Curr Neurol Neurosci Rep (2014) 14:488
DOI 10.1007/s11910-014-0488-y

INFECTION (ML SOLBRIG, SECTION EDITOR)

Endogenous Retrovirus-K and Nervous System Diseases

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Published online: 20 August 2014

Abstract A new appreciation of the microbiome is changing the way we perceive human health and disease. The holobiontic nature of humans is even etched into our DNA in the form of viral symbionts. Empirical evidence for the presence of endogenous retroviruses (ERVs) in the human genome and their activity in homeostatic and pathologic states has accumulated; however, no causal relationship with human disease has been established to date. In this review, we will focus on the role of endogenous retrovirus-K in neurologic disease. Specifically, we will attempt to reconcile the pathologic contribution of ERVK in disparate neurologic diseases by providing evidence as to inter-individual differences in ERVK genotypes, addressing the molecular regulation of ERVK, and provide detailed examples of ERVK-mediated processes in nervous system diseases.

Keywords Endogenous retrovirus · Human endogenous retrovirus-K · Polymorphism · Transcription factor · Inflammation · Amyotrophic lateral sclerosis · Schizophrenia · Bipolar disorder · Multiple sclerosis · Human immunodeficiency virus · Prion disease · Antivirals

Introduction

The DNA provirus hypothesis—where viral DNA integrates into a host genome—was proposed by Nobel laureate Howard M. Temin in the 1960s [1]. Indeed, over 8 % of human DNA is the result of retrovirus integrations scattered throughout the genome. Among the 31 lineages of endogenous retroviruses (ERVs) within the human genome, (which are spread among several Retroviridae subfamilies), the betaretrovirus ERVK (alias human endogenous retrovirus-K, HERV-K) is the most recently endogenated ERV. The ERVK (HML-2) clade is estimated to have been active as recently as 250,000 years ago [2••], and is considered the most transcriptionally active ERV. Several insertions in the human genome are relatively intact, permitting the expression of viral RNA and proteins. Full-length ERVK elements retain a classical retroviral genome structure, with core genes *gag* (group-specific antigen), *pr* (protease), *pol* (polymerase), and *env* (envelope) flanked by long terminal repeats (LTRs) [3]. Regulatory proteins within ERVK have also been described [4, 5]. There is even evidence of ERVK virion production in HIV infection [6] and lymphoma [7]. Unlike canonical retroviruses, Dube et al have recently proposed that ERVK virions can contain either infectious viral RNA or viral DNA genomes [8], thus changing how ERVK expression and replication should be viewed in the context of health and disease pathology.

This article is part of the Topical Collection on *Infection*

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Genotypic Interindividual Differences in ERVK

There are approximately 1000 ERVK (HML-2) integrations in humans, based on the human reference genome. Of these, all are considered replication-defective, with only 24 fixed loci retaining the capacity to encode viral proteins from at least 1 of their genes [3, 9]. However, evidence suggests that this is a fraction of the entire ERVK presence within individual human genomes [2••, 10, 11•].

Polymorphic ERVK insertions (unfixed proviruses) have been identified in several cohort studies [2••, 3, 9, 12, 13], with considerable variation between ethnic groups, as well as distinct inter-individual profiles. These studies indicate that people carry a distinctive ERVK signature based on individual genotypes. For a given locus, ERV polymorphism can occur as

integration of a full-length ERV (with varying degrees of coding capacity), a solitary LTR, or an unoccupied pre-integration site [3, 9, 14]. Recently, Belshaw's group has performed Next Generation Sequencing on individual human genomes revealing that several unfixed ERVK (HML-2) loci are absent from the human reference genome annotation [2••]. Moreover, the frequency of unfixed ERVK (HML-2) loci varied dramatically in the populations tested [2••], further supporting the idea that specific ERVK signatures may be associated with inter-individual differences in ERVK expression, pathology and disease states.

Sequence variation, resulting in ERV alleles, may also alter the function of viral proteins. For example, the ERVK-18 envelope protein is a superantigen that is encoded by 3 distinct alleles, which can alter the amino acid sequence of the protein [15], with predicted but uncharacterized biological effects. Among these 3 ERVK-18 *env* alleles, the K18.3 form is the minor allele with a frequency of 10.8 % within the Caucasian population [15]. The ERVK-18 *env* polymorphism has been shown to be a risk factor for Multiple Sclerosis (MS); homozygous carriers of the K18.3 allele had a significantly increased risk of this disease, suggesting that ERVK-18 may influence the genetic susceptibility to MS [16, 17]. ERVK-18 has also been associated with enhanced risk of Type 2 diabetes (T2D) in individuals with schizophrenia (SCZ) [18], with a risk haplotype comprised of 2 single nucleotide polymorphisms (SNPs) in the *env* region (rs558648 and rs1090799). These results remain controversial, as several cohort studies disagree over whether ERVK-18 polymorphisms are risk factors in T2D and SCZ [18, 19].

Phenotypic Variation in the Expression of ERVK

Current research indicates that not all ERVs remain silent passengers within our genomes; re-activation of ERVK is associated with many inflammatory diseases, such as cancers [7], HIV infection [4], rheumatoid arthritis [20], systemic lupus erythematosus [20] as well as neurologic conditions including multiple sclerosis (MS) [21], schizophrenia (SCZ) [22], bipolar disorder (BD) [22], amyotrophic lateral sclerosis (ALS) [23•], and Creutzfeldt-Jakob disease (CJD) [24]. While there is ubiquitous ERV expression in many tissues, regardless of health or disease, it has been shown that individuals largely exhibit distinct ERV expression signatures [22]. A difficulty in understanding these individual profiles and their association with disease states is a lack of appreciation for the biological control of ERVs.

At the molecular level, there is limited experimental evidence to indicate the cellular state or signals that are required to control the expression of ERVK. Accumulating evidence points to the importance of epigenetic mechanisms in the control of transposable elements including ERVs, and has been reviewed elsewhere [25]. The transcription of ERVK is under the control of viral promoters called long terminal repeats (LTRs), which flank either side of the provirus. To date, only transcription factors Sp1 [26], Sp3 [26], YY1 [27], MITF-M [28], and steroid hormone receptors [29, 30] have been experimentally shown to induce ERVK activity in human cells. Our group has recently focused on examