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Contrasting microbiotas between Finnish and Estonian infants: exposure to *Acinetobacter* may contribute to the allergy gap

Short title: Nasal, skin and gut microbiotas in Finnish and Estonian infants

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Author contributions

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The study was conceived by LR, LvH, TH and MK. LR, AP, AK, and HS analysed the data. APe, AMH, VT, KK, SMV and ON contributed to data collection. LR and AP wrote the manuscript. All authors were involved in editing and final approval of the paper.

Conflicts of interest

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Abstract

Background: Allergic diseases are more common in Finland than in Estonia, which—according to the biodiversity hypothesis—could relate to differences in early microbial exposures.

Methods: We aimed at defining possible microbial perturbations preceding early atopic sensitisation. Stool, nasal, and skin samples of 6-month-old DIABIMMUNE study participants with HLA susceptibility to type 1 diabetes were collected. We compared microbiotas of sensitised (determined by specific IgE-results at 18 months of age) and unsensitised Estonian and Finnish children.

Results: Sensitisation was differentially targeted between populations, as egg- and birch pollen-specific IgE was more common in Finland. Microbial diversity and community composition also differed; the genus *Acinetobacter* was more abundant in Estonian skin- and nasal samples. Particularly, the strain level profile of *Acinetobacter lwoffii* was more diverse in Estonian samples. Early microbiota was not generally associated with later sensitisation. Microbial composition tended to differ between children with or without IgE-related sensitisation, but only in Finland. While land-use pattern (i.e. green areas vs. urban landscapes around the children's homes) was not associated with microbiota as a whole, it associated with the composition of the genus *Acinetobacter*. Breastfeeding affected gut microbial composition and seemed to protect from sensitisation.

Conclusions: In accordance with the biodiversity hypothesis, our results support disparate early exposure to environmental microbes between Finnish and Estonian children and suggest a significant role of the genus *Acinetobacter* in the allergy gap between the two populations. The significance of the observed differences for later allergic sensitisation remains open.

Keywords: atopic sensitisation, IgE, intestine, microbiota, respiratory, skin

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1. Introduction

Immune-mediated diseases such as allergies and autoimmune diseases represent one possible cost of our urbanised lifestyle. Reduced contact with diverse microbiotas of natural environments contributes to the rising epidemic of immune-mediated diseases, as explained by the biodiversity hypothesis of allergic disease.[1] Many factors, such as living on a farm, having older siblings, having pets, etc., have been linked to decreased risk for allergy and atopy.[2-4] All of these factors, which either vanish or become less likely with an urban lifestyle, could be traced back to increased exposure to environmental microbes,[5] which potentially translates into increased microbial colonisation to the body. Importantly, some of these environmental microbes have been linked to increasing immune tolerance.[5-7]

A living laboratory to test the biodiversity hypothesis exists across the Finnish borders. Westernized living conditions in Finland meet a more traditional lifestyle on the Russian side. Studying these contrasting populations, a three- to ten-fold increased prevalence of asthma or allergic sensitisation,[4,8] a four-fold increased prevalence of coeliac disease,[9] and a six-fold increased incidence of type 1 diabetes has been reported in Finland,[10] as compared to Russian Karelia. Estonia is a country of intermediate level of westernization compared to Finland and Russia. It has also witnessed a rapid socioeconomic growth since its independence in 1991. Type 1 diabetes and celiac disease are three times more common in Finland compared to Estonia,[11,12] and wheezing during the past year has been reported in 7-10% of Estonian compared to 15-20% of Finnish children.[13,14] Similarly, atopic dermatitis and skin prick test positivity are more prevalent among Swedish infants (24%) as compared to Estonian infants (~14%).[15] According to the DIABIMMUNE study, allergic sensitisation to any allergen by the age of 3 years was more common in Finland compared to Estonia (45.6 vs. 32.4%, *P* = .001; Mustonen N, personal communication).

These reported differences in epidemiology have been shown to parallel differences in the human microbiotas,[8,16] which is likely to reflect differential exposure to environmental microbes. Gut microbial composition has been shown to differ significantly between Finnish and Russian children, but not substantially so between Finnish and Estonian children.[16] Also, skin and nasal microbiotas were contrastingly different in composition between adolescents from Russian and Finnish Karelia.[8] This is in line with many other observations that dysbiosis – abnormal/imbalanced microbiota – is associated with the development of inflammatory disorders.[17]

Many previous studies have compared either school children or young adults. However, environmental exposures crucial to later development of allergic diseases may occur during early life.[3,18] Accordingly, in this study we compared skin, nasal mucosa, and gut microbiotas from infants from Finland

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and Estonia. Our aim was to search for early differences in the commensal microbiota that might predict early allergic sensitisation and the gap in the prevalence of allergic diseases between these two populations.

