-up. Skin microbiota, as well as bacterial and fungal communities in nasal mucosa was contrastingly different between the populations, best characterised by the diversity and abundance of genus *Acinetobacter*; more abundant and diverse in Russia. Overall, diversity was significantly higher among Russian subjects ($P_{\text{skin}} < 0.0001$, $P_{\text{nasal-bacteria}} < 0.0001$, and $P_{\text{nasal-fungi}} < 0.01$). Allergic diseases were not associated with microbial diversity in Finnish subjects.

Conclusions & Clinical Relevance: Differences in allergic phenotype, developed in early life, remain between populations. A parallel difference in the composition of skin and nasal microbiota suggests a potential underlying mechanism. Our results also suggest that high abundance and diversity of *Acinetobacter* might contribute to the low allergy prevalence in Russia. Implications of early life exposure to *Acinetobacter* should be further investigated.

1. Introduction

Allergies and other chronic inflammatory disorders have steadily increased in the Western world during the past fifty years [1–4]. This megatrend has been related to changes in lifestyle and environment [1,2,4,5]. The border between Finland and Russia marks one of the sharpest boundaries between modern and developing health and living standards in the world. The Republic of Karelia in North-Western Russia (Russian Karelia) was almost completely closed to visitors during the Soviet era until 1991. Even today, people are living in a largely rural environment in the midst of cattle and other domestic animals, and they produce a great deal of their food in their own gardens. The household water is often taken from their own wells and nearby springs. The natural environment is relatively well preserved, although pulp mills, mining and deforestation have had some adverse effects on the environment. Highly developed and built environments are scarce in Russian Karelia. In contrast, the environment and lifestyle of people in North Karelia in eastern Finland (Finnish Karelia) are notably more western and modern than in Russian Karelia, although a large part of this region remains rural. The Russian Karelia and the Finnish Karelia have the same climatic and geographical features, and hence comparison of their populations provides an opportunity to examine allergies and individual microbiota in contrasting living conditions. The populations share partly the same ancestry, and about 15% of the current population of the Karelian Republic are Finns or Karelians.

In Finland, the recent allergy and asthma trends resemble those in other developed countries. The prevalence of allergy has increased for decades [2,6,7], though the increase may now be levelling off, at least for asthma [8]. In 2003, schoolchildren from Finnish and Russian Karelia were examined [9–11], and the results showed that increased serum IgE levels and self-reported allergic symptoms were manifold in Finland. For example, Finnish children showed skin prick test positivity to timothy grass in 28.2 %, as compared with 4.8 % in Russian children, and hay fever was reported in 15.6 % of Finnish and 1.1 % of Russian children. Ten years later, a part of the same children were re-examined for symptoms and sIgE levels. In addition, the skin and nasal microbiota on both sides of the border were explored to see how lifestyle and living environment affect microbial colonization.

2. Methods

Study cohorts

In 2003, randomly selected children, aged 7–16 years, were examined in North Karelia, Finland (Finnish Karelia; $n = 344$) and in Pitkäranta, the Republic of Karelia (Russian Karelia; $n = 427$) [9–11]. A follow-up study was conducted some ten years later. Children who were 7–11 years old in 2003 and had participated in the first study were invited to the follow-up (Finnish Karelia $n = 205$, Russian Karelia $n = 209$). This age group was invited because many of the older subjects (12–15 years old in 2003) had moved away from home.

The follow-up consisted of 118 Finnish (participation rate 58%) and 93 Russian (participation rate 45%) subjects. Only those participants for whom specific IgE (sIgE) measurements were available for both study periods were included in the present analyses of questionnaire data and serum samples (Finnish Karelia $n = 98$: 38 males, 60 females; Russian Karelia $n = 82$: 39 males, 43 females). The

study protocol was approved by the Ethics Committee of the Helsinki University Central Hospital, a study permission for the Russian Karelia was obtained from the Petrozavodsk State University in the Republic of Karelia, and written informed consent was obtained from all participants.

