Early Childhood Gut Microbiomes Show Strong Geographic Differences Among Subjects at High Risk for Type 1 Diabetes

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OBJECTIVE
Gut microbiome dysbiosis is associated with numerous diseases, including type 1 diabetes. This pilot study determines how geographical location affects the microbiome of infants at high risk for type 1 diabetes in a population of homogenous HLA class II genotypes.

RESEARCH DESIGN AND METHODS
High-throughput 16S rRNA sequencing was performed on stool samples collected from 90 high-risk, nonautoimmune infants participating in The Environmental Determinants of Diabetes in the Young (TEDDY) study in the U.S., Germany, Sweden, and Finland.

RESULTS
Study site–specific patterns of gut colonization share characteristics across continents. Finland and Colorado have a significantly lower bacterial diversity, while Sweden and Washington state are dominated by *Bifidobacterium* in early life. Bacterial community diversity over time is significantly different by geographical location.

CONCLUSIONS
The microbiome of high-risk infants is associated with geographical location. Future studies aiming to identify the microbiome disease phenotype need to carefully consider the geographical origin of subjects.
Continuous variables were tested using the one-way ANOVA or Kruskal-Wallis test for differences in means. Generalized estimating equations for longitudinal correlated data were used to assess the association between geographical location and bacterial abundance and diversity adjusting for demographic, clinical, and dietary variables. Separate models were examined for each bacterial genus under study. A permutation test and the F statistic were used to determine whether SDI differed among the six TEDDY study sites, as previously described (16). P values < 0.05 were considered significant.

RESULTS

Sex (P = 0.0092) and age at first introduction to oats (P = 0.0042), gluten (P = 0.0001), and milk products (P < 0.0001) were the only clinical characteristics significantly different by geographical location. To characterize the development of the gut microbiome over time, 16S rRNA sequencing read values of bacterial genera were grouped according to age of subject (in months) at the time of sample collection (Fig. 1A). Bacteroides was the predominant genera at all sites (average abundance 22.7%, SE 0.7%). The abundance of Bifidobacterium (P = 0.0172), Veillonella (P = 0.0048), Faecalibacterium (P = 0.0122), Streptococcus (P = 0.0003), and Akkermansia (P = 0.0196) was significantly different by geographical location after adjusting for significant clinical and dietary variables. Although Bacteroides abundance was not significantly different (P = 0.0530) by site, Colorado had a significantly higher abundance than all other sites (P = 0.0126), except Finland.

The permutation test of the SDI of bacterial genera identified at each site showed that all sites differ from each other across time (Fig. 1B). Furthermore, the difference remained significant after adjustment for delivery mode and age at first introduction to milk products (P = 0.0045). Colorado and Finland had a significantly lower SDI than all other sites (P = 0.0258). Georgia/Florida and Germany had a more diverse profile, characterized by a relative abundance of Clostridium, Bifidobacterium, and Veillonella of > 8.0% each (Fig. 1A). In Sweden and Washington state, the fecal profile was dominated by Bifidobacterium until 8 and 10 months of age, respectively, and the overall abundance was significantly higher than in Colorado and Finland (P = 0.0199).

CONCLUSIONS

This study highlights the great variability in the composition and diversity of gut microbiomes among the six TEDDY study sites. So far, studies examining the association between the gut microbiome and type 1 diabetes autoimmunity have focused on small geographical regions (2–6). Country- and lifestyle-specific factors are a major player in shaping the composition of the gut community (7–11), but the extent to which they affect the microbiome of infants at high risk for type 1 diabetes was previously unknown. Our study is the first to investigate the fecal microbial profile of high-risk children across two continents and multiple countries.

Our data suggest that children at high risk for type 1 diabetes have study site–specific patterns of gut colonization showing intercontinental similarities but intracontinental differences. Geographical origin significantly associated with the diversity of bacterial communities and the relative abundance of numerous bacterial genera (Fig. 1A). Low bacterial diversity was characteristic of subjects from Finland and Colorado. Previous studies have reported similar reductions in microbiome diversity in northern European infants compared with infants from southern European countries (Sweden vs. Spain and Finland vs. Germany, respectively) (9, 10). While Finland has the highest incidence of type 1 diabetes in children (17), it remains to be seen whether SDI is associated with disease incidence. Perplexing differences, not explained by clinical characteristics, exist between the neighboring countries Sweden and Finland. Compared with their Swedish neighbors, Finnish subjects had a significantly higher abundance of Bacteroides (P = 0.0508) and Veillonella (P = 0.0160), and a lower abundance of Bifidobacterium (P = 0.0199), Akkermansia (P = 0.0014), and Ruminococcus (P = 0.0248). The fecal profile of Swedish subjects was more similar to those from Washington state (both groups of subjects were dominated by Bifidobacteria at early time points), suggesting a
are different and cultural factors that, on their own, may influence the underlying environment. Geography represents a cultural factor with geographical location, even in a more typical colonization pattern of the infant gut (18).

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Figure 1—A: A heat map of the relative abundance of the most abundant bacterial genera shows a distinct pattern of development at each study site. 16S rRNA read values were grouped according to age of subject (in months) at the time of sample collection. If a subject had more than one sample within 1 month, the read values were averaged to prevent over-representation of a single individual. Bacterial genera denoted in black font are represented in the top 10 most abundant genera at all sites, and those denoted in blue font represent genera from the 10 most abundant at only some sites. Symbols indicate a statistically significant difference in bacterial abundance by geographical site after adjusting for age at stool collection and other significant covariates, as follows: *P < 0.05, **P < 0.01, ***P < 0.001, +P = 0.053. B: Changes in the SDI of genus-level microbial communities over time differ significantly at each site (P < 10^−5). Diversity remains significantly different after adjusting for mode of delivery and age at first introduction to milk products (P = 0.0045). The left panel depicts a histogram of 10,000 F statistics obtained after randomly permuting the site labels. The blue line indicates the 95% quartile of this F statistic, and the arrow indicates the observed F statistic. The right panel depicts, for every site, a polynomial of degree 3 adjusted to the observed SDI, days after birth.

exist even after adjusting for numerous early-life and dietary variables. Future studies must carefully consider the impact of geographical location on the microbiome of children who are genetically at higher risk for type 1 diabetes as location may confound analyses of disease-associated microbiome states. Furthermore, whether the microbiome is causative or merely an indicator of underlying type 1 diabetes etiology, these microbiome differences suggest a geographically tailored approach to diagnostics or preventative therapies.

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