2. Materials and Methods

2.1 Subjects and sample collection

The DIABIMMUNE birth cohort (BC) was a prospective study in Finland, Estonia and Russia following children with HLA susceptibility to type 1 diabetes from birth until the age of 3 years from September 2008 to October 2013.[12] Blood and swab samples were collected at the study center visits (3, 6, 12, 18, 24, and 36 months of age).

For this study, children observed to be sensitised according to the IgE results by the age of 18 months were included from the Finnish (n=387) and Estonian DIABIMMUNE cohorts (n=330). Unfortunately, not enough Russian samples were available for this study. The allergen-specific IgE levels tested at 18 months of age were cat, hen's egg, cow's milk, peanut, birch, timothy, and dust mite (*Dermatophagoides pteronyssinus*). To include only children with a definite allergic sensitisation, a cut-off of IgE≥0.7 kU/L in at least one serum allergen-specific IgE level was used for the identification of cases instead of the standard cut-off of 0.35 kU/L. In the statistical analyses, we classified subjects as sensitized, based on an empirical cut-off of the maximum specific IgE value, set at –0.5 on $\log_{10}$ scale (Fig. S1 in supplementary material). Table S1 shows the proportion of Finnish and Estonian study participants with positive sIgE responses (≥0.35 kU/L) to the seven allergens tested at the age of 18 months.

The inclusion criteria resulted in the identification of 70 Finnish children (18.1%) but only 28 Estonian children (8.5%) with allergic sensitisation, reflecting the existing gap in allergy prevalence between these two populations. For the 70 Finnish children, we included 70 country-matched controls with no allergen-specific IgE levels above 0.35 kU/L at the age of 18 months. Due to the lower number of sensitised children in Estonia, we included two controls for the 28 Estonian children. In total, this study comprised 224 children (117 boys), 98 children with definite allergic sensitisation by allergen-specific IgE levels at 18 months of age, and 126 controls. Table S2 presents the demographic and other characteristics of the Finnish and Estonian study participants and Table S3 the corresponding characteristics in the sensitised and non-sensitised participants.

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Stool, skin and nasal samples were collected at the age of 6 months. Nasal swab samples were available for 217 children (n_{Fin} = 135, n_{Est} = 82), skin swab samples for 172 children (n_{Fin} = 111, n_{Est} = 61), and stool samples for 218 children (n_{Fin} = 135, n_{Est} = 83). A complete set of samples was obtained from 162 children (n_{Fin} = 102, n_{Est} = 60). For those children without a stool sample at 6 months of age, a sample at 5 months was selected (n=14), and for those lacking both samples, a sample at 7 months was selected (n=2).

The local ethics committees approved the DIABIMMUNE study protocol, and legal guardians of the study participants gave their written informed consent.

2.2 Land-use patterns

Land use was calculated with the CORINE2012 land-cover data, using five land-use types: agricultural land, built area, forest, water bodies and wetland. We used a buffer with a radius of 3 km around the homes.[19]

2.3 DNA extraction and sequencing

Bacterial composition was determined after DNA extraction by amplifying V1-V3 regions of bacterial 16S rRNA genes by polymerase chain reaction (PCR) (see online supplement).

2.4 Bioinformatics

The bioinformatics methods have been described in the online supplement.

2.5 Statistics

We tested the ability of microbial composition to classify individuals according to national identity, using random forest analysis, as implemented in the randomForest package[20]. This analysis was also used to identify characteristic operational taxonomic units (OTUs) of each group. Prior to analysis, in order to limit the number of feature included in the analysis, we selected only those OTUs having a variance higher than the median variance across all OTUs. An optimal number of trees was selected based on classification error and an optimal number of randomly selected variables used in the analysis was sought using the train function in the caret package[21], based on 10-fold cross validation. Due to imbalanced sample sizes – larger sample size of Finnish children would skew the prediction so that the Estonian children could not be

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predicted reliably, we took a random sample of n_{skin}=60, n_{nasal}=80, and n_{stool}=80 samples from each population to be used in the analysis. The number of features used were 1,391 for skin and nasal microbiota, and 1,387 for stool microbiota. For skin, nasal, and stool samples we used 100, 100, and 200 trees, respectively, and 100, 197, and 100 random features, respectively.