In Finnish Karelia, the follow-up study was performed in 2010, when the children were 14–18 years old. In Russian Karelia, the follow-up study was conducted, due to logistic reasons in 2012, when the participants were 16–20 years old. All surveys were carried out in the autumn. In 2012, additional samples were collected from the Finnish cohort ($n = 76$). In Russia, in addition to the original cohort, samples from an extra cohort of 88 subjects aged 15–19 years (34 males, 54 females) were collected. For the microbial analyses only 2012 data from the original cohorts (aged 16–20 years) and the extra Russian cohort (aged 15–19 years) were used.

Measurements

Data from survey questionnaires as well as total and allergen-specific sIgE measurements were analysed. The questionnaire was an extended phase I of the International Study of Asthma and Allergies in Childhood (ISAAC) questionnaire on demographic factors, home/living conditions, and on the occurrence of atopic symptoms and diseases in children. In the baseline study the parents, but in the follow-up study the participants themselves filled in the survey questionnaires. Here we use data from seven questions from the baseline and the follow-up survey: physician-diagnosed and self-reported asthma, self-reported hay fever and atopic eczema at any time as well as self-reported rhinitis during the last 12 months (sneezing /runny /blocked nose not associated with flu or common cold).

Serum IgE (sIgE) antibodies were measured against eight common inhalant allergens (Phadiatop®: timothy grass, birch, mugwort, *Cladosporium herbarum*, horse, cat, dog and house dust mite i.e. *Dermatophagoides pteronyssinus*) and six common food allergens (cow's milk, hen's egg, cod-fish, soy, wheat and peanut) as well as Phadiatop® for inhalant and food allergens were measured using a radioallergosorbent test (UniCAP 1000 v.2; Pharmacia Upjohn, Uppsala, Sweden). To classify individuals to either healthy or sensitized, based on total sIgE, a cut-off of 110 kUA/l was used. For specific sIgE concentrations two cut-offs were used: 0.35 and 2.5 kUA/l.

The skin microbiota was sampled, during the later follow-up in 2012, by lightly pressing a sterile nylon swab (Copan Diagnostics) dipped in sterile 0.15 M NaCl + 0.1% Tween 20 solution against the skin on a 5 × 5 cm area of the volar surface of the forearm of the subject's writing hand, midway between the wrist and elbow. The nasal microbiota was sampled using sterile nylon swabs, moistured as above, by gently pressing and rotating the swab 5 times in the right anterior nostril. All samples were kept in –70°C for further analyses. The sequencing of these samples is described in Text E1 in the Supporting Information.

Statistical analyses

Clinical data

We calculated the frequencies and 95% confidence intervals of physician-diagnosed and self-reported symptoms as well as subjects with total sIgE ≥ 110 kUA/l and specific sIgE ≥ 0.35 kUA/l. The difference between the study areas in 2003 and in 2010/2012 were analysed with chi-square tests. We also calculated the frequency of subjects with the higher cut-off sIgE ≥ 2.5 kUA/l for selected allergens. Interactions between study area and year were tested in logistic models for asthma, allergic symptoms and sensitization to allergens. Statistical analyses for tables 1 and E1 and Fig. 1 were performed with the Stata statistical software package, version 11.0 (Stata Corp., College Station, TX, USA).

Log-transformed IgE values for inhalant allergens (the sum of specific sIgEs for house dust mite, other animals and pollens), and for food allergens (the sum of measured specific food sIgEs) were calculated and values in 2003 were plotted against values in 2010/2012 to observe intrapersonal changes in sensitization (Fig. 2). The distributions of log-transformed sums of sIgE are presented by study area and round (Fig. E1). These analyses were run and the figures drawn with the statistical software package R [12].

The IgE values for the original Russian cohort and the additional sample (88 subjects, only used in the microbial analyses) in 2012 were compared. No notable differences between these samples were observed.

Microbial data

Before any further analysis, raw sequence counts were normalised with cumulative sum normalisation in the metagenomeSeq package in R [13]. This method aims to remove biases in the count data resulting from sample-specific amplification patterns. CSS normalisation is an adaptive extension of the quantile normalization approach where raw counts are divided by the cumulative sum of counts up to a percentile determined using a data-driven approach [14]. This sort of sample-specific normalisation is recommended over sample rarefaction [15].

