Skin microbiota and allergic symptoms associate with exposure to environmental microbes

Lehtimaki, Jenni

2018-05-08


http://hdl.handle.net/10138/235355
https://doi.org/10.1073/pnas.1719785115

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.
Skin microbiota and allergic symptoms associate with exposure to environmental microbes

Jenni Lehtimäki1,2, Hanna Sinkko1,2, Anna Hielm-Björkmanb, Elina Salmela1,2, Katriina Tiira3, Tiina Laatikainen4,5, Sanna Mäkeläinen6, Maria Kaukonen7, Liisa Uusitalo8, Ilkka Hanski2,9, Hannes Lohi2,9, and Lasse Ruokolainen3

*Faculty of Biological and Environmental Sciences, University of Helsinki, 00014 Helsinki, Finland; †Department of Equine and Small Animal Medicine, Faculty of Veterinary Medicine, University of Helsinki, FI-00014 Helsinki, Finland; ‡Research Programs Unit, Molecular Neurology, University of Helsinki, FI-00014 Helsinki, Finland; †National Institute for Health and Welfare, FI-00029 Helsinki, Finland; ‡Institute of Public Health and Clinical Nutrition, University of Eastern Finland, FI-70211 Kuopio, Finland; †Joint Municipal Authority for North Karelia Social and Health Services, North Karelia Central Hospital, FI-80210 Joensuu, Finland; ‡Department of Veterinary Biosciences, University of Helsinki, FI-00014 Helsinki, Finland; and †Folkhälso Institute of Genetics, Folkhälso Research Center, FI-0014 Helsinki, Finland

Edited by Karen E. Nelson, J. Craig Venter Institute, Rockville, MD and approved March 22, 2018 (received for review November 13, 2017)

A rural environment and farming lifestyle are known to provide protection against allergic diseases. This protective effect is expected to be mediated via exposure to environmental microbes that are needed to support a normal immune tolerance. However, the triangle of interactions between environmental microbes, host microbiota, and immune system remains poorly understood. Here, we have studied these interactions using a canine model (two breeds, n = 169), providing an intermediate approach between complex human studies and artificial mouse model studies. We show that the skin microbiota reflects both the living environment and the lifestyle of a dog. Remarkably, the prevalence of spontaneous allergies is also associated with residential environment and lifestyle, such that allergies are most common among urban dogs living in single-person families without other animal contacts, and least common among rural dogs having opposite lifestyle features. Thus, we show that living environment and lifestyle concurrently associate with skin microbiota and allergies, suggesting that these factors might be causally related. Moreover, microbes commonly found on human skin tend to dominate the urban canine skin microbiota, while environmental microbes are rich in the rural canine skin microbiota. This in turn suggests that skin microbiota is a feasible indicator of exposure to environmental microbes. As short-term exposure to environmental microbes via exercise is not associated with allergies, we conclude that prominent and sustained exposure to environmental microbiotas should be promoted by urban planning and lifestyle changes to support health of urban populations.

Our health tends to be affected by our living environment, green space being the promoter of our well-being (1). Supporting evidence is provided by studies showing that allergies are increasing due to urbanization, especially in Western countries, while allergies remain rarer in developing countries as well as in rural areas (2, 3). As the urban and rural environmental microbiota, i.e., the diverse microbial community in a defined ecosystem, differ (4), it is suggested that increased exposure to microbes from natural environments, directly or indirectly through animals or family members, is protective against the development of allergic diseases (5). This idea is further strengthened by findings that the microbiota differs between healthy and allergic people (reviewed in ref. 6). While there is good evidence for associations between the living environment and allergic diseases (7, 8) as well as between host microbiota and allergies (e.g., refs. 6 and 9), it is unclear whether all these factors are related, and if so, how.

Unfortunately, a multitude of confounding factors associated with human life, such as a constant change both in lifestyle patterns and in living areas due to moving and traveling, makes it difficult to establish a causal relationship between living environment and lifestyle and health (8). To overcome this problem, several studies linking allergies to microbiota have been carried out in the mouse model (e.g., refs. 10 and 11). However, controlled laboratory experiments are not ideal for studying the effect of complex living environments, as they are practically impossible to replicate in artificial conditions. In an attempt to establish a feasible compromise between problematic human studies and overly simplistic laboratory models, we adopted a canine model (pet dogs). We consider dogs to be relevant model animals, as they suffer increasingly from diseases analogous to inflammatory disorders in humans, such as allergies (e.g., in ref. 12). Importantly, dogs develop these diseases spontaneously unlike in a mouse model. Also, dogs share the living environment with and are influenced by the lifestyle of their owners. However, dogs still live simpler and shorter lives than humans, excluding several confounding factors common in studies involving human subjects.

