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Analysis of single-nucleotide polymorphisms in the *APOBEC3H* gene of domestic cats (*Felis catus*) and their association with the susceptibility to feline immunodeficiency virus and feline leukemia virus infections

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ABSTRACT

Feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) are widely distributed retroviruses that infect domestic cats (*Felis catus*). Restriction factors are proteins that have the ability to hamper retroviruses' replication and are part of the conserved mechanisms of anti-viral immunity of mammals. The APOBEC3 protein family is the most studied class of restriction factors; they are cytidine deaminases that generate hypermutations in provirus DNA during reverse transcription, thus causing hypermutations in the viral genome, hindering virus replication. One of the feline *APOBEC3* genes, named *APOBEC3H*, encodes two proteins (APOBEC3H and APOBEC3CH). In other mammals, *APOBEC3H* single-nucleotide polymorphisms (SNPs) can alter the stability and cellular localization of the encoded protein, thus influencing its subcellular localization and reducing its anti-viral effect. In cats, the association of *APOBEC3H* SNPs with susceptibility to retroviral infections was not yet demonstrated. Therefore, this study aimed the investigation on the variability of *APOBEC3H* and the possible association with FIV/FeLV infections. DNA obtained from whole blood of fifty FIV- and/or FeLV-infected cats and fifty-nine FIV- and/or FeLV-uninfected cats were used as templates to amplify two different regions of the *APOBEC3H*, with subsequent sequencing and analysis. The first region was highly conserved among all samples, while in the second, six single-nucleotide variation points were identified. One of the SNPs, A65S (A65I), was significantly correlated with the susceptibility to FIV and/or FeLV infections. On the other hand, the haplotype analysis showed that the combination "GGGGCC" was positively correlated with the lack of FIV and/or FeLV infections. Our results indicate that, as previously shown in other mammals, variability of restriction factors may contribute to susceptibility of domestic cats to retroviral infections; however, these results should be confirmed by more extensive analysis and *in vitro* experiments.

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1. Introduction

The feline immunodeficiency virus (FIV) is a lentivirus that belongs to the *Retroviridae* family. This virus is widely distributed among domestic cats and induces an important immunosuppressive effect upon infection (Lutz, 1990, pp. 131–146). The last stage of FIV infection is characterized by an acquired immunodeficiency syndrome, named feline acquired immunodeficiency syndrome—FAIDS (Goto et al., 2000), which is similar to that seen in humans infected with the human immunodeficiency virus (HIV). The outcome of FIV infections is variable, and death occurs in approximately 18% of infected animals within the first 2 years of infection (Barr, 2000, pp. 433–438).

The feline leukemia virus (FeLV) belongs to the genus *Gammaretrovirus*, within the family *Retroviridae*. Like FIV, FeLV is also a worldwide, cosmopolitan distributed virus, which is associated

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with immunosuppression, leukemia, lymphosarcoma, and myeloproliferative syndromes in domestic cats (Costa et al., 2000; Cotter, 1991; Pontier et al., 1998). For a long time, FeLV was considered to account for most tumoral disease-related deaths, and it was responsible for more clinical syndromes than any other single agent in cats (Hartmann, 2012).

In addition to innate and adaptive immune responses to viruses, humans and other mammals produce specific anti-retroviral proteins that interact with virions during their replication, blocking the completion of the virus replication cycle (Berthoux et al., 2005; Kirchhoff, 2010; Virgen and Hatziioannou, 2007). They are highly conserved proteins, named restriction factors, which are constitutively expressed in some cellular types and/or interferon-induced cells (Berthoux et al., 2005; Kirchhoff, 2010; Virgen and Hatziioannou, 2007).

The apolipoprotein B mRNA editing enzyme catalytic polypeptide 3 (*APOBEC3* or *A3*) genes encode the most studied class of restriction factors, which belongs to the superfamily of cytidine deaminases (Stremlau et al., 2004; Wedekind et al., 2003). These genes are found in different numbers in mammals; while primates contains seven of these genes (*A3A* to *A3H*), the cat genome presents one *A3H* gene and three *A3C* genes. Of these four, only the A3H product displays anti-retroviral activity (Münk et al., 2008). In addition to these, a fifth transcript, designated *A3CH*, is expressed by read-through alternative splicing, and its product was also shown to restrict feline retroviruses (Münk et al., 2008). The latter is the most evolutionary divergent member of the *A3* family (Conticello et al., 2005). The *A3H* gene is organized in five exons. The first is a noncoding exon. The second and third exons fall in the region that code the catalytic motifs, characterized by the following sequence: His-X-Glu-X_{23-28}-Pro-Cys-X_{2-4}-Cys (Jarmuz et al., 2002; Münk et al., 2008).