Microbial diversity in each sample was quantified as the 'true' or Hill diversity [16,17]:

$${}^qD_{ij} = \left(\sum_i^S p_{ij}^q \right)^{1/(1-q)}, \quad (1)$$

where p_{ij} is the proportional abundance for OTU i in sample j , and q is the so-called Hill's parameter specifying the weight given to abundance. The Hill diversity of order 1 ($q = 1$) is related to the Shannon diversity index (in this case ${}^1D = \lim_{q \rightarrow 1} {}^qD$, approximated as $\exp(-\sum p_{ij} \log(p_{ij}))$). When $q = 0$, eqn. (1) returns species richness, but the diversity estimate becomes increasingly unreliable when $q < 1$, which is a general problem with diversity estimates that do not consider species abundances [17].

To compare community composition between samples, we calculated the Bray & Curtis dissimilarity [18] for microbial samples, based on normalised OTU-counts (OTU = Operational Taxonomic Unit). This was used to visualize (using Principal Coordinate Analysis; PCoA) and to numerically analyse (with multiple regression on distance matrices, MRM, as implemented in the vegan package in R

[19], where the statistical significance was calculated based on 9999 random permutations) differences between study populations, controlling for subject age, sex, and sensitization. To identify OTUs best characterizing different populations, Random Forest (RF) classification was used, using 1000 trees, as implemented in the Random Forest package in R [20], where the Mean Decrease in Accuracy for each group was used as a feature selection criterion.

Based on the RF-classification, we selected OTUs best differentiating the populations for further oligotyping, as described in [21]. This allowed us to look beyond the OTU level classification and to compare differences between sensitized and healthy subjects in both populations. The nasal samples were chosen here, because of the better quality in data coming from MiSeq, as compared to 454 reads. The MiSeq sequences were also longer (median 470 bp) than 454 reads after trimming (400 bp). Briefly, all sequences matching a given OTU were extracted and aligned with MAFFT [22]. Oligotyping parameters were tuned based on the entropy plots. We also calculated oligotype diversity for each sample in each OTU, by setting the Hill's parameter $q = 2$ in eqn. (1) (equivalent to the inverse Simpson index). To compare differences in oligotype profiles between groups, we calculated the between-sample Bray & Curtis dissimilarity, as above.

3. Results

Populations remain different in their symptom and sensitization profiles

Both physician-diagnosed and self-reported asthma, hay fever, and atopic eczema were rare in Russian Karelia compared to Finnish Karelia in both study periods (**Fig. 1, Table E1 in Supporting Information**). In schoolchildren in Russian Karelia, no physician-diagnosed hay fever was reported by parents in 2003, and only one subject reported it in 2012. No significant changes in asthma, hay fever and atopic eczema were observed in either area, and there was no significant interaction between study area and year. The prevalence of self-reported rhinitis during the last 12 months was higher in Russian Karelia than the other symptoms and increased significantly in both areas during the follow-up.

The sIgE measurements specific to inhalant allergens in 2003 and in 2010/2012 were highly correlated in both study areas (FIN: $r = 0.83$, $P < 2.2e^{-16}$; RUS: $r = 0.71$, $P = 9.8e^{-14}$), indicating that the level of sensitization had remained relatively constant in the study subjects. For food allergens the comparable correlation was high in Finland ($r = 0.76$, $P < 2.2e^{-16}$) but in Russian Karelia there was a slight decrease in the level of sensitization and no significant correlation ($r = 0.13$, $P = 0.24$; note that there were only a few positive values).

