Genetic diversity of feline leukemia virus

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Abstract. Feline Leukemia Virus (FeLV) belongs to retrovirus family, causing various proliferative and immunosuppressive diseases in felines. There are two forms of FeLV: endogenous (enFeLV) and exogenous (exFeLV), the latter has 4 subgroups: A, B, C and T with different receptor specificity. The FeLV-A is the most abundant transmissive form. The FeLV-B emerged as a recombinant between provirus FeLV-A and endogenous virus of domestic cats. The FeLV-C appeared as a result of accumulation of mutations in the env FeLV-A gene. The chimeric FeLV-T virus was obtained as recombination event between 61E and 61C viruses. This review also covers two new recently described subgroups - FeLV-D и TG35.

1. Introduction
Feline leukemia virus, (FeLV) is an RNA virus of Gammmaretrovirus genus, Retroviridae family. The spherical virion is 8-100 nm in diameter with 8 nm surface spikes. The FeLV is first described feline retrovirus associated with lymphoma, macrocitary anemia, aplastic anemia, that could be fatal. In Moscow area, 9% of cats are infected [1,2,3]. This review describes all known subgroups and new variants of FeLV. The work was funded by State Project 0578-2019-0003-C-01.

2. Genomic structure of FeLV
The FeLV genome is a linear single stranded 8.3 kb RNA molecule. It has major structural and nonstructural genes gag, pol, env, flanked by long terminal repeats (LTR) (figure 1) [1, 4, 5]. Transcription is regulated by 5'-LTR. The FeLV genome contains two ORFs: one for gag and pol, another for env genes.

Figure 1. Genomic organization of FeLV.
The gag gene is coding for structural proteins p15, (matrix protein, MA), p12, p27 (capsid protein, CA) and p10 (nucleocapsid protein, NC). The pol gene is coding for catalytic proteins: p14 (protease, PR), p80 (reverse transcriptase, RT), and p46 (integrase, IN). The env gene is coding for outer capsid proteins, such as gp70 (SU) and transmembrane (TM) proteins p15E [5,6]. Apart from the described proteins, the glycosylated polypeptide pr80\textsuperscript{Ag} is produced with a unique N-terminal 15 kD fragment and all components from Gag (pr65\textsuperscript{Ag}) polypeptide. The synthesis and post-translation modifications of pr80\textsuperscript{Ag} are independent from synthesis of pr65\textsuperscript{Ag}. In contrast to pr65\textsuperscript{Ag}, the pr80\textsuperscript{Ag} is glycosylated, cleaved by cellular protease and released from FL infected cells. The 40 kD cleavage product (gp40\textsuperscript{Ag}) contains epitopes CA, p12, NC, but not MA. The gp40\textsuperscript{Ag} protein is not found in the virion, it may represent a membrane protein type II with signal/anchor domain. The function of this glycosylated protein is not clear. However, research on Gag of MuLV (Murine Leukemia Virus) suggest the role of this protein in cell penetration [4,5,6].

3. Endogenous form of FeLV
Endogenous FeLV (enFeLV) is a replication defective provirus found in Felis, genus species including domestic cat (Felis catus). Expression of provirus DNA is limited to subgenomic transcripts preventing the assembly of infectious virus. However, DNA fragments of the enFeLV can recombine with exogenous FeLV resulting in recombinant viruses causing leukemia. It is suggested that endogenous retroviruses could play a role in exogenous retroviral infection either by suppression of the exogenous virus reproduction, or by enhancing the exogenous viral infection [7,8]. The endogenous and exogenous FeLV are approximately 86% similar by nucleotide sequences. The differences between the enFeLV and exFeLV are found in the gag and env genes as single nucleotide changes as well as insertions and deletions (INDELs). There are also silent mutations accumulated in unique 3′ end regions of the LTR.

4. Exogenous form of FeLV
Exogenous FeLV (exFeLV) exits as a replication-competent horizontally transmitted form [4,6]. The exFeLV isolates that are capable of replication, are divided into 4 major subgroups: A, B, C, T. They differ in gene env nucleotide sequences and by receptor specificity (Table 1).

| FeLV subgroups | Cellular receptor | Receptor function |
|----------------|------------------|-------------------|
| FeLV-A         | THTR1            | Thiamin transportation protein |
| FeLV-B         | Pit1 или Pit2    | Na-dependent transporter of inorganic phosphates. |
| FeLV-C         | FLVCR            | Heme carrying protein |
| FeLV-T         | Pit1             | Na-dependent transporter of inorganic phosphates. |