A3 proteins act in retroviruses by deaminating cytidine into uridine in the minus-sense single-strand viral DNA, which results in the extensive G-to-A hypermutations in the plus-strand viral DNA (Bishop et al., 2004; Dang et al., 2006; Luo et al., 2004; Schäfer et al., 2004). In addition to the resulting high levels of mutations, deamination-independent mechanisms, e.g., interference on the reverse transcription process (Sakai et al., 2006), also add to the anti-viral effect of A3 proteins (Wedekind et al., 2003). On its turn, FIV evolved the viral infectivity factor (Vif) protein, whose function is to counteract the A3 proteins effect (Münk et al., 2008). Vif interacts with these restriction factors and triggers their ubiquitination and degradation via the proteasomal pathway (Bishop et al., 2004; Dang et al., 2006; Luo et al., 2004; Münk et al., 2008). As a consequence, the replication of Vif-deficient FIV (ΔvifFIV) is highly restricted (Münk et al., 2008). Although the feline A3H displays activity against ΔvifFIV, its effect on FeLV replication is barely marginal (REFS). A3CH was also shown to display a pronounced antiretroviral activity against ΔvifFIV and FeLV, also by generating hypermutations (Münk et al., 2008).

In humans, *A3* haplotypes are distributed differently among individuals of Asian, European, and African origin. The different genetic backgrounds in allele frequencies and haplotype architecture observed among different populations may influence the outcome of HIV infection and progression of disease (An et al., 2004; Bizinoto et al., 2011; Harari et al., 2009). Different reports have analyzed the effect of *A3* single-nucleotide polymorphisms (SNPs) on the susceptibility of human subjects to HIV infection and progression. Indeed, some SNPs, which may alter the level of A3 activity or expression, are associated with a higher or lower risk of HIV infection (An et al., 2004; Bizinoto et al., 2011; Harari et al., 2009). In felines, there are no such reports, even though the importance of A3 proteins in feline retroviruses restriction is unequivocal.

2. Theory

Once the A3H protein shows a restrictive role against feline retroviruses, our objective is to search for polymorphisms in two genomic regions of the *A3H* of FIV- and/or FeLV-infected as well as uninfected domestic cats. In addition, we aim to identify a possible association between variations in this gene with susceptibility to infection.

3. Materials and methods

3.1. DNA samples

DNA samples were extracted from peripheral blood of domestic cats collected at the Hospital de Clínicas Veterinárias/Universidade Federal do Rio Grande do Sul (UFGRS). The blood samples were derived from male and female cats, with ages ranging from 1 month to 13 years old, who were displaying different clinical syndromes suspected of FIV and/or FeLV infections. These animals are outdoor, non-pedigree cats, characterized as a genetically homogeneous population. Blood samples were stored individually in tubes and kept refrigerated during transport to the Virology Laboratory of UFRGS, where DNA was extracted using buffer-saturated phenol and submitted to two polymerase chain reactions (PCRs) to detect provirus DNA based on the amplification of the *gag* region of the virus genome (unpublished data). Briefly, to detect FIV genomes, the amplification reaction targeted a conserved region of the gene *gag* using the primers (IDT) FIVF 5′-AAAATGGTGTC-CATTTTTATGG-3′ and FIVR 5′-GCTTCTGCTTGTTGTTCTTG-3′. To detect FeLV genomes, the amplification reactions targeted a conserved region of the *gag* gene using the primers FeLVF 5′-AACTAAC-CAATCCCCACGC-3′ and FeLVR 5′-AATGGCTGTCCCACTAGAG-3′. The primers used in the PCR assays were originally designed to avoid annealing to endogenous retroviruses' sequences.

The sensitivity of the PCRs was determined by titration of internal control (IC) templates (DNA fragments that are amplified with the same primers and under the same PCR conditions). Both FIV and FeLV PCRs were able to detect between 25 and 250 IC molecules, implying that the lower detection limit of these PCRs was about 25 IC molecules (unpublished data).

In total, 50 positive samples (comprising 18 FIV-positive, 15 FeLV-positive, and 17 positive for both viruses) and 59 samples from uninfected animals were obtained and used in the present study.

3.2. Determination of polymorphisms in the APOBEC3H gene

All of the DNA samples were used as templates to amplify two different regions of the *A3H* gene. The first region comprises the first 300 bp of the translated portion of the gene. The second targeted region corresponds to a 590 bp product between nucleotides 707–1296, comprehending exon 3 and part of introns 2 and 3 (Fig. 1). According to previously published data, both regions analyzed here correspond to portions of the A3H transcript that

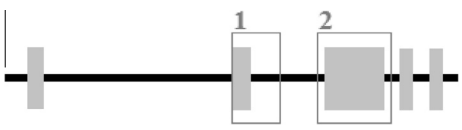

Fig. 1. Schematic structure of the feline *A3H* gene. The exons are represented by the gray boxes, and the introns are represented by the black lines. The first targeted region corresponds to the initial 300 bp of the translated portion of the gene, and the second one corresponds to a 590 bp product, corresponding to exon 3 and part of introns 2 and 3.

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