

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



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)

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-(COMT),Methyltransferase tryptophan hydroxylase 1 and 2 (TPH1 and TPH2), and oxytocin receptor (OXTR).

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

F.cat/1-1178	561 SVEEST <mark>PPLASPRESVEGSAPPLTGPTSIPTPRESLNQSMASREKASVAGHMFDVVVIGGGISGLSAAKLLAEHEVNVLV</mark> 6	40
H.sap/1-527	1MENQEKASIAGHMFDVVVIGGGISGLSAAKLLTEYGVSVLV	41
M.mus/1-526	1MTDLEKPSITGHMFDVVVIGGGISGLAAAKLLSEYKINVLV	41
F.cat/1-1178	641 LEARDRVGGRTYTVRNEHVNYVDVGGAYVGPTQNRILRLSKELGLETYKVNVCERLVQYVKGKTYPFRGAFPPVWNPVAY	720
H.sap/1-527	42 LEARDRVGGRTYTIRNEHVDYVDVGGAYVGPTQNRILRLSKELGIETYKVNVSERLVQYVKGKTYPFRGAFPPVWNPIAY	121
M.mus/1-526	42 LEARDRVGGRTYTVRNEHVKWVDVGGAYVGPTQNRILRLSKELGIETYKVNVNERLVQYVKGKTYPFRGAFPPVWNPLAY	121
F.cat/1-1178	721 L DYNNLW <mark>R TMDNMGK E I PADAPWEAPHAK EWDK MTMK E LIDKI CWTKTARQFAYL FVNI NVTSEPHEVSALWFLWYVKQC</mark> 8	00
H.sap/1-527	122 L DYNNLWR TIDNMGK E I PTDAPWEAQHAD KWDK MTMK E LIDKI CWTKTARR FAYL FVNI NVTSEPHEVSALWFLWYVKQC	01
M.mus/1-526	122 L DYNNLWR TMD DMGK E I PVDAPWQARHA E EWDK I TMKDLIDKI CWTKTARE FAYL FVNI NVTSEPHEVSALWFLWYVRQC	01
F.cat/1-1178	801 GGTTR IFSVTNGGQERK FVGGSGQVSER IMDRLGDRVKLKRPVTYVDQSGDSIIIETLNHEVYECRYVISAIPPTLTAKM	880
H.sap/1-527	202 GGTTR IFSVTNGGQERK FVGGSGQVSER IMDLLGDQVKLNHPVTHVDQSSDNIIIETLNHEHYECKYVINAIPPTLTAKI	281
M.mus/1-526	202 GGTSR IFSVTNGGQERK FVGGSGQISEQIMVLLGDKVKLSSPVTYIDQTDDNIIIETLNHEHYECKYVISAIPPVLTAKI	281
F.cat/1-1178	881 HFK PELPSER NOLIOR LPMGSIIKCMMYYKEAFWKK NDYCGCMIIQDEEAPISITLDDTK PDGSLPAIMGFILARKADRL	960
H.sap/1-527	282 HFR PELPAER NOLIOR LPMGAVIKCMMYYKEAFWKKKDYCGCMIIEDEDAPISITLDDTK PDGSLPAIMGFILARKADRL	961
M.mus/1-526	282 HFK PELPPER NOLIOR LPMGAVIKCMVYYKEAFWKKKDYCGCMIIEDEEAPISITLDDTK PDGSMPAIMGFILARKAERL	961
F.cat/1-1178	961 AKLHKEIRKKKICELYAKVLGSQEALHPVHYEEKNWCEEQYSGGCYTAYFPPGIMTQYGRVIRQPVGRIYFAGTETATHW 10	40
H.sap/1-527	362 AKLHKEIRKKKICELYAKVLGSQEALHPVHYEEKNWCEEQYSGGCYTAYFPPGIMTQYGRVIRQPVGRIFFAGTETATKW 4	41
M.mus/1-526	362 AKLHKDIRKRKICELYAKVLGSQEALSPVHYEEKNWCEEQYSGGCYTAYFPPGIMTLYGRVIRQPVGRIYFAGTETATQW 4	41

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

variants

250

ပိ 200

150

100

50

VNTR SNP 1

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
350		
300		

Homozygous

Heterozygous

Reference

DRD5 SNP

F.cat/1-1178 H.sap/1-527 M.mus/1-526	1041 SGYMEGAVEAGERAAREVLNALGKVAKKDIKVQEPESKVSPAASPPLTSNCVKNCAALGHMHASTWRIRSA 442 SGYMEGAVEAGERAAREVLNGLGKVTEKDIWVQEPESK 442 SGYMEGAVEAGERAAREVLNALGKVAKKDIWVQEPESK	RPCPCEREL 1120 479 479 479
F.cat/1-1178	1121 SANDLCSFVQDVPAVEITRTFWERNLPSVSGLLKIIGFTTSVTAVWIVVHKFRLLSRS	1178
H.sap/1-527	480 DVPAVEITHTFWERNLPSVSGLLKIIGFSTSVTALGFVLYKYKLLPRS	527
M.mus/1-526	480 DVPALEITHTFLERNLPSVPGLLKITGFSTSVALLCFVLYKFKQPQS-	526

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

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. **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).

VNTR SNP 2 DRD4 LoF SNP

√ariant



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
2. Hoehe MR, Morris-Rosendahl DJ. Dialogues Clin Neurosci. 2018;20: 169–177. doi:10.31887/dcns.2018.20.3/mhoehe
3. AVMA. Schaumburg, IL; 2022.
4. Travnik I de C, Machado D de S, Gonçalves L da S, Ceballos MC, Sant'anna AC. Animals. 2020;10: 1–23. doi:10.3390/ani10091516
5. Faraone S, Perlis R, Doyle A, Smoller J, Goralnick J, Holmgre M, et al. Bio Psy. 2005;57: 1313-1323. doi:10.1016/j.biopsych.2004.11.024

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