Our aim was to describe associations between the exposure to environmental microbes, skin microbiota, and allergic symptoms, utilizing the canine model. Skin microbiota was selected instead of widely studied gut microbiota, because it has more immediate contact with the environment. Recent findings also suggest that the skin microbiota can have a systemic influence on immune tolerance (10, 13, 14). We defined three factors concurrently influencing the exposure of the skin to environmental microbes: (i) land use in the residential environment, (ii) contact with different land-use microbiomes, and (iii) biodiversity hypothesis. Urbanized lifestyle, featuring restricted animal contacts and small family size, is allergy promoting both in rural and urban dogs. Hence, both environment and lifestyle seem to influence the microbiota and, probably consequently, immune tolerance.


The authors declare no conflict of interest.


Published online April 23, 2018.

www.pnas.org/cgi/doi/10.1073/pnas.1719785115

PNAS May 8, 2018 | vol. 115 | no. 19 | 4897–4902

Significance

Urban, Westernized populations suffer extensively from noncommunicable diseases such as allergies. However, the overlapping effects of living environment and lifestyle are difficult to separate. Intriguingly, also our fellow animals, dogs, suffer from analogous diseases. Therefore, we suggest that pet dogs, sharing their environment and lifestyle with humans but having a comparatively simple life, provide a valuable model for understanding origins of noncommunicable diseases. We show that living environment and lifestyle concurrently, but still independently, shape both the skin microbiota and the risk of allergic disease in dogs. Urbanized lifestyle, featuring restricted animal contacts and small family size, is allergy promoting both in rural and urban dogs. Hence, both environment and lifestyle seem to influence the microbiota and, probably consequently, immune tolerance.


The authors declare no conflict of interest.


*This open access article is distributed under Creative Commons Attribution-NonCommercial-NoDerivatives License 4.0 (CC BY-NC-ND).

Data deposition: All 165 RNA-gene sequences have been deposited in the National Center for Biotechnology Information (accession no. SRP133457).

1L.J. and L.S. contributed equally to this work.
2To whom correspondence should be addressed. Email: jenni.lehtimaki@helsinki.fi.
3Deceased May 10, 2016.
4This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1719785115/-/DCSupplemental.
types during exercise, and (iii) lifestyle of the dog owner (Fig. 1). We tested how these factors relate to the skin microbiota, characterized by bacterial 16S rRNA gene sequences, and allergies, quantified by a large questionnaire developed by canine dermatologists. We also tested the relation of residential environment at the time of birth with skin microbiota and allergies in adult dogs.

**Results**

**Exposure to Environmental Microbes Shapes the Skin Microbiota.** All three factors characterizing the total exposure of a dog to environmental microbes—i.e., residential environment, exercise environment, and lifestyle (Fig. 1)—were related to skin microbiota. Interestingly, microbial communities tended to cluster according to both the residential environment and lifestyle (Fig. 2), while the effect of exercise environment was marginally significant (SI Appendix, Fig. S1). In other words, dogs living in an urban environment, exposed to urban lifestyle (i.e., living in a single-person family without other pets and having many dog-related hobbies such as agility or tracking, SI Appendix, Fig. S2), differed in their skin microbiota from dogs living in the same environment but having owners with a rural lifestyle (i.e., living in a large family with other pets, SI Appendix, Fig. S2). However, it is important to note that these three explanatory variables were somewhat correlated (SI Appendix, Fig. S3), which makes interpretation challenging.

While the effects of residential environment and lifestyle were partly overlapping (these variables were correlated; $r=0.48, P=0.001$), they also tended to be associated with independent aspects of the skin microbiota, as can be seen in Fig. 2 with separate but partly overlapping groups. More specifically, the first axis of principal component analysis (PCA1) of the land-use types correlated slightly but significantly with the second axis of principal coordinates analysis (PCoA2) of skin microbiota (Pearson’s correlation: $–0.36, P_{adj}=0.00001$). Instead, PCoA1 of lifestyle correlated with PCoA4 of skin microbiota (Pearson’s correlation: $0.25, P_{adj}=0.003$). While these correlations were low, they indicate that lifestyle (i.e., passive exposure) and the residential environment (i.e., passive exposure) independently influenced the composition of the skin microbiota community.

