Identification of Candidate Polymorphisms for Feline Behavior Differences

Christine A. Kemmerly, 99 Lives Consortium, Leslie A. Lyons

Department of Veterinary Medicine and Surgery, College of Veterinary Medicine, University of Missouri, Columbia, Missouri

Veterinary Research Scholars Program
University of Missouri

Introduction

Nature versus nurture is the everlasting argument around behavior. However, many behavioral traits and psychiatric disorders have some level of heritability, indicating genetics play a role in behavioral expression [1, 2]. In domestic animals, finding genetic polymorphisms linked to specific behavioral traits can help with decisions on treatment, service animal use, and evaluating potential of animals for adoption. Behavioral genetics research is much more prevalent in dogs compared to cats, despite more than 60 million pet cats in the US [3]. In cats, most of the behavior research has investigated the physiology of behavior, association of behavior with other phenotypic traits, such as coloration, and the heritability of behavior [4]. This study initiates investigation into specific mutations and genes associated with different behaviors in felines. Due to their association with behavior in humans and other animals, polymorphisms will be investigated in *monoamine oxidase A* (*MAOA*), *serotonin transporter/solute carrier family 6 member 4* (*SLC6A4, 5-HTT*), *dopamine receptor subtypes D1-D5* (*DRD1-DRD5*), *catechol-O-Methyltransferase* (*COMT*), *tryptophan hydroxylase 1 and 2* (*TPH1* and *TPH2*), and *oxytocin receptor* (*OXTR*).

Methods

- Evaluated variant data from sequence data from 336 cats **(Figure 1)**
- Software (GoldenHelix VarSeq) was used to identify variants in the 11 candidate genes
- Prioritized variants most likely to be deleterious and reliable based on data **(Figure 2-3)**
- Three variants were further investigated via Sanger sequencing
- Variants will be genotyped in cat breeds associated with specific behavioral phenotypes and in cats from a research colony with observable differences in behavior

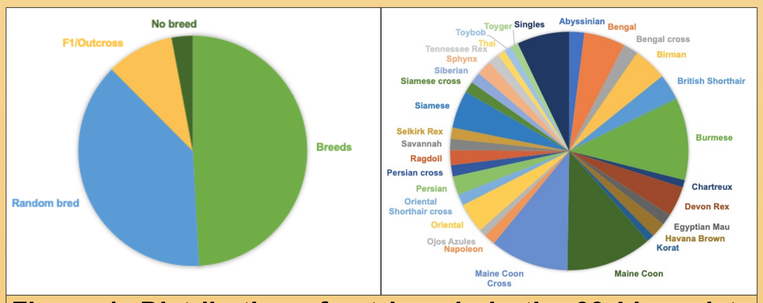

Figure 1: Distribution of cat breeds in the 99 Lives data set. (Left) Cats divided into larger signalment of random bred (n=129), breeds (n=165), F1/outcross (n=32), and no breed provided (n=10). **(Right)** Breakdown of specific breeds and outcross breeds in dataset. 14 cats were grouped as "singles" with only one individual from that breed in the dataset.

Results

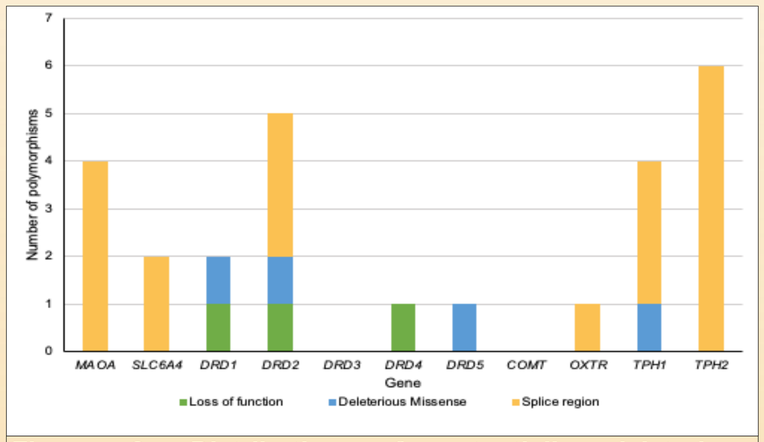

Figure 2: Distribution of potentially deleterious polymorphisms within each candidate gene investigated.