4.1. Subgroup A
The FeLV-A is major horizontally transmitted form [4,9]. It is thought to be less pathogenic than other forms, nevertheless, this subgroup is associated with macrocitranemia, immunosuppression and lymphoma [6]. All cats that are infected with FeLV-B и FeLV-C, are co-infected with FeLV-A. Thus it is hypothesized that FeLV-A serves as required parental virus for development of more pathogenic groups, and as a helper virus for other subgroups. FeLV-A transmits within Felis genus through saliva via licking, joint feeding, through bites. It also transmits transplacentally and by milk feeding. The FeLV-A is specific to thiamin transportation protein (ThTR-1) as a receptor for cell entry, thus limiting itself within the Felis genus. However, research data on the pseudo-type of FeLV-A (chimeric virus construct with MSV envelope proteins substituted by env FeLV) suggest its specificity for broader range of hosts. The pseudo-type of FeLV-A can infect cell cultures derived from rabbit, pig, mink, dog and humans, although less efficient as feline cells [9]. Interesting to note, that primary infection with FeLV-B or FeLV-C blocks the subsequent infection with the pseudo-type of FeLV.
4.2. Subgroup B
The FeLV-B is a result of recombination between the provirus FeLV-A DNA and endogenous FeLV DNA that is located in host DNA. About half of cats infected with FeLV-A are normally infected with FeLV-B causing higher morbidity and mortality due to development of leukemia and lymphoma. The FeLV-B is tumorogenic and is thought to be incapable to horizontal transmission except rare events when it transmits with FeLV-A. For cell entry, the FeLV-B is specific to Na-dependent transporter of inorganic phosphates (Pit1 and closely related Pit1) as receptors for cell entry. These receptors are also used by Gibbon ape leukemia virus and some murine leukemia viruses. The FeLV-B can use Pit1 and homologous receptors of feline and human origin, but only feline Pit2 receptors. Comparative analysis of FeLV-A and FeLV-B genomes revealed 10 variable regions, the level of diversity depending on the source of the virus. The variable regions 1-5 (vr1-5) and C-terminal domain are believed to be responsible for cell tropism changes due to changes in the receptor binding protein gp70. Amino acid changes located in these two variable regions (VRA and VRB), affect the binding capability of the FeLV-B to the receptors Pit1 or Pit2. Apart from changes in the env gene, there are recombinants of the FeLV-B, that include nucleotide sequences from enFeLV in LTR and gag genes [6,9].

4.3. Subgroup C
The FeLV-C virus has appeared as a result of multiple mutations in the gene env (SU) of the FeLV-A virus. Sequencing of naturally circulating isolates of FeLV-C revealed accumulation of amino acid changes in the N-terminal part of the surface protein. Heme carrying protein (FLVCR) serves as a receptor for FeLV-C. It is homologous to human FLVCR. Genomic analysis of the FeLV-C established the link between changes in the 3′pol and 5′env genes (3′-end of pol (73 amino acids) to 5′-end of env (241 amino acids)) with aplastic anemia and broader range of sensitive cell cultures including cross species. There is also a link between virulence and three codon deletions within first variable region vr1 of the 5′env gene and nine adjacent substitutions in the same area [4].

4.4. Subgroup T
In addition to the mentioned subgroups of FeLV, there is a separate one, that is capable of inducing a fatal immunosuppressive disorder FAIDS (Feline acquired immune deficiency syndrome). This type of FeLV was originally isolated from complex mixture of FeLV variants from domestic cats that were originally infected with the cloned transmissive strain of FeLV-A [4,6,9]. Two isolates were cloned from this mixture: replication-defective clone 61C and replication-competent but non-virulent clone 61E. Co-infection with 61C and 61E viruses resulted in FAIDS in cats. The chimeric replication-competent virus (EECC) inducing FAIDS in cats, was obtained as a result of recombination between 5′-LTR-gag-pol region of 61E and env-3′LTR region of 61C clone. The RRCC virus demonstrates t-cell tropism, thus it has been designated as FeLV-T. The nucleotide sequence of surface protein genes of the FeLV-T (EECC) is closely related to one from FeLV-A. This gene contains N-terminal 6 amino acid deletion, C-terminal 6 amino acid insertion and different changes in 11 amino acid positions comparing with the corresponding gene from parental non-virulent clone FeLV-A 61E. These changes in Env protein result in lack of resistance to superinfection, accumulation of large amount of non-integrated provirus DNA in the infected cells leading to their death. Besides, there is one more subtype of FeLV-T discovered carrying 4 amino acid insertion (81T). The FeLV-T uses Pit1 for a receptor, as well as does FeLV-B, however, the FeLV-T alone is not capable to induce membrane fusion using this receptor, because of substitution of histidine asparagate at N terminus. The infection is only possible with help from FeLIX protein, a truncated surface protein. The FeLIX protein is produced by the enFeLV, and is more than 90% identical to the env protein from FeLV-B [4,8].

4.5. New subgroups
Two recently described additions to the FeLV family include less abundant types FeLV-D and TG35. The FeLV-D virus was first identified simultaneously with the discovery of a novel endogenous retrovirus from domestic cat (ERV-DC) that was different from previously known enFeLV. Insertion of the gene env ERV-DC into FeLV would result in appearance of the FeLV-D. It is thought that
reproduction of the FeLV-D is regulated by an anti-retrovirus factor called Refrex-1[4,10]. This factor is a soluble truncated Env protein, coded for by provirus loci ERV-DC7 and ERV-DC16. Such truncated protein does not attach to cell membrane, thus it can be effectively secreted from cells in different tissues. Today it is not completely clear how the Refrex-1 down-regulate viral infection. According to one suggestion the Refrex-1 competes with the FeLV-D for cell receptors, thus preventing the virus from penetration. Nevertheless, there is no doubt that the Refrex-1 plays an important role in restricting viral reproduction and protection of cats from retroviral infection [10].

The FeLV-TG35 virus was first isolated from a one year-old castrated cat. Even though the genomic sequences of the FeLV-TG35 were similar to the ones from FeLV-A, analyses of virus interference suggested that there were changes in the Env protein. The changes were found in the vr1 region resulting in altered Env TG35-2 protein that has seven amino acid changes. This changes viral receptor specificity and potentially leads to forming of a new subgroup [10].

5. Conclusion
In this review we described the known and recently discovered genetic subgroups of Feline leukemia virus. Some variants appeared as a result of rare point mutations. At the same time, there are subtypes that appeared after recombination events affecting almost 30% of viral genomes. The existing genetic diversity of FeLV provides for potential for generation of new FeLV variants during infection both in vivo and in vitro. There is a need for more research of interaction between endogenous and exogenous retrovirus forms. The FeLV is a useful model for understanding the mechanisms of evolution of the retroviruses.

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