Questionnaire data on animal contact was converted to binary form (by coding high/prominent contact with 1 and low/infrequent contact with 0). The resulting data was used to calculate an aggregate estimate of the intensity of animal contact, with principal component analysis (PCA), where the first PCA axis was used in the analysis.

A more detailed description of the methods can be read in the online supplement.

3. Results

3.1 Between-population differences in allergic sensitisation

For the statistical analyses, we used the maximum specific IgE values, measured at the age of 18 months, to classify individuals to either 'healthy' or 'sensitised', setting the cut-off at –0.5 on $\log_{10}$ scale (0.61 on arithmetic scale; based on the frequency distribution of IgE values, Fig. S1). The maximum IgE at 18 months correlated relatively well with that at 6 months (n_6=210; Spearman ρ = 0.69, P < .0001) and 36 months (n_{36}=198; Spearman ρ = 0.74, P < .0001). These correlations did not differ markedly between population cohorts.

The IgE values of individual allergens were generally very low; the medians of all measured allergens were less than 0.1 kU/L. As low measures below 0.1 kU/L are not considered to be trustworthy, we used this level as cut-off. The data suggests egg- and birch-specific IgE is elevated in Finland (egg: $OR_{Finland}$ = 2.22, CI = [1.25, 3.95], P = 0.0068; birch: $OR_{Finland}$ = 4.69, CI = [1.04, 21.17], P = 0.045). Interestingly, house dust mite-specific IgE was elevated in Estonia, but the difference was not statistically significant ($OR_{Finland}$ = 0.36, CI = [0.11, 1.14], P = 0.083). These results suggests that the immune response might be differently targeted between the populations, even at this early age, which is in accordance with previous observations between Finnish and Russian Karelia[8].

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3.2 Patterns in the composition of microbiota

The sequencing resulted in 2,782 OTUs shared between both sequencing runs in the 607 samples from skin, nasal and stool, obtained at the age of 6 months. Microbial diversity was highest in the skin samples (P = .0004) and lowest in the stool samples (P < .0001) (Fig. 1). Across all samples, Estonian children tended to have higher microbial diversity, as compared to Finnish children (P < .0001), and especially so when considering either skin or stool samples. Considering diversity between the populations, as well as healthy and sensitised children (assessed at 18 months), the only significant difference was that the Estonian children had more diverse microbiota in their stool compared to Finnish children (P = .007). In the skin samples from Estonia, diversity tended to be higher in sensitised children as compared to healthy children (P = .028) (Fig. 1).

Microbial community composition differed most clearly between populations when considering skin samples [multiple regression on distance matrices (MRM) on weighted UniFrac: R^2 = .08, P < .001; Fig. 2a)], followed by the nasal samples (R^2 = .03, P < .001; Fig. 2b), and stool samples (R^2 = .02, P < .001; Fig. 2c). Significant differences between healthy and sensitised children were observed both in the nasal and stool samples, but only among Finnish children (MRM on Bray&Curtis: nasal: R^2 = .02, P < .001; stool: R^2 = .01, P = .013). These analyses were corroborated by random forest classification (RFC). The overall classification accuracy (based on 10-fold cross validation of a random subsample of the data) was highest for skin microbiota (accuracy = 92.5%), followed by nasal (accuracy = 91.9%), and stool microbiota (accuracy = 81.3%). In all cases, subject recall was higher for Finnish than for Estonian subjects: nasal samples Fin = 92.5%, Est = 91.3%; skin samples Fin = 95%, Est = 90%; stool samples Fin = 86.3%, Est = 76.3%.

Using RFC, we were able to assess the most important OTUs differentiating between Finnish and Estonian children (Fig 2.). In both nasal and skin samples the most important OTU belonged to genus *Acinetobacter* (OTU-108) (Fig. 2a,b). This OTU was more abundant in Estonian children as compared to Finnish children – on average over 20 times more abundant on the skin (P_{skin} < .0001) and four times more abundant on the nasal epithelium (P_{nasal} = .0025) (Fig. 3a). Furthermore, it could be identified to species *A. lwoffii* (a blast search against the NCBI 16S ribosomal RNA sequence database gave a 99% match over 486 nucleotides). The "strain" level profile was also found to be different between Finnish and Estonian children using an oligotyping pipeline (R^2 = .06, P < .001, based on Bray&Curtis dissimilarity, correcting for sample type; Fig 3b). Again, the strain profile of OTU-108 was more diverse (measured by Shannon diversity) among Estonian children (P_{nasal} & P_{skin} < .0001). While the strain profile seems to differ

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especially between healthy and sensitised Finnish children, these differences were not statistically significant.