The sIgE profiles were clearly different between the Finnish and Russian Karelia (**Table 1, Figs 1, 2 & E1 in the Supporting Information**). The sIgE levels for inhalant allergens were higher in Finnish Karelia for positive sIgE values (**Figs. 2, E1**). House dust mite was an exception, with a higher prevalence in Russian subjects in both study periods, though this difference was not statistically significant. Similarly, the mean sIgE value against the house dust mite was higher in Russia (11.5 kUA/l in 2003 and 4.2 in 2012) than in Finland (1.0 kUA/l in 2003 and 0.8 in 2010) (difference between the study areas $P = 0.06$ in 2003, $P = 0.036$ in 2010/2012). Considering sIgE specific to food allergens, the differences between the study areas were even greater, though the number of subjects with positive sIgE values was much smaller than for inhalant allergens. Sensitization to

peanut, a common food allergen, was practically non-existent in Russian Karelia, as only one subject had a positive IgE response in 2003 and none in 2012 (**Table 1**).

Skin and nasal microbial profiles differ across the border

At the later follow-up in 2012, microbial samples were collected from both populations. Subjects from the Finnish and Russian Karelia clearly differed in their skin ($R^2 = 0.04$, $P = 0.0001$; **Fig. 3a**) and nasal ($R^2 = 0.03$, $P = 0.0001$; **Fig. 3b**) bacterial community compositions. The (Shannon) diversity was also higher in Russian samples than in Finnish samples for both skin ($P = 2.7e^{-8}$) and nasal ($P = 7.0e^{-6}$) microbiota (**Fig. E2**). Random forest feature selection and classification analysis of the microbial composition of samples indicated that the Finnish microbiota was enriched in *Micrococcus* and *Corynebacterium* in both cases (**Fig. 3a,b**), while *Acinetobacter*, *Aerococcus*, and *Jeotgalicoccus* were characteristic of the microbiota in Russian Karelia (**Fig. 3a,b**). For example, the abundance of *Acinetobacter* OTU-72 was on average 3 times higher on the skin ($P = 3.1e^{-5}$) and 4 times higher on the nasal epithelium ($P = 1.2e^{-21}$) in Russian Karelia, as compared to Finnish subjects. In addition to bacterial communities, fungal communities also differed between Finnish and Russian Karelia ($R^2 = 0.01$, $P = 0.0001$, **Fig. 3c**), the Russian fungal flora being more diverse ($P = 0.003$, **Fig. E2**). Based on the random forest classification analysis, the most discriminative features for the Russian Karelia belonged to the genera *Aspergillus* and *Phoma* (**Fig. 3c**).

Next we used oligotyping to compare the “strain” level diversity on the top discriminative bacterial OTUs between the countries. The analysis included OTU-72 (100% identity to *Acinetobacter lwoffii*) and OTU-142 (100 % identity to *A. johnsonii*), which were both more abundant in Russian subjects than in Finnish subjects (comparison of sqrt-transformed relative abundance: $P = 3.7e^{-9}$ and $P = 3.7e^{-10}$, respectively; **Fig. 4a,b**), while differences between healthy and sensitized subjects were not significant. We also considered OTU-231 (100 % identity to *Micrococcus luteus* or *M. flavus*, depending on the oligotype), which tends to be more abundant among Finns ($P = 1.5e^{-6}$, **Fig. 4c**).

In addition to the differences in abundance, we found that the oligotype diversity of *Acinetobacter* OTUs 72 and 142 were higher in Russian subjects than in Finnish subjects ($P = 7.6e^{-8}$ and $P = 7.6e^{-5}$, respectively), while the opposite was true for *Micrococcus* OTU-231 ($P = 2.4e^{-5}$). Furthermore, the oligotype profile of *Acinetobacter* OTU-72 in samples from sensitized Finns differed from all other samples ($R^2 = 0.014$, $P = 0.020$, **Fig. 4a**). The other two OTUs (*Acinetobacter* OTU-142 and *Micrococcus* OTU-231) had also a different oligotype profiles between Finns and Russians ($R^2 = 0.02$, $P = 0.002$ and $R^2 = 0.04$, $P = 0.004$, respectively). No significant difference was found between sensitized and healthy subjects for *Acinetobacter* OTU-142, whereas for *Micrococcus* OTU-231 oligotype profiles tended to differ between healthy and sensitized among the Russian subjects ($R^2 = 0.02$, $P = 0.043$; **Fig.4c**).