Rural lifestyle and environment were associated with the increasing abundance of microbes from environmental sources on skin microbiota (Fig. 2B). In contrast, both urban lifestyle and urban living environment were associated with enrichment of taxa often found in built environments, such as *Chroococcidiopsis* (Fig. 2B). In addition, (human) skin-related taxa such as *Propionibacterium* and *Friedmanniella* were enriched on dog skin in an urban environment, which, in turn, was associated with a homogenization of urban dog microbiota (the within-group dissimilarity of skin microbiota was smaller in urban dogs than in rural dogs; $P=0.0001$). On the contrary, urban lifestyle seemed to increase within-group dissimilarity ($P=0.0026$).

**Allergic Symptoms Associated with Exposure to Environmental Microbes and Skin Microbiota.** In addition to microbial composition, land use and lifestyle were also associated with the prevalence of allergic symptoms in dogs (Fig. 3), while the exercise environment was not associated with allergic symptoms. Allergies were most common in dogs living in urban environments and having an urban lifestyle, whereas the lowest prevalence was associated with rural environments and rural lifestyle (Fig. 3A). Urban-type lifestyle seemed almost to double the prevalence of allergies in dogs living in rural environments compared with dogs with rural-type lifestyle in rural environments (Fig. 3B). Furthermore, not just the prevalence of allergic symptoms ($P=0.006$) but also the severity of the symptoms was positively associated with urban lifestyle ($P=0.0001$). We also tested classical biomarkers of allergy and inflammation, Immunoglobulin E (IgE) and C-reactive protein (CRP) (SI Appendix, Table S1), against environment and lifestyle, but we did not discover any robust associations. Moreover, the severity and prevalence of allergic symptoms were not associated with IgE or CRP levels.

Interestingly, the predicted prevalence of allergic symptoms and the skin microbiota changed concurrently along the land use of the residential environment (Fig. 4A). An increasing area of forest and arable land correlated with a greater amount of different Proteobacteria and other soil-related taxa on skin. In urban areas, in turn, general skin-related bacteria such as Actinobacteria, especially of the genera *Friedmanniella* and *Microlunatus* (*Propionibacteriales*), were enriched on skin (Fig. 4B). In other words, these results suggest that the living environment, skin microbiota, and allergic diseases were interrelated. This was also supported by both differing skin microbiota and prevalence of allergies in groups based on residential environment and lifestyle (Figs. 2A and 3B).

The microbial communities of allergic dogs resembled each other more than the communities of healthy dogs ($P=0.0001$), indicating commonalities in the skin microbiota in allergic individuals. However, the variation among dogs was still so high that no clear key taxa in microbiota of allergic dogs could be identified in the random forest (RF) analysis, apart from the enrichment of human-derived skin microbiota. However, the summed abundance of microbes relevant in the skin microbiota of urban individuals (defined by the RF analysis of land use against microbiota) was significantly higher in allergic dogs, but individual-level variation remained large (SI Appendix, Fig. S4).

**Early-Life Environment Partly Associated with the Current Skin Microbiota and Allergic Symptoms.** Interestingly, we found that the skin microbiota differed between dogs belonging to healthy and allergic dog families (i.e., dog families with only healthy puppies vs. at least one allergic puppy, $P=0.0158$, SI Appendix, Fig. S5A). This compositional difference seemed to relate to both early-life and current residential environment. Microbiota of dogs belonging to healthy dog families was significantly associated with increasing area of arable land and forest in the surroundings of the birthplace ($P=0.0007$).
The first weeks of life also had an influence on the prevalence of allergies. Allergies seemed to be most common in dogs that had spent their entire life in an urban environment, and rarest among dogs that were born and lived only in rural environments (SI Appendix, Fig. S5B). It is noteworthy that dogs from healthy dog families, those born and living in rural environments, had a similar skin microbial composition (i.e., “the triangle of healthy individuals” delineated by the land-use axes in SI Appendix, Fig. S5A). Mothers and their puppies tended to be more similar in their microbial composition than random pairs between adults (dog mothers) and puppies (P = 0.0001). Additionally, the dissimilarity between dogs was smaller in allergic than in healthy dog families, indicating, again, the homogenization of skin microbiota (SI Appendix, Fig. SSD, P = 0.0001). These findings suggest that puppies receive bacteria from the early-life living environment (most likely via their mother), which can affect their future health. However, we were unable to exclude the effect of genetic predisposition on the prevalence of allergies, which can be important as an allergic mother dog more likely had allergic puppies than a healthy mother (P = 7.5e−5).