- The diverse breed representation suggested breed specific variants could be identified
- 744 variants were identified
- *MAOA, SLC6A4, OXTR, TPH1*, and *TPH2* had significant variants only in splice regions
- *COMT* and *DRD3* had no predicted deleterious variants

Three variants were investigated **(Figure 3)**

- *DRD4* variable number of tandem repeats region (VNTR) in exon 3 and two single nucleotide polymorphisms (SNPs) identified within the VNTR
- *DRD4* stop gain - loss of function variant (LoF)
- *DRD5* SNP - arginine to histidine change

Variant Description	Variant	Change
DRD4 VNTR SNP 1	C/T	Pro>Leu
DRD4 VNTR SNP 2	C/T	Pro>Leu
DRD4 LoF	C/A	Cys>Ter
DRD5 SNP	C/T	Arg>His

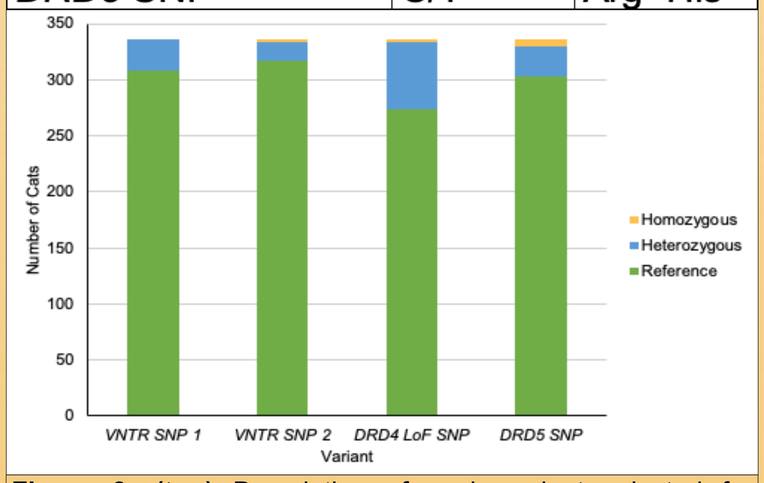

Figure 3: (top) Description of each variant selected for sequencing in more cats. **(bottom)** Genotypes of cats in database for the variants being sequenced. *DRD4* VNTR SNP 1 (homozygous (homo) variant = 0, heterozygous (het) = 28). *DRD4* VNTR SNP 2 (homo = 2, het = 17). *DRD4* LoF SNP (homo = 3, het = 59), *DRD5* SNP (homo = 6, het = 27).

Other Findings

- Cats do not have an *SLC6A4* and *MAOA* VNTR as in primates
- A 5 bp repeat area in the promoter of *MAOA* and *DRD2* was identified
- Two splice region variants in *TPH1* seem to be associated with Rex breeds
- From alignment of the protein sequences, major differences in the cat compared to the mouse and human were identified in *MAOA*, *TPH1* and *DRD2* **(Figure 4)**

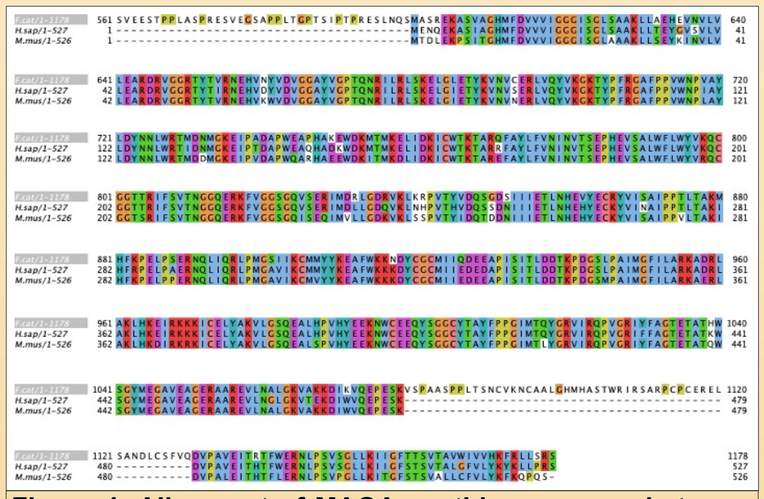

Figure 4: Alignment of MAOA peptide sequence between species. Note the start of the alignment at residue 600 of the cat. The aligned cat sequence has 90% and 87% homology with the human and mouse, respectively

Future work

- Genotyping these variants in more cats can lead to defining associations with behavior traits, such as attention-deficit/hyperactivity disorder-like behavior [5]
- Additional variants, such as splice region variants and a potential *DRD2* frameshift variant should also be investigated
- More investigation into polymorphisms in the regulatory regions is suggested

References: **1.** Salonen M, Vapalahti K, Tiira K, Mäki-Tanila A, Lohi H. Sci Rep. 2019;9: 1–10. doi:10.1038/s41598-019-44324-x
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