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A novel deletion in the LTR region of a Greek small ruminant lentivirus may be associated with low pathogenicity

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Abstract

Greek small ruminant lentivirus (SRLV) strains remain relatively uncharacterized at the molecular level, despite the fact that lentiviral diseases of small ruminants are known to be widespread in the country. In the present study, we investigated the sequence diversity of the LTR region in Greek SRLV strains from sheep with and without disease symptoms, since sequence differences within this genomic area have been shown to lead to SRLVs with distinct replication rates. The AP-4 and AML (vis) motifs and the TATA-box were highly conserved among Greek strains, whereas the two AP-1 sites exhibited some substitutions. Pairwise comparisons with reference strains revealed that Greek LTR sequences were closer to the ovine strains (25.7% average divergence) rather than the caprine strain CAEV (59.1% average divergence). The most striking difference observed between the two groups of animals was a 13–14 nucleotide deletion in the strains obtained from the asymptomatic sheep. The deletion was located within the R region of LTR, which was also found to be much less homologous (39.6% average divergence) than the U3 and U5. Taken together, our data suggest that the R region of LTR may be involved in virus transcriptional activation. Furthermore, a specific deletion within this region may, at least in part, be associated with low pathogenicity of some SRLV strains.

Keywords: SRLV; MVV; CAEV; LTR; R region

1. Introduction

The small ruminant lentiviruses (SRLV) – which consist of maedi-visna virus of sheep (MVV) and caprine arthritis encephalitis virus of goats (CAEV) – constitute a divergent group of the lentivirus genus (Shah et al., 2004). They induce a lifelong infection, which can cause inflammatory and degenerative disease in various target organs including the mammary glands, lungs, synovia and brain (Pépin et al., 1998), often after a long latent period. The resulting lesions involve chronic inflammatory changes characterized by lymphoid hyperplasia and interstitial infiltration of mononuclear cells (Georgsson and Palsson, 1971; Pépin et al., 1998). Sheep and goats can become infected at an early age, but clinical signs are rarely evident before the second or third year of age (Berriatua et al., 2003). Following a long asymptomatic period, SRLV infections result in a multisystemic disease, the major manifestations of which are interstitial pneumonia, mastitis, encephalitis and arthritis (Houwers et al., 1988; Narayan and Clements, 1989; Van der Molen and Houwers, 1987).

SRLVs have a genetic organization that is typical of lentiviruses. Their genome is comprised of the *gag*, *pol* and *env* genes, and the *tat*, *rev* and *vif* open reading frames (Clements and Zink, 1996). Long terminal repeats (LTRs) – divided into the U3, R and U5 regions – flank the proviral DNA and provide the signals required for transcription, integration and polyadenylation of viral RNA (Pépin et al., 1998). They have also been shown to be responsible for the cellular tropism of the virus (Agnarsdottir et al., 2000). Lentiviral genomes are among the most rapidly evolving known. An elevated substitution rate is attributed to the low fidelity of the lentiviral reverse transcriptase, which has no proofreading exonuclease activity (Leroux et al., 1997).

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Table 1
Origins and characteristics of the Greek MVV strains

Sheep number	Flock	Symptoms	Virus strain	GenBank accession number
6	I	None	MVV-Gr-LTR6	DQ084441
7	I	None	MVV-Gr-LTR7	DQ084442
12	I	None	MVV-Gr-LTR12	DQ084443
19	I	None	MVV-Gr-LTR19	DQ084444
22	I	None	MVV-Gr-LTR22	DQ084445
58	II	Weight loss/dyspnoea	MVV-Gr-LTR58	DQ084446
63	III	Weight loss/dyspnoea	MVV-Gr-LTR63	DQ084447
80	VI	Weight loss/dyspnoea	MVV-Gr-LTR80	DQ084448