Discussion

Our results suggest a vital role of residential environment and lifestyle in dog health. Exposure to environmental microbes, both passively (via the living environment i.e., where one is) and actively (through lifestyle i.e., what one does), related to the composition of skin bacterial communities. Remarkably, both passive and active exposure were also associated with the prevalence of allergies, as shown previously (15). Finally, the composition of skin microbiota differed in healthy and allergic dogs. Therefore, our results suggest a triangle of associations between skin microbiota, allergies, and exposure to environmental microbes. Recent studies trying to relate the living environment and allergic disease have produced somewhat contrasting results, most likely due to several confounding factors associated with complexities of human lifestyles (8). While previous studies

![Fig. 2. Land use in residential environment (Env) and lifestyle of dogs is associated with skin microbial communities. (A) The distance-based redundancy analysis (db-RDA) of the skin microbiota constrained by the rural–urban categories of residential environment (P = 0.0006) and lifestyle (P = 0.0024). Each line represents the distance of microbiota from a centroid of a group of an individual dog. Different colors indicate dogs as follows: blue, rural dog with rural lifestyle; purple, rural dog with urban lifestyle; red, urban dog with rural lifestyle; and gray, urban dog with urban lifestyle. Footnotes “en” and “li” mark residential environment and lifestyle, respectively. (B) Tukey boxplots of summed and square root transformed abundance (normalized counts) of taxa, which best predicted groups in A in an RF analysis. Outlier data points are not shown. Colors of the boxplots correspond to the groups in A. These groups were used to visualize the clustering of dogs’ microbiota due to the effect of constraining variables.](image)

![Fig. 3. Residential environment and lifestyle together shaped the prevalence of allergic symptoms in dogs. (A) The prevalence of allergy (lines), predicted from data. Red and light blue symbols indicate allergic and healthy dogs, respectively. (B) The prevalence of allergic symptoms in dogs having similar combinations of living environment and lifestyle (rural environment, RUen; rural lifestyle, RUi; urban environment, URen; and urban lifestyle, URI).](image)
on the relationships between the living environment, microbiota, and health have failed to find a robust link between environment-associated patterns in microbiota and allergic sensitization (3, 16), our simplified canine model has provided explicit empirical evidence of such interaction, as predicted by the biodiversity hypothesis of allergic diseases (5).

The combined effect of passive (living environment) and active (lifestyle) contact with environmental microbes on allergies in humans or dogs has previously received little attention. However, the contrasting allergy prevalence in farm and nonfarm children in rural areas (17), and in children predisposed to traditional and modern farming (18) suggest that active contact to the environment is important. Here we show that dogs living in urban environments and exposed to urban-type lifestyle—characterized by various hobbies and by living in apartments and in a single-person family without other pets—are most susceptible to allergic diseases. In opposite circumstances, i.e., when dogs are living in rural environments with a large family and frequent animal contacts, their allergies are rare. Interestingly, an urban-type lifestyle almost doubled the prevalence of allergies in dogs living in rural environments compared with dogs with rural-type lifestyle in rural environments. Furthermore, the skin microbiota of dogs differed between all combinations of rural and urban residential environment and lifestyle, suggesting that microbiota can have a role in the differing allergy prevalence in each group. This assumed interplay between microbial exposure, host microbiota, and allergies, which rises from findings here but also from previous work (3, 16–18), highlights the importance of natural environments for health. It also suggests that the exposure to a diverse microbial world can be a protective factor both in humans and dogs.