3.3 Living environment affecting skin & nasal microbiota

Land-use patterns, characterised as the first principal component of land-use types, differed significantly between populations (P = 9.1e–8); Estonian subjects lived in more rural surroundings than the Finnish subjects. This is reflected in the analysis of dissimilarities, such that for both skin and nasal microbiota, land-use did not remain significant after correcting for the significant effect of population (P = .001). As reported before,[19] land use was not associated with sensitisation in this data. Also, (taking a random sample of subjects from each population: n_{skin} = 50, n_{nasal} = 70), only 18% of variation along the land-use gradient could be predicted with skin microbiota (using 150 trees and 225 features) and 11% based on nasal microbiota (using 150 trees and 500 features), using random forest regression. Interestingly, while land-use was not associated with microbiota as a whole, there was a significant association between *Acinetobacter* composition in the nasal samples and land-use. After accounting for the population effect (P = .001), both the amount of agricultural land (P = .002) and forest cover (P = .029) were associated with similarity in *Acinetobacter* composition between samples (Bray&Curtis dissimilarity on sqrt transformed OTU-level counts).

3.4 Stool microbiota & other lifestyle factors

To account for possible dietary effects, we considered the influence of breastfeeding patterns on stool microbiota. While the duration of breastfeeding did not differ between populations (Table S3), those children that were exclusively breastfed for at least the first month of life were less likely to be sensitised at the age of 18 month (P = .00015), after accounting for population, mode of delivery, and the month to start on solid foods (Table 1). This could not be explained by between-population differences in exclusive breastfeeding (P = .59), indicating that this protective effect occurs across populations. Interestingly, stool microbial diversity was significantly reduced by breastfeeding at the time of sampling (P <.0001), again accounting for population, mode of delivery, and the month to start on solid foods (Table 1). Moreover, breastfeeding at time of sampling was also significantly associated with variation in between-sample (weighted) UniFrac distances (P = .002; Table 1), indicating that breastfeeding influenced the composition of the gut microbial community. Particularly, breastfeeding was associated with an increase in the abundance of Lactobacillaceae (P = .0003, when accounting for population effect).

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Finally, we tested whether the amount of animal contact in early life (6 months) was associated with later sensitisation and microbial composition (measured at 18 months). While animal contact was significantly more intensive in Estonia as compared to Finland (P = 1.44e–7), it was not associated with allergic sensitisation (P = .94), after accounting for the between-population difference (P = .041). However, stool microbiota was significantly associated with animal contact (MRM on weighted UniFrac, R^2 = .02, P = .003; corrected for between-population differences), which might be reflected in later health. When comparing individuals with low (1st quartile) and high (4th quartile) animal contact, a few OTUs identified by RFC, were significantly associated with animal contact. These OTUs represented the genera of *Anaerostipes* (Kruskal-Wallis test, P = 0.0023), *Blautia* (P = 0.0074), and *Bacteroidetes* (P = 0.014), all more abundant in the high-contact group.

4. Discussion

In this study of 6-month-old Finnish and Estonian infants, our objective was to compare patterns in microbiota between populations and discover potential microbial predictors of early sensitisation to allergens. Indeed, Finnish and Estonian infants were found to differ in their skin, nasal, and stool microbiota. The role of these differences for later developing allergic sensitisation could not be confirmed at this stage. However, the results clearly indicate—in agreement with earlier findings—that observed between-population differences in epidemiology could be partly explained by differential exposure to environmental microbiota. Such a conclusion is supported by the fact that the Estonian cohort is exposed to more rural environment and they also tend be in closer contact with farm animals as compared to the Finnish cohort.

The contrasting microbial community composition between Finnish and Estonian children on the skin and the nasal epithelium was best characterised by differential abundance of several OTUs from the genus *Acinetobacter* (Fig. 2a,b). In particular, we found that *A. lwoffii* (and the Genus in general) was substantially more abundant, and genetically more diverse, in the skin and nasal samples from Estonian than Finnish infants (Fig. 3). This resonates with our earlier observation that adolescents in the Russian Karelia have significantly higher abundance of more diverse *A. lwoffii* on their skin and nasal epithelium than their Finnish counterparts.[8] Importantly, the present results indicate that these differences are detectable already during infancy and precede the development of allergic sensitisation. This suggests sustained exposure to *Acinetobacter* in these populations, which is likely to begin already before birth and range into adulthood.

