Screening a puppy's DNA methylome may help predict how energetic or fearful they will be

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Anyone who's ever had a dog knows how different one can be from another. For example, they can be reserved or friendly, playful or calm,
fearful or bold, and prone to bark or fetch or not. Research has shown that some of these differences are genetically determined. But even within dog breeds, where line breeding and artificial selection have led to the loss of much of the original genetic variation, the behavior of individuals can differ widely.

Now, researchers show that part of the differences in temperament—in particular their 'energy' level and fear-related behaviors—depend on acquired differences in the epigenome. The epigenome, an individual's unique set of chemical tags on DNA and its associated histone complexes (nucleosomes), can dial the expression of local genes up or down. Because the epigenome is known to depend on age, diet, exercise, training, socialization, and other environmental factors, it can impact the activity and wiring of nerve cells and thus alter behavior.

Corresponding author Dr. Matteo Pellegrini, a professor at the University of California at Los Angeles, said, "Here we show that the behavior of dogs is associated with their epigenome, in particular DNA methylation. Our results open the door to using epigenetics to screen and select for desired behavioral traits in companion or service dogs."

Behavioral questionnaire

Pellegrini and his colleagues quantified the epigenetic, genetic, and behavioral differences between 46 female and male dogs from 31 different breeds, with an age between one and 16 years.

Behavioral differences were quantified based on how the owners rated their dog in the Canine Behavioral and Research Assessment Questionnaire (C-BARQ) questionnaire, a widely used standardized tool that consists of 101 questions. The researchers used a form of machine learning, Partial Least Squares (PLS) regression, to identify significant associations between genetic or epigenetic variants and behavioral traits.
The epigenome is known to differ strongly between tissues. In principle, **nervous tissue** would be the best place to look for associations between behavior and the epigenome. But for ease of collection, the researchers studied the epigenome of epithelial and **immune cells** obtained from swabs inside the dogs' cheeks. For this proof-of-concept study, they focused on DNA methylation at 3,059 CG sites, as this is easier to quantify than other types of epigenetic marks, for example methylation or acetylation of histones.

Heat map of the dog phenotype data and breed using Python Seaborn Clustermap (Waskom, 2021). The map depicts dog's breeds and their scores from 0 to 4 in...
the 14 behavioral phenotypes. White colors indicate a score of 0 for a behavioral phenotype while darkening shades of green indicate a behavioral score close to 4. Related breeds and traits are clustered close to one another on nodes of the hierarchical trees. These nodes are determined by hierarchical clustering. Credit: Frontiers in Psychology (2022). DOI: 10.3389/fpsyg.2022.1025494

**Epigenetics are more informative than genetics for behavior**

The results showed that the epigenome was a better predictor of behavior than the genotype at the sites they measured. Even within the most represented breed—Australian shepherds with 12 dogs in the sample—only two of the 930 selected Single-Locus Polymorphisms (SNP) examined were strongly associated with behavioral traits. Two SNPs on chromosome 12 could partly predict a dog's degree of stranger-directed fear (ie, fear of unfamiliar people).

But when the authors corrected for the potential confounding effect of differences in age, differences in DNA methylation between dogs explained a far greater proportion of observed variation in energy, attention seeking, nonsocial fear, and stranger-directed fear than genetic differences did. These results imply that the epigenome helps to shape behavioral differences in dogs, even in tissues that aren't part of the nervous system.

**Surprising results**

"These associations between the DNA methylation of cells in the mouth and dog behaviors were surprising, and suggests that future studies that examine DNA methylation in nerve tissues may identify similar patterns," said Pellegrini.
"We plan to conduct much larger studies in the future, with the goal of developing biomarkers that allow us to better identify dogs with specific behavioral predispositions."

He concluded, "Ultimately, we would be very interested in examining the epigenomes of highly specialized dogs such as guide dogs or sled dogs, to be able to assist in the selection of dogs that might be more likely to successfully complete their training."

The paper is published in the journal *Frontiers in Psychology*.


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