As a control, we also analysed the oligotype diversity of some of the most abundant OTUs found in both populations, in addition to the top discriminative OTUs. The oligotype- diversity in these OTUs did not differ between any groups (**Fig. E2**), which indicates that the observed differences for the discriminative OTUs are biological rather than technical artefacts or random noise.

4. Discussion

Hay fever and food allergies were almost non-existent among the youth in Russian Karelia and the difference in prevalence of allergic symptoms and atopic sensitization has remained largely unchanged compared with the Finnish youth during the roughly 10-year follow-up period. Asthma, hay fever, or atopic eczema at any time, and self-reported rhinitis (not associated with flu) were substantially more common among schoolchildren and young people in Finnish than Russian Karelia. The same was true for atopic sensitization assessed by measuring allergen-specific sIgE levels. As expected, the prevalence of symptoms and positive sIgE responses increased somewhat in both areas as the subjects grew older, but the difference between the two populations remained. In the second study period, the Russian subjects were two years older (16–20 years) than the Finnish subjects (14–18 years) thus having had more time to become sensitized. This makes our analysis conservative.

Our study supports previous findings indicating that early life exposure to beneficial environmental microbiota may confer protection against allergic manifestations in later life [23–25]. Our recent work similarly highlights the importance of the living environment in developing atopy [26]. The ‘westernization/urbanization’ process has been slow in Russian Karelia in 2003–2012. In a previous study among adult Finns, the prevalence of sensitization to inhalant allergens increased with successive birth years from 1944 to 1983 [10]. This was in striking contrast to successive Russian cohorts, where no marked change was observed. Based on the present results, we cannot assess any recent changes in allergies among younger cohorts due to possible changes in lifestyle and the living environment. For that a comparison across birth cohorts would be needed. This would also be required to better link early-life exposure to later allergic outcomes. Here we can only assume that the exposure has remained similar through life, but this is practically impossible to control apart from the immediate living environment remaining the same through the study period.

A noteworthy finding was that sensitization to house dust mite was more common among the Russian than Finnish subjects, whereas sensitization to pollens and animal allergens showed the opposite pattern. We have suggested previously that monosensitization to mite is possibly a marker of exposure and natural defence in mite rich environment and not necessarily associated with clinical disease [27]. Indeed, the exposure to mite was much stronger in Russia, where the microscopic analysis showed high house dust mite counts (mean 125 mites/g dust), whereas mites were virtually absent in Finnish dust samples. In general, the evidence from Russian Karelia does not support the concept that increased exposure to house dust mite in early life leads to increased risk of asthma and related symptoms later in life. Furthermore, it was remarkable that sensitization to peanut, a common food allergen, was lacking among the Russian schoolchildren and young people.

Some allergic symptoms such as hay fever were practically non-existent among children and young people in Russian Karelia both in 2003 and 2012. The differences in health care systems and practices between Russia and Finland may explain some of the contrast, but the self-reported symptoms were as infrequent as physician-diagnosed conditions in Russian Karelia, which suggests that there are no major diagnostic biases.

It is recognised that early-life exposure to environmental microbiota can protect against later allergies [28]. This exposure is likely to depend on the living environment and lifestyle, which have been shown to affect the composition of human microbiota [26,29,30]. In this light, the contrasting differences in the composition of microbiota between Finnish and Russian Karelia may be biologically related to the different sensitization profiles, as both the living environment and lifestyle have mostly remained unchanged in the study subjects. While this does not prove a causal relationship between the observed contrasting differences in allergic sensitization and clinical symptoms, and composition of microbiota between the populations, it is a plausible explanation. For example, previous analyses of the same individuals have found that exposure to environmental chemicals or epigenetic modifications are unlikely explain the allergy gap [31,32].