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Within the genus *Acinetobacter*, *A. lwoffii* seems to be the most common commensal on human skin and nasal mucosa.[22,23] These bacteria are found in soil,[24] but they are also relatively abundant in cowshed microflora.[7] This indicates that the living environment and lifestyle might contribute to the colonisation of this bacterium to the body, which could explain the observed between-population differences. This is in turn supported by the previous observation that exposure to *A. lwoffii* in the living environment is associated with reduced risk of allergic sensitisation(25). In the present context, it would be extremely interesting to follow health outcomes of the participants in later life to see whether this is indeed the case.

Remarkably, *A. lwoffii* has been shown to polarize the immune system towards Th1 responses in a mouse model.[7,26] Furthermore, even prenatal exposure to *A. lwoffii* can significantly prevent the development of an asthmatic phenotype in the progeny in the mouse model.[27,28] For example, Brand et al.[28] showed that the exposure of the mother to *A. lwoffii* leads to epigenetic modifications in the progeny, promoting the production of IFN-γ. More recently, Fyhrquist et al.[26] demonstrated that exposure to *A. lwoffii* through the skin is associated with elevated expression of IL-10 and IFN-γ, and suppression of IL-5 and IL-13 expression. This local Th1-polarisation of the immune response was also associated with systemic protection from allergic sensitisation and inflammation on allergen challenge. These experimental findings, together with our observations, suggest that the Estonian and Russian children are being protected against allergic sensitisation via both pre- and postnatal exposure to *Acinetobacter.*

Also, the faecal samples at the age of 6 months differed between populations, but the differences were less clear. While the relatively small between-population difference is understandable due to the selectivity of the gut habitat,[17,29] Estonian children had a more diverse gut microbiota compared to Finnish children. Lower diversity of gut microbiota has been associated with development of allergic diseases,[30] which is in line with our findings of reduced diversity in a population sampled from a country with higher prevalence of allergic diseases. However, it is not clear why this difference arises. In both cohorts, breastfeeding was found to be protective against sensitisation. Breastfeeding was also associated with an increase in the abundance of Lactobacillaceae, in agreement with previous reports.[31] This could relate to the protective role of breastfeeding via increased colonic production of short-chained fatty acids,[32] which can shape the immunological environment in the lung and influence the severity of allergic inflammation.[33] Notably, the compositional change in gut microbiota in the context of breastfeeding was also associated with reduced microbial diversity. We interpret this as the functional role of the microbial community members being more important for the host than mere diversity.

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We searched for predictors of early allergic sensitisation at the age of 18 months and found the detected differences in microbiotas to be somewhat surprising. Microbial diversity tended to be higher in sensitised children as compared to healthy children (Fig. 1), although these differences were not statistically significant. Additionally, *A. lwoffii* tended to be more abundant in sensitised compared to healthy infants (Fig. 3). Based on the assumption that higher microbial diversity is associated with protection from allergic diseases, and the fact that *A. lwoffii* being more abundant in Estonian samples was proposed to play a role in a lower allergy prevalence in this population, one might have expected to see an opposite pattern. It should be kept in mind that it is not clear that higher microbial diversity in the gut *per se* would be beneficial.[17] Patterns in diversity likely arise from interactions between taxa, which are affected by factors such as diet and medication.[17]

Another possible explanation could be that our selected endpoint, sensitisation at 18 months of age, was not detecting true atopic development in our study population but rather reflected exposure of these infants to different allergens. Consequently, a child displaying an IgE response to allergens could merely have been exposed to a larger array or higher loads of these allergens, which might also be evident as a more diverse microbiota. According to Lynch et al.,[34] such a scenario can actually protect against, rather than predispose to, allergic disease. It is possible that at the age of 18 months, the children sensitised do not necessarily represent children who will later develop allergic diseases but rather this IgE response will later dissolve. For example, in early life, sensitisation towards food allergens is more frequent (here the maximum IgE at 18 months came from a food allergen in 78% of the subjects) than in later life, and for example in a German study, two-thirds of the children with sensitisation by the first year of life did not develop any atopic disease by the age of six years.[35]

Also, sensitisation to dust mite—especially monosensitisation—might not be related to later atopic diseases but could even provide protection from such conditions.[36] Only three children, however, one Estonian and two Finnish, were sensitised to dust mite at the level of 0.35 kU/L. None of these children were monosensitised, although dust mite IgE levels higher than 0.1. kU/L were more common among Estonian than Finnish children. However, breastfeeding was a significant factor potentially contributing to the development of sensitisation; infants exclusively breastfed for at least one month were less likely to be sensitised at the age of 18 months. While this corroborates many earlier studies describing breastfeeding as a protective factor for allergic diseases,[37] there are also several studies that do not agree.[38,39]