The effect of lifestyle on the skin microbiota has been suspected previously, but “lifestyle” is difficult to define. A recent study found that the living environment clearly differentiated the skin microbiota of children, but the effect disappeared in teenagers, which was likely due to shared lifestyle patterns regardless of living environment in this age group (16). Our findings highlight that where a dog lives and what a dog does can jointly influence microbiota and health. Surprisingly, the exercise environment (where a dog moves) was only marginally related to skin microbiota and did not associate with allergies. Obviously, living environment and lifestyle are more

Fig. 4. Living environment is associated with skin microbiota and the prevalence of allergies. (A) Distance-based redundancy analysis (db-RDA) of canine microbiota uses land-use gradients (PC1 and PC2) as explanatory variables to constrain microbial dissimilarity. Shown are skin microbiota changes along the land-use gradient from forested to urban areas (P = 0.001 for PC1) and from arable lands to forested areas (P = 0.002 for PC2). Each dot corresponds to microbial community of an individual dog. A color gradient of dots marks the proportion of different land-use types in the residential environment of an individual dog. Purple lines mark the predicted severity of allergic symptoms showing increase along the urbanization. (B) Abundance of skin microbial taxa along the land-use gradients. The appointed taxa in are among those that best explained the corresponding land-use gradients in the RF analysis. In each panel, a dot marks the summed abundance of appointed taxa in microbiota of individual dogs. The abundance of Clostridia in the lowest panel was square root transformed. The color gradient of dots marks the proportion of different land-use types in the residential environment of an individual dog as in A. Blue lines demonstrate the fitted values.

<table>
<thead>
<tr>
<th>taxa</th>
<th>rank</th>
<th>abundance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Friedmanniella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microlunatus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alphaproteobacteria</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bradyrhizobium</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sphingomonas</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clostridia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptostreptococcus</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lachnospiraceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proteiniclasticum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peptosteptococcus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
sustained factors than exercise environment, which may change on a day-to-day basis and the exposure lasts only for short periods of time. Hence, we suggest that prominent, sustained exposure is needed for health-promoting changes in skin microbiota to arise or last normal immune function.

We observed that certain common members of the human skin microbiota, for example Actinobacteria, were enriched in urban canine skin microbiota. On the contrary, soil-related and other microbes from environmental sources, such as Bradyrhizobium (Alphaproteobacteria) (19), were abundant in the rural skin microbiota, indicating that skin microbiota is a relevant indicator of exposure to environmental microbes. Moreover, the urban dogs were more alike than the rural ones, which indicates homogenization of skin microbiota in urban environments. Our observations resonate with previous research, showing that urban environmental microbiota is more homogenous than rural environmental microbiota (4), and that inside a city, the built areas are homogenous compared with parks (20). Moreover, microbiota in closed indoor spaces, such as apartments and subways, are dominated by human skin-origin microbes (21, 22). Interestingly, human-derived microbes were enriched in allergic dogs compared with healthy dogs and that the skin microbiota in allergic dogs was more alike than in healthy dogs, pointing again to microbial homogenization. These findings indicate that either certain rural-dwelling microbes or a constant exposure to heterogeneous environmental microbial communities, or both, support normal immune function.

Previous studies have demonstrated the importance of early-life exposures in the development of allergic diseases in humans. The composition of gut microbiota during the first weeks of life tends to differ between those who do and do not develop allergies later on refs. 23 and 24. Importantly, there seems to be a limited window of opportunity, during which immune education by microbiota is most decisive (24). When we studied microbiota of individuals from healthy and allergic dog families, we found that in addition to the current living environment, early-life environment was associated with later composition of skin microbiota. Excitingly, we observed that the genus Actinobacteria was more prevalent in healthy dog families. These bacteria have previously been associated with protection against allergies in humans (25–27) and in a mouse model (10, 28, 29). In allergic dog families, human skin-related Friedmanniella was also more prevalent. Both genera associated with land use in early-life environment, demonstrating that early-life environment can influence the microbiota. Nevertheless, a dog’s current environment seems to be more important in development of allergy even though early environment also leaves a mark. The skin microbiota was more homogenous in allergic than in healthy dog families, which can be a primary driver in the development of allergy. Based on these observations, we propose that lack of certain early-life exposure may create a susceptibility to develop allergy, but that later lifestyle and exposure can change the final outcome of allergy in dogs. Because dogs move from their breeder to the owner at a young age (7–8 wk old), the window of opportunity regarding immune development may still be open at that time. This complicates the interpretation of importance of early versus later life exposure. Previous studies have reported associations between the environment and allergies in dogs (12, 30), but early-life environment was not important (15). Another limitation is that canine allergy is not a well-defined disease. Allergies in dogs are commonly clustered to specific breeds, indicating that the genetic background.

Also, in our data the Labrador retrievers were more allergic than the Finnish lapphunds, indicating the importance of genetics (12). However, the patterns we found in microbiota and the prevalence of allergies were similar in both breeds, indicating that the environmental component operates similarly regardless of the genetic background.

We have demonstrated here that pet dogs can be a convenient model for understanding the relation between living environment and inflammatory diseases. While free of most confounding factors associated with human life, pet dogs suffer increasingly from inflammatory diseases similar to those in humans, and they share the environment and lifestyle with their owners. This means that our results are likely to apply also to humans. However, the validation of this canine model requires comparative research efforts on dog and human microbiota and health. Finally, we want to emphasize the need for longitudinal or interventional studies, focusing on the triangle of interactions between health, microbiota, and the exposure to environmental microbes. Future studies should concentrate on better understanding the right timing, quality, and duration of exposure to environmental microbes, as well as the contribution of the microbiota in different body parts to health. Experimental studies should follow these, to confirm causality that cannot be established in our cross-sectional study. We suggest that model animal-based research is needed to speed up the definition process of optimal exposure, with pet dogs serving as a valuable real-life model with spontaneous allergy.

Materials and Methods

Data Collection. Breeders of either Labrador retrievers or Finnish lapphunds were invited in the study. These breeds are equally common in rural and urban areas and their large size eases blood sampling. The owners of puppies from selected litters were recruited via the breeders. Our sample included 169 dogs (39 were mother dogs). Skin microbial (swab) samples were taken from the inner side of the front leg, at about carpus level (3 × 3 cm2 area, SI Appendix, Form S1). Next, DNA was extracted and the V1–V3 region of 16S rRNA gene was sequenced as previously described (16). Blood serum was collected for the analysis of IgE and CRP. Owners filled out a questionnaire (SI Appendix, Form S1), concerning their dogs’ environment, lifestyle, and allergic symptoms. A subset of owners (n = 96) followed their dogs’ movement for a week with a passive tracker (Trail, SleuthGear). Details about participants and data collection are provided in SI Appendix, SI Methods.

Bioinformatic Analysis. The processing of 16S rRNA gene sequences, formation of OTUs, and taxonomic classification are described in SI Appendix, SI Methods. The processed sequence counts were normalized with cumulative scaling (37) and contaminant OTUs were removed as described in SI Appendix, SI Methods. Finally, our data included 45,905 normalized OTUs that were used in further statistical analysis. The large number of OTUs is explained by the strict similarity threshold we used. All 16S rRNA gene sequences have been deposited in the National Center for Biotechnology Information (accession no. SRP 133457).

Simplification of Complex Data. The quantification of residential (birth and current) and exercise environment land use was done as described in Lehtimäki et al. (16), using PCA. From each PCA, the first or second component, or both, were extracted for further analysis (SI Appendix, Table S2). Higher values along the first axis indicated more rural, especially forested areas, while lower values indicated a higher proportion of urban such as built environments (SI Appendix, Table S2). Higher values along the second axis indicated a transition from forested areas to agricultural areas.

As no single question regarding allergic symptoms was considered a reliable measurement of canine allergy, these questions (questions 5.1–6.14, SI Appendix, Form S1), containing several mutually correlating variables, were summarized to a single continuous variable using PCA (based on Gower distance, accepting a mixture of numeric and categorical variables). The first PCA axis captured the variation in the severity of allergic symptoms, with higher values indicating more severe symptoms. Similarly, we selected a set of family- and lifestyle-related questions (marked with * in SI Appendix, Form S1), which, according to our knowledge, influence how dogs are exposed to environmental microbes, to create a lifestyle variable. The first PCA axis allowed us to allocate dogs according to the lifestyle of the owner, which showed a gradient from rural to urban lifestyle. Briefly, rural lifestyle correlated with living in bigger (human) families, having more animal contacts, and living in town houses,
while urban lifestyle correlated with having many hobbies, traveling a lot, and
family history (ILAR J 63: 208–214). However, the role of diet in the development of allergic disease is less clear. The composition of the gut microbiota has been shown to be influenced by diet, with Western diets generally containing a higher amount of pro-inflammatory bacteria than traditional diets (123). This suggests that dietary changes could potentially be used as a strategy for preventing or treating allergic diseases.

In conclusion, the current research aims to understand the relationship between the gut microbiota and allergic disease. By using a combination of techniques such as 16S rRNA sequencing and bioinformatic analysis, we can identify potential biomarkers that may be useful for diagnosis and treatment. Future studies should focus on validating these findings in larger, more diverse populations and on developing effective interventions to modulate the gut microbiota for prevention and treatment of allergic diseases.