One clear example of the contrasting differences in microbial composition between the populations is the significantly higher abundance of genus *Acinetobacter* in Russian subjects, as compared to Finnish subjects. Furthermore, the results indicate that *Acinetobacter lwoffii* is significantly less diverse among sensitized Finns than the rest. Bearing in mind that Russian subjects classified as sensitised are unlikely to develop any allergies, contrary to Finnish subjects, this suggests that the abundance and diversity of *A. lwoffii* could be of clinical importance. This is, in turn, in accordance with our previous experimental results, demonstrating a prominent, systemic immunoregulatory role for *A. lwoffii* [33]. Still, establishing a causal relationship between the microbiota and health requires more elaborate immunological experiments.

An indication of higher microbial exposure in Russian Karelia is that skin and nasal microbiota was found to be significantly more diverse than in Finnish Karelia. Recently, Birzele et al. [34] have reported that higher microbial exposure to the airways from mattress dust is inversely associated with asthma. In the present case, however, no association between nasal microbial diversity and asthma (~12% prevalence among Finnish subjects) could be found ($P = 0.23$; controlling for age and sex). There was also no significant difference in skin ($P = 0.21$), nor nasal ($P = 0.11$) diversity between healthy subjects and those with atopic eczema (~25% cumulative prevalence among Finnish subjects). While this is in agreement with results by Kong et al. [35], there might be differences in other body sites [35]. We did not sample faecal microbiota (namely due to bureaucratic and logistic difficulties), but previous studies suggest that low gut microbial diversity might be associated with both asthma [36] and eczema [37], as well as allergic sensitization [38]. Between-population comparison would have been problematic, however, as diet is a major confounding factor affecting gut microbial composition [39]. Also, while reduced diversity in early life can be important [36,38], it is unclear whether diversity in later life affects disease onset/severity.

The disparity of allergic diseases between Finnish and Russian Karelia children remained the same during a follow-up from 2003 to 2010/2012. Allergies were still infrequent among the Russian study subjects even at the age of 16–20 years. The lifestyle and environment are slowly changing in Russian Karelia, but the effects remain to be seen. The skin and nasal microbiota was richer and more diverse in young people living in Russian Karelia. Interestingly, both the abundance and strain-level diversity of genus *Acinetobacter* was higher among Russian subjects. These findings support the notion that early life exposure to environmental microbiota may be biologically related to allergic manifestations in young age.

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Table 1. Percentage of study subjects in Finnish and Russian Karelia with allergen-specific IgE level ≥ 0.35 kU_A/l and total IgE level ≥ 110 kU_A/l.