A limitation of our study is that the included children represent selected cases in terms of HLA class II genetics. Accordingly, the results might not directly be generalizable to the whole population and possibly some differences existing in the whole population are missed. We compared microbial

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samples at the age of 6 months, which in the light of recent findings might already be late. Especially the alterations in stool microbiota have often been described very early in infancy: Arrieta et al.[18] reported a transient intestinal microbial dysbiosis at 3 months of age in infants at high risk of later asthma development, which had mostly disappeared already at 1 year of age. Even earlier differences in association with later development of allergic disease have been reported,[30,40] and in the DIABIMMUNE cohort also differences in umbilical cord blood transcriptomics have been described.[41]

Another limitation is that the number of subjects is small. This practically prevents any thorough adjustment of results with various demographic factors,[42] such as breastfeeding, age at solid food introduction, number of siblings, day care attendance, exposure to smoking and pets, living on a farm, land-use, and mode of delivery (Table S3). Especially factors associated with the living environment and lifestyle are very likely to affect individual skin/nasal microbiota.[43,44] While we cannot establish causalities in this study, we feel that previous experimental work[7,26,27] provides a solid mechanistic foundation that supports our results.

To conclude, in this nested case-control study from DIABIMMUNE infants with signs of allergic sensitisation at the age of 18 months, we described differing nasal and skin microbiota between Finland and Estonia. Namely, *Acinetobacter lwoffii* was more abundant and diverse in Estonian infants already at the age of 6 months, which could provide one potential explanation for the allergy gap between these populations. Differences in gut microbiota or between children later developing allergen-specific IgE responses were less clear. Possibly, studies from different time points of sample collection or a longer follow-up to detect development of the allergic phenotype could provide a more conclusive answer, regarding the role of the early commensal microbiota in the development of allergic sensitisation.

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Table 1. (a) Exclusive breastfeeding at 1 month of age reduced the risk (OR) of allergic sensitisation (logistic regression model). (b) Breastfeeding at the time of sampling reduced microbial (Shannon) diversity (generalised least squares model, account for between-group residual variation) and community composition (weighted UniFrac distance, permutational regression on distance matrices) in stool samples. All analyses were controlled for country, delivery mode (vaginal or caesarean section = CS), number of siblings, and the month when solid foods were included in the diet.

Response	Predictor	OR/Estimate/R^2	*P*
(a) Sensitisation	Country (Estonia)	0.47	0.018
	Delivery mode (CS)	0.47	0.12
	Number of siblings	0.67	0.009
	Month to start solid food	1.13	0.45
	Exclusive breastfeeding at 1 mon	0.29	0.0003
(b) Diversity	Country (Estonia)	0.40	<.0001
	Delivery mode (CS)	–0.10	0.70
	Number of siblings	–0.01	0.30
	Month to start solid food	–0.06	0.0001
	Breastfeeding at 6 mon	–0.30	<.0001
(c) Composition	Country	0.02	0.003
	Delivery mode	0.02	0.002
	Number of siblings	0.01	0.14
	Month to start solid food	0.02	0.003
	Breastfeeding at 6 mon	0.024	0.001

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Legends for the figures

Figure 1 Microbial diversity (Shannon's index) in different sample types (skin, nasal, and stool), comparing healthy and sensitised subjects among the Finnish and Estonian cohorts.

Figure 2 Ordination of community composition and the top discriminative OTUs for each cohort in either the (a) skin, (b) nasal, or (c) stool samples. Most important OTUs differing between Finnish and Estonian children were found with random forest classification. Ordinations were based on the Bray&Curtis dissimilarity.

Figure 3 (a) Proportional abundance (b) and the oligotype profile of OTU-108 (*Acinetobacter lwoffii*) in healthy and sensitised Finnish and Estonian children, in both skin and nasal samples. An oligotype is a group of reads that are binned together based on the nucleotides they possess by using Shannon entropy.

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Figures

Figure 1

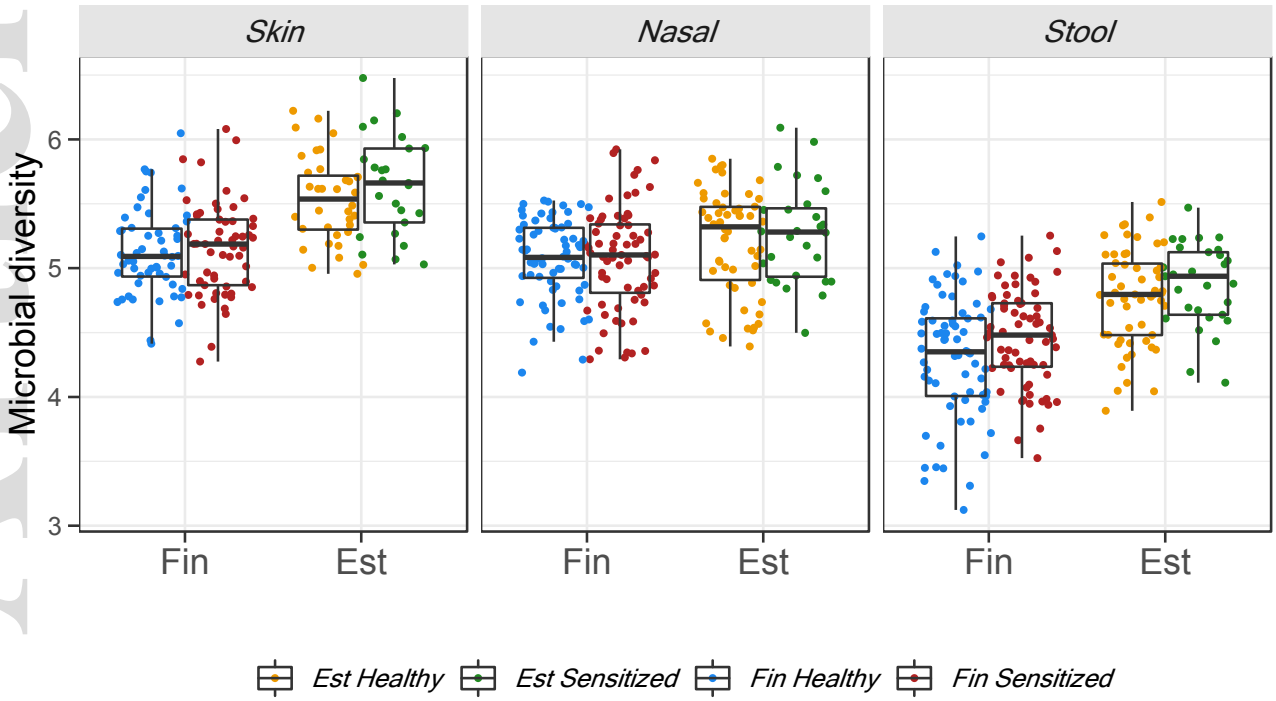

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Figure 2

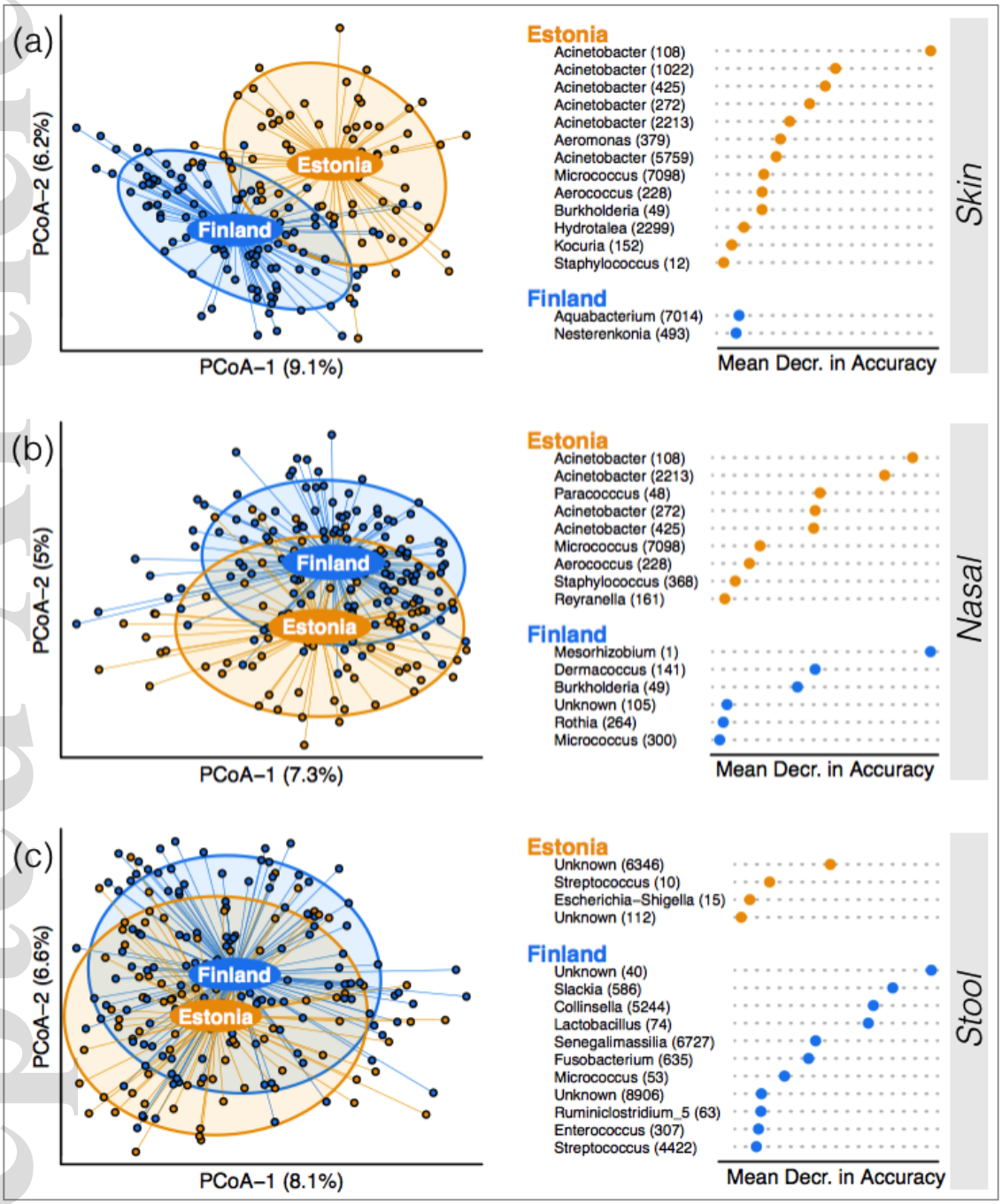

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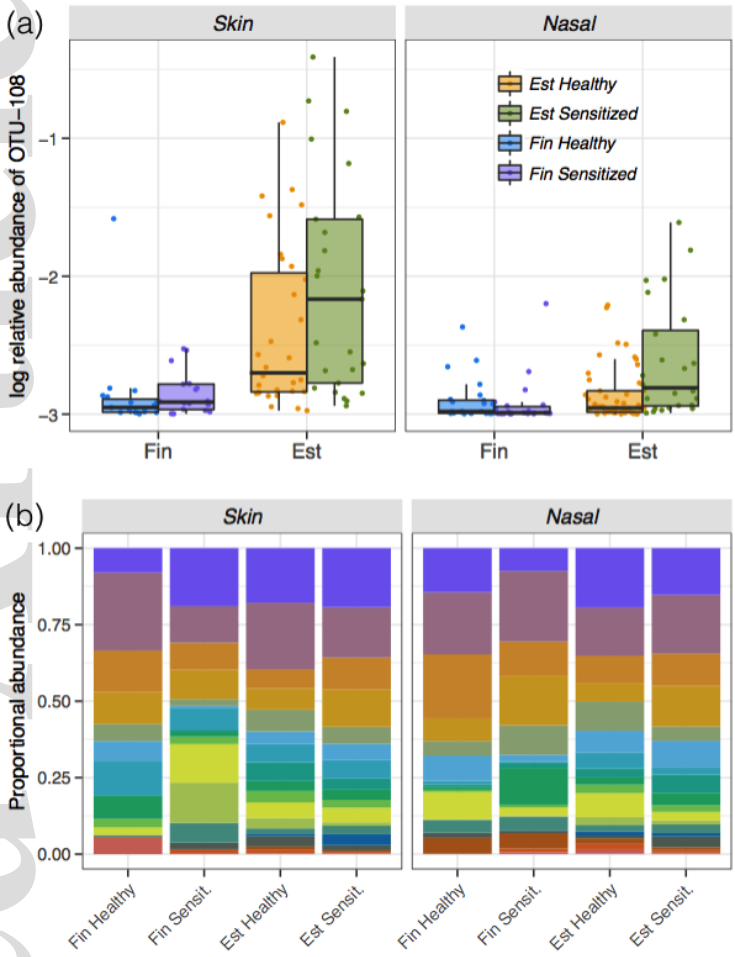
Figure 3



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