# Feline Leukemia Virus Inhibits Thiamine Uptake, With Pathological Consequences

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Feline leukemia virus (FeLV) is a horizontally transmitted retrovirus that can cause disorders of hematopoiesis, a term that describes development of the different cellular components of blood. Cats infected with FeLV may exhibit immune suppression, anemia, lymphomas and leukemias (Willett and Hosie, 2013). Four subgroups of FeLV (A, B, C and T) have been identified. FeLV-A, the most abundant subgroup, is transmitted between cats during natural infections. Subgroups B, C and T arise *de novo* from FeLV-A within infected cats through recurrent mutation and/or by recombination with 'endogenous' retroviruses that have been integrated into the genome of domesticated cats. Chronically infected cats often possess a mixture of FeLV-A and one or more of the other subgroups.

Working in the laboratory of Dr. Julie Overbaugh (Human Biology Division), Dr. Ramon Mendoza and Maria Anderson previously showed that FeLV-A enters target cells by binding to a feline ortholog of the human thiamine transporter protein 1, or THTR1 (Mendoza *et al.*, 2006). Two genes, or the proteins they encode, are said to be orthologous if they diverged in molecular structure after the split between two evolutionary lineages, such as the lineages leading to humans and cats. Thiamine, known also as vitamin $B_1$, is an essential nutrient for all mammals, yet it was unclear if the interaction between FeLV-A and the newly-characterized feline thiamine transporter contributes to the pathologies resulting from FeLV-A infection. To test the effects of FeLV-A on thiamine uptake and growth of feline cells, Drs. Mendoza and Overbaugh teamed up with Dr. Dusty Miller, who leads his own lab group in the Human Biology Division.

There are two known thiamine transporters in mammals, THTR1 and THTR2. Studies in mice and humans show that these transporters have distinct, non-overlapping roles in thiamine uptake and homeostasis. Thus, to fully understand potential interactions between FeLV infection and thiamine uptake, Mendoza cloned and sequenced the cat ortholog corresponding to the second transporter (THTR2) as part of his Ph.D. thesis research. He then tested whether feline THTR2 (feTHTR2) could function as a receptor for FeLV-A. He expressed feTHTR2 in cultured mouse fibroblasts and showed that these cells did not allow the entry of FeLV-A. In the same experiment, mouse fibroblasts expressing feline THTR1 (feTHTR1) were highly permissive of FeLV-A infection. Mendoza then

measured the uptake of thiamine by mouse fibroblasts expressing feTHTR1, feTHTR2, human THTR1 (for reference) or feline inorganic phosphate transporter (as a negative control). Fibroblasts expressing feTHTR1, feTHTR2 or human THTR1 showed similar thiamine uptake activities, whereas thiamine uptake by cells expressing the phosphate transporter were similar to that of unmodified cells. Thus, feTHTR1 and feTHTR2 both function in thiamine uptake at levels similar to that of human THTR1, but only feTHTR1 functions as a cell-entry receptor for FeLV-A in cats. Moreover, the researchers demonstrated that thiamine uptake was severely disrupted in FeLV-A-infected mouse fibroblasts expressing feTHTR1.

To understand which feline cells might be most susceptible to the inhibitory and possibly toxic effects of FeLV-A on thiamine uptake, the authors examined the expression patterns of feTHTR1 and feTHTR2 in several different cat tissues. As with the corresponding human and mouse orthologs, feTHTR1 is ubiquitously expressed, while feTHTR2 is narrowly expressed. Importantly, feTHTR2 was not found at all in T-cells, skeletal muscle, heart, small intestine or large intestine, suggesting that active thiamine uptake by these cells and tissues is mediated solely by feTHTR1. Finally, Mendoza examined the effects of FeLV infection on growth of a feline embryonic fibroblast cell line (AH927) that naturally expresses feTHTR1 but not feTHTR2. At physiologically-relevant extracellular concentrations of thiamine, growth of the AH927 cells was inhibited by FeLV-A infection, but not by FeLV-B infection. The toxic effect of FeLV-A on cell growth was alleviated at extracellular concentrations of thiamine that were high enough to drive passive diffusion of this nutrient into the cells. In summary, the researchers' findings suggest that inhibition of thiamine uptake by FeLV-A in some tissues may contribute to FeLV pathogenicity in cats.

Many cat species in the wild are among the most endangered animals on Earth, and some members of the cat family (Felidae) are now being threatened by FeLV in addition to habitat loss and/or poaching. On its latest Red List of Threatened Species, the International Union for Conservation of Nature (IUCN) lists 16 of the 41 known cat species as vulnerable, endangered or critically endangered (IUCN, 2012). In all 16 cases, total population sizes are on the decline. FeLV originating from domestic cats have caused outbreaks resulting in multiple deaths of Iberian lynx, which is at extremely high risk of extinction in the wild, and Florida panthers (see figure). Given these multiple cross-species transmission events, other cat species are likely susceptible to FeLV-A infection as well. The findings of Mendoza *et al.* support a prediction that could have important consequences for veterinary medicine and cat conservation: Raising the plasma levels of thiamine in FeLV-infected cats may reduce some of the pathological effects of this virus, perhaps benefitting the survival of some threatened cat species.

Mendoza R, Miller AD, Overbaugh J. 2013. Disruption of thiamine uptake and growth of cells by feline leukemia virus subgroup A.*J. Virol.* Epub ahead of print.

Also see: IUCN. 2012. The IUCN Red List of Threatened Species. Version 2012.2. Available at http://www.iucnredlist.org. Downloaded on 2 January 2013.

Mendoza R, Anderson MM, Overbaugh J. 2006. A putative thiamine transport protein is a receptor for feline leukemia virus subgroup A. *J. Virol.* 80:3378-85.

Willett BJ, Hosie MJ. 2013. Feline leukaemia virus: Half a century since its discovery. *Vet. J.* 195:16-23.

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Different species of cat susceptible to feline leukemia virus (FeLV). Above: The Florida panther (Puma concolor coryi) on the left and the Iberian lynx (Lynx pardinus) on the right, an endangered sub-species of cougar and a critically endangered species of lynx, respectively. Below: The domesticated cat (Felis catus), the primary global reservoir of feline leukemia virus. FeLV infection has also been reported in the jaguarundi (Puma yagouaroundi) and could very well afflict populations of one or more of the six other species of Felis, which are even more closely related to domesticated cats than are pumas and lynx. These other Felis species have not yet been surveyed for FeLV infection in the wild.