	Finland, 2003 (n = 98)		Russia, 2003 (n = 82)		P value	Finland, 2010 (n = 98)		Russia, 2012 (n = 82)		P value
	sIgE+ (%)	CI (95%)	sIgE+ (%)	CI (95%)		sIgE+ (%)	CI (95%)	sIgE+ (%)	CI (95%)	
Timothy grass	24.5	(15.8–33.2)	6.1	(0.8–11.4)	<0.001	31.6	(22.3–41.0)	12.2	(5.0–19.4)	0.002
Birch	20.4	(12.3–28.5)	6.1	(0.8–11.4)	0.006	28.6	(19.5–37.7)	7.3	(1.6–13.1)	<0.001
Mugwort	14.3	(7.2–21.3)	3.7	(0–7.8)	0.015	19.4	(11.4–27.4)	7.3	(1.6–13.1)	0.02
<i>Cladosporium herbarum</i>	3.1	(0–6.5)	1.2	(0–3.6)	0.627**	3.1	(0–6.5)	0		0.252**
Horse	5.1	(0.7–9.5)	1.2	(0–3.6)	0.222**	13.3	(6.4–20.1)	2.4	(0–5.8)	0.009
Cat	20.4	(12.3–28.5)	7.3	(1.6–13.1)	0.013	25.5	(16.7–34.3)	7.3	(1.6–13.1)	0.001
Dog	26.5	(17.6–35.4)	4.9	(0.1–9.6)	<0.001	29.6	(20.4–38.8)	7.3	(1.6–13.1)	<0.001
House dust mite*	8.2	(2.6–13.7)	14.6	(6.8–22.4)	0.169	19.4	(11.4–27.4)	22	(12.8–31.1)	0.672
Peanut	12.2	(5.6–18.9)	1.2	(0–3.6)	0.004	10.2	(4.1–16.3)	0		0.002**
Cow's milk	13.3	(6.4–20.1)	6.1	(0.8–11.4)	0.11	2	(0–4.9)	2.4	(0–5.8)	0.999**
Hen's egg white	8.2	(2.6–13.7)	7.3	(1.6–13.1)	0.833	2	(0–4.9)	3.7	(0–7.8)	0.661**
Soybean	4.1	(0.1–8.1)	0		0.127**	6.1	(1.3–11.0)	0		0.032**
Wheat	10.2	(4.1–16.3)	3.7	(0–7.8)	0.091	9.2	(3.4–15.0)	3.7	(0–7.8)	0.139
Fish (cod)	0		0			0		0		
Any†	42.9	(32.9–52.8)	24.4	(14.9–33.9)	0.009	51	(40.9–61.1)	34.1	(23.7–44.6)	0.023
Any (not house dust mite)‡	39.8	(29.9–49.7)	20.7	(11.8–29.7)	0.006	45.9	(35.9–56.0)	26.8	(17.0–36.6)	0.008
Phadiatop® (inhalant allergens)	36.7	(27.0–46.4)	20.7	(11.8–29.7)	0.019	53.1	(43.0–63.1)	36.6	(25.9–47.2)	0.027
Phadiatop® (foods)	27.6	(18.5–36.6)	18.3	(9.7–26.8)	0.144	13.3	(6.4–20.1)	6.1	(0.8–11.4)	0.11
Total IgE	48	(37.9–58.0)	40.2	(29.4–51.1)	0.3	43.9	(33.9–53.9)	35.4	(24.8–45.9)	0.246

* House dust mite = *Dermatophagoides pteronyssinus*

† One or more positive (≥ 0.35 kU_A/l) responses out of the 14 allergens included.

‡ One or more positive (≥ 0.35 kU_A/l) responses excluding house dust mite.

** Fisher's Exact Test

Figure Captions

Figure 1. (a) Prevalence of physician-diagnosed asthma, hay fever or atopic eczema. Sensitization (with sIgE level ≥ 0.35 kUA/l or ≥ 2.5 kUA/l) to (b) inhalant (animals and pollen), (c) house dust mite, and (d) food allergens in Finnish (in 2003 and 2010, $n = 98$) and Russian (in 2003 and 2012, a: $n = 81$, b–d: $n = 82$) Karelia. The asterisks indicate the significance of the difference between the study areas. *** = $P < 0.001$, ** = $P < 0.01$, * = $P < 0.05$.

Figure 2. Distributions of IgE values specific to inhalant allergens (top row) and food allergens (bottom row) in Finnish and Russian Karelia. Inhalant IgE is the sum of specific IgEs to house dust mite, other animals, and pollen, and food IgE is the sum of specific food IgEs. The n_+ gives the number of non-zero observations. Sample sizes: FIN = 98, RUS = 82.

Figure 3. Ordination diagrams (PCoA, Bray&Curtis distance) showing a difference in the composition in (a) skin microbiota ($n_{\text{FIN}} = 76$, $n_{\text{RUS}} = 84+76$), (b) nasal microbiota ($n_{\text{FIN}} = 71$, $n_{\text{RUS}} = 82+74$), and (c) nasal fungal community ($n_{\text{FIN}} = 71$, $n_{\text{RUS}} = 82+74$), between Finnish and Russian subjects. The panels on the right indicate those OTUs that best characterize each population, based on random forest classification.

Figure 4. Relative abundance and mean oligotype profiles of three OTUs best differentiating between populations. OTUs (a) 72 and (b) 142 are more abundant in Russian subjects, while the third OTU (c) 231 is more abundant among Finnish subjects. Analysis is based on nasal microbial samples. Sensitized individuals were defined as having the sum of IgE specific to inhalant allergens > 1 . *** = $P < 0.001$ and ** = $P < 0.01$ for between-population comparisons, correcting for age and sex.