SRLV isolates may be classified as rapid/high or slow/low according to their replication rate in vitro. The rapid/high strains replicate rapidly, inducing cell lysis and reaching high titers, whereas the slow/low grow slowly and to low titers (Barros et al., 2004; Lairmore et al., 1987; Querat et al., 1984; Woodward et al., 1995). Transcription of the proviral genome plays a crucial role in the virus life cycle, as it provides the template for synthesis of structural and regulatory proteins, and results in the generation of new copies of viral RNA. Transcription is regulated by the binding of various cellular proteins to DNA sequences, which reside within the LTR of the proviral genome. Several studies have demonstrated that AP-1, AP-4 and AML (vis) binding sites, which are located in the U3 promoter region of LTR, have a central role in the regulation of viral transcription (Barros et al., 2004, 2005; Campbell and Avery, 1996; Gabuzda et al., 1989; Gdovin and Clements, 1992; Hess et al., 1986; Sutton et al., 1997). Repeated sequences, which span the AP-1, AP-4 and AML (vis) motif-containing region, have been identified within the U3 of several virus isolates (Agnarsdottir et al., 2000; Barros et al., 2004, 2005; Campbell and Avery, 1996; Hess et al., 1989; Sargan et al., 1995). Due to the multiplication of these transcription factor binding sites, the repeats have been implicated in enhanced LTR-mediated transcription and subsequent increased virus replicative potential and accelerated disease progress. Proviral gene expression is also transactivated by the virus protein Tat, which mediates an increase in viral RNA synthesis (Hess et al., 1989) and RNA stability (Gdovin and Clements, 1992), through sequences located in U3. Therefore, sequence variations in SRLV LTRs may affect interactions with cellular transcription factors and may lead to an altered viral gene expression and replication. Indeed, it has been shown that sequence divergence between SRLV strains leads to LTRs with distinct transcriptional activities (Barros et al., 2004; Sargan et al., 1995).

In Greece, although lentiviral diseases of small ruminants have been described since 1967 (Exarchopoulos, 1967), SRLV strains remain relatively uncharacterized at the molecular level. Viral DNA sequences have only lately been detected by PCR in blood samples of sheep and goats from flocks with history of SRLV infections (Karanikolaou et al., 2005), and a partial sequence analysis of the *gag* gene of Greek strains has been recently published (Angelopoulou et al., 2005). Since sequences that control viral transcription are located in the LTR, and sequence differences (including sequence repeats) within this region have been shown to lead to SRLVs with distinct replication rates; the aim of the present study was to characterize this genomic area in Greek ovine lentivirus strains.

2. Materials and methods

2.1. Animals and blood samples

Fifty sheep (2–12 years of age) from flocks in Northern Greece with history of MVV infections were used in this study. Forty-seven of them, which were from the same flock (flock I; Table 1), had no clinical signs of infection. The other three suffered from weight loss and dyspnoea, and each one of them belonged to a different flock (flocks II, III, IV; Table 1). Whole blood (10 mL) was collected in EDTA tubes by jugular venipuncture. Blood samples were centrifuged at $400 \times g$ for 10 min, and buffy coats were separated and washed twice with a 0.83% NH_4Cl solution. Peripheral blood mononuclear cells (PBMC) were stored at −20 °C until DNA extraction.

2.2. DNA extraction

DNA was extracted from PBMC using the NucleoSpin Blood kit (Macherey-Nagel, Germany). Manufacturer's instructions were followed throughout. DNA concentration and quality were determined spectrophotometrically, and the samples were stored at −20 °C until analysis.

2.3. PCR

Primers that anneal at positions 8990–9013 (LTR2s) and 153–176 (LTR2a) of the MVV strain K1514 were used to amplify a 291 bp region of the long terminal repeat of the proviral genome according to Extramiana et al. (2002). Primer LTR2s resides within a 37 bp sequence that is known to be repeated in the U3 region (Barros et al., 2004). Changes to the published temperature cycling protocol were proved to be necessary in order to achieve optimum amplification. The protocol on a Techgene Thermal Cycler (Techne Limited, Cambridge, UK) consisted of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s and extension at 72 °C for 40 s. The cycling was repeated 35 times. PCR amplified DNA fragments were analyzed by electrophoresis on 1.5% agarose gels stained with ethidium bromide and visualized on a UV transluminator.