

# Association between Gene Variants and Aggressive Behavior in Purebred and Mixed Breed Canines

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## Abstract

In 2017, one in sixty-nine people were bitten by dogs. This sparked our exploration of the genetic roots of aggression and how it correlates to certain breeds of dogs. Previous studies have identified gene variants for dog aggression using genome wide associations. It is hypothesized that differences within DNA amongst certain breeds of dogs are associated with dog aggression. In our study we mined data from Darwin's Ark. We developed a multiple linear regression model using R studio to see if there is a correlation between mixed vs. purebred, the presence of this variant, and if the dog is homozygous, heterozygous or neither for the variant. The dog aggression score is the dependent variable while variant 18:20272961:A:C (number of alleles) is the independent variable. We also created a multiple regression model with 30 variants from Darwin's Ark Data, including breed (purebred or not purebred). We then calculated the coefficient of determination to see how much the dependent variable is explained by the independent variable. We predict that the mixed breeds will be associated with genetic variants, including 18:20272961:A:C, that correlate with aggressive behavior. This research enables us to find a possible explanation behind dog aggression by applying a genetic perspective behind a dog reactions.

## Background

### Gene variant 18: 20272961:A:C

- Located on chromosome 18
- A gene variant is a permanent alteration in the nucleotide sequence of a chromosome
- According to the genotype data set for Vassay et al. variant 18:20272961:A:C increases the risk of fear and aggression
  - Homozygous - two identical alleles of a particular gene
  - Heterozygous - two different alleles of a particular gene
- Specifically variant 18:20272961:A:C is associated with
  - Dog-oriented fear and aggression
  - Stranger-oriented fear and aggression



### Darwin's Ark Project

- An organization working with teams of researchers at Broad Institute of Harvard and MIT, the University of Massachusetts Medical School, and the International Association of Animal Behavior Consultants and pet owners
  - Want to understand the interaction of genetics and environment, and work toward bettering the lives of people and pets
  - 1,529 dog owners have sent their dog's DNA to the Darwin's Ark project
- Participants can be involved by answering survey questions about their pet, and can receive a DNA kit as well

## Review of Literature

### 1: Managing the risk of aggressive dog behavior (Williams et al. 2019)

- Purpose of the study is to find what influences a dog owners to use outdated punishment techniques and what prevented and drove them to using positive reinforcement solutions
- Researchers will systematically examine the factors influencing an owner's choice of training technique, as well as the impact of this behavior upon the owners of these dogs.
- Positive reinforcement based behaviour techniques are humane and effective in the treatment of aggressive behaviour in dogs
- The use of punishment-based techniques are likely to be detrimental to the welfare of the dog and can lead to an increase in aggression
- This provides a foundation for future research can further explore the influence of different psychological factors on an owner's decision to use positive reinforcement techniques to manage their dog's aggressive behaviour.

### 2: A Common Underlying Genetic Basis for Social Behavior in Dogs and Humans (vonHoldt et al. 2017)

- The purpose of this article is to exemplify how there is a genetic basis for hypersocial behavior for dogs and humans.
- The researchers sequenced a region of chromosome 6 in dogs and found multiple sections of canine DNA that were associated with differences in social behavior.
- The researchers also collected and analyzed the data of 18 domesticated dogs and 10 captive human-socialized wolves, as well as the biological samples used to sequence their genomes.
- The domesticated dogs displayed more human-directed behavior and spent more time in proximity to humans than the wolves.
- The research was able to understand the history of dogs behavior by comparing their genetic data to that of the human-socialized wolves.

### 3: Canine Aggression and Genetic Control (Norwegian School of Veterinary Science et al. 2010)

- The control of different behaviors is a complex idea because of the influence of both genetics and social interaction and activity
- The connections between dog aggression and the genes that are involved in the mental aspect of the brain and aggression (neurotransmission in the brain)
- The species of dogs can differentiate each other from their level of aggression and their mental state, however, the study of the human brain helps in the comparison to a dog's brain
- The study showed a variety of genes related to serotonin and dopamine. The study describes the genetic activity in different areas of the brain and how that relates to a dog's aggression and non-aggression

## Purpose and Hypothesis

**Purpose:** To investigate the association between aggressive behavior, breed, and the presence of a gene variants including chromosome 18:20272961:A:C.

**Hypothesis:** It is hypothesized that being pure or mixed breeds and the presence of a gene variant, specifically chromosome 18:20272961:A:C, can be used to predict and explain aggression.

## Methods

**Step 1** We collected the data from the Darwin's Ark project and then summarized the data using graphs relating to: mixed vs purebred for the particular gene, the presence of chromosome 18:20272961:A:C, and how many were homozygous vs heterozygous

**Step 2 Part A** We created a linear regression model using R-studio to see if there is a correlation between breed (mixed vs. purebred) and the presence of the chromosome 18:20272961:A:C, and if it is homozygous vs heterozygous or neither. The dog aggression score was the dependent variable while chromosome 18:20272961:A:C and breed (mixed or purebred) were the independent variables.

**Step 2 Part B** We were most interested in chromosome 18:20272961:A:C, but we also tested other variants recorded by Darwin's Ark (over 100 variants identified). We tested every single variant to see if they individually predict aggression score (loop)

- We created separated tables with the selected variants that are significantly associated with aggression

**Step 3** Variants were selected from the histogram shown in Figure 2. We filtered half of the variants that were highly correlated with each other (correlation coefficient > 0.9). We did this to prevent overfitting the regression model. We calculated the coefficient of determination (r squared) to see how much the dependent variable is explained by the independent variables. This number needed to be adjusted to account for the high number of variables.

**Step 4** Created multiple regression model with filtered variables (30 variables including breed (purebred or not purebred)

- We made a bar chart that shows the coefficient for each significant variant
- We made a box plot to show the relationship between zygosity for variant X18 and aggression score

## Significance Of Study

In 2017, one in sixty-nine people were bitten by dogs. This sparked our exploration of the genetic roots of aggression and how it correlates to certain breeds of dogs. Previous studies have identified gene variants for dog aggression using genome wide associations.

**Controls**

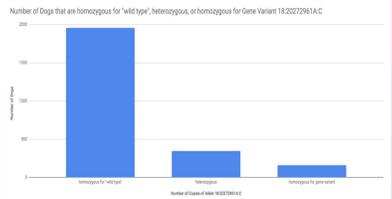
- Environment
- Age of the dogs
  - Between ages 2-6
- Number of Dogs: 2471

**Independent Variable**

- Gene Variant (we will be testing 60 variants)
  - Specifically interested in variant 18:20272961:A:C located on chromosome 18.
- Breed (mixed or pure)

**Dependent Variable**

- Dog aggression score
  - Agnostic Threshold factor: the reactivity of the dog and their response to distressing or annoying stimuli (ex. approached by a stranger)



Example from data set of how many are homozygous/heterozygous for the gene variant chromosome 18: 20272961:A:C

## Results

```
> lmodelr::lm(data = dogData, aggression_score ~ X18_20272961_A_C_A)
> summary(lmodelr)

Call:
lmFormula = aggression_score ~ X18_20272961_A_C_A, data = dogData

Residuals:
    Min:    30 Median:    30     Max:
-2.8777 -0.4526  0.1462  0.6709  2.4793

Coefficients:
(Intercept)      Estimate Std. Error t value Pr(>|t|)
            -0.28722      0.00386   -74.884 <2e-16 ***
X18_20272961_A_C_A  0.13344      0.00533   25.043 <2e-16 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9728 on 1538 degrees of freedom
[2 observations deleted due to missingness]
Multiple R-squared:  0.00501, Adjusted R-squared:  0.004981
F-statistic: 0.664 on 1 and 1538 DF, p-value: 0.41625

> lmodelr::lm(data = dogData, aggression_score ~ purebred + X18_20272961_A_C_A)
> summary(lmodelr)

Call:
lmFormula = aggression_score ~ purebred + X18_20272961_A_C_A,
data = dogData

Residuals:
    Min:    30 Median:    30     Max:
-2.8876 -0.5614  0.1378  0.6382  2.4662

Coefficients:
(Intercept)      Estimate Std. Error t value Pr(>|t|)
            -0.49758      0.13750   -3.595 0.00025 **
purebred         0.18876      0.07721   2.458 0.0145 *
X18_20272961_A_C_A  0.07562      0.07720   0.980 0.3295

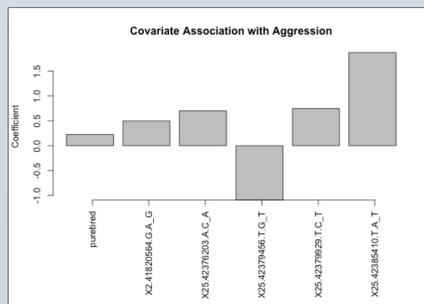
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 0.9377 on 1575 degrees of freedom
[2 observations deleted due to missingness]
Multiple R-squared:  0.02124, Adjusted R-squared:  0.00549
F-statistic: 3.664 on 2 and 1575 DF, p-value: 0.02823
```

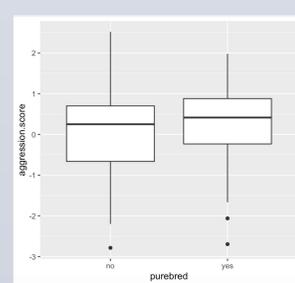
A. Aggression Score = m1("X18"variant) + b B. Aggression Score = m1(Purebred) + m2("X18"variant) + b

**Figure 1A:** shows that the chromosome 18 variant is significantly associated with aggression when singly included in the regression model.

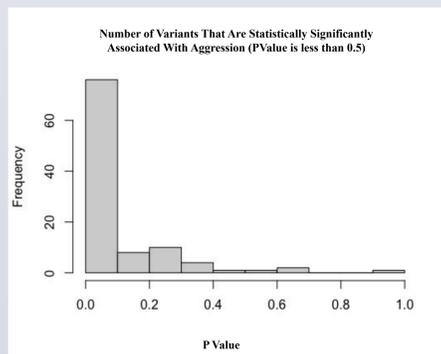
**Figure 2B:** shows that purebreds are associated with higher aggression scores.



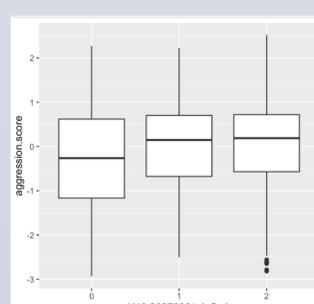
**Figure 3:** This graph shows the coefficient for breed and gene variants (covariates) that were significantly associated with aggression in the regression model described in methods - "step 4".



**Figure 4:** This figure shows the aggression score for purebreds who have variant X18.



**Figure 2:** This histogram shows the number of gene variants identified by Darwin's Ark that are significant predictors of dog aggression most specifically with the p value below 0.5. These values were corrected to account for multiple hypothesis testing.



**Figure 5:** This graph focuses on the specific variant: X18. This graph shows the number of alleles on the horizontal axis and the aggression score on the vertical axis. The dog will either be homozygous or heterozygous ("zygosity") for one of these variants. The number of alleles does make a difference towards aggression (two alleles, one allele, or no alleles). Two alleles would mean that the dog appears to be a little more aggressive.

## Review of Literature (Continued)

### 4: Endogenous Oxytocin, Vasopressin, and Aggression in Domestic Dogs (MacLean et al. 2019)

- Behavioral Data was recorded in C-BARQ (Canine Behavioral Assessment and Research Questionnaire - obtained from University of Pennsylvania)
- Heritability Analysis: Randomly sampled 25 individuals from each breed without replacement
- Genome wide association: identified 131 SNPs (single nucleotide polymorphism- type of genetic variation) associated with breed differences in behavior through conducting Gene Ontology enrichment analysis test
- Genotype difference accounts for more than 50% of behavioural variation across breeds - up to 25% higher than heritability estimates from genetic studies within breeds
- Also discovered that SNPs (Single-nucleotide polymorphism) associated with breed differences in behaviour tend to fall in genes that are disproportionately expressed in the brain

### 5: Genetic Mapping of Canine fear and aggression (Zapata et al 2017)

- The purpose of this study was to examine whether breed stereotypes of fear and aggression could be mapped by cross-breed GWA and how these breed stereotypes play into the factor of dog aggression.
- The scientists did a genomewide association (GWA) to map the different breeds of dogs and their stereotypes of aggression and fear.
- Through the GWA mapping scientists identified that variants on chr18 are associated with fear and aggression in dog breeds. This shows how aggression can be detected biologically.

## Discussion

Through our study, we proved our hypothesis, by examining the association between dog's aggressive behaviour of purebred vs mixed-breed and the gene variants enabled us to uncover how variant 18:20272961:A:C is associated with aggressive behavior but only when included in linear regression as a single independent variable. When examining our data we came to the conclusion that purebreds are associated with higher aggression scores. When included with other gene variants identified in Darwin's Ark, there was a non-significant association. In the creation of our final regression model we utilized variant 18 along with other variants found to be associated with aggression (figure 3). By mining data from the Darwin's Ark Project and by performing a linear regression we were able to confirm association between dog's aggression and how not only is their aggression based from training but also comes from the genetics of the dog's breed.

## Conclusion

This research enables us to find a possible explanation behind dog aggression by applying a genetic perspective behind a dog's reactions. This study not only looks at the genetic basis but a comparison between purebred dogs and mixed breed dogs. This research will promote further research for dog breeders since now there is a connection between the dogs genetics and the behaviors recorded amongst multiple participants of the survey, similarly this study promotes the choosing of service dogs.

## Mentor

- Kathleen Morrill
- PhD candidate at the University of Massachusetts Chan Medical School (September 2016 - present)
  - University of Massachusetts Chan Medical School
  - Kathleen's Research
    - "Darwin's Ark": medical school team work with pet owners to understand the interaction of genetics and environment, and work toward bettering the lives of people and pets



## References

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- Norwegian School of Veterinary Science. "Canine aggression and genetic control." *ScienceDaily*. ScienceDaily, 26 May 2010. <www.sciencedaily.com/releases/2010/05/100525090552.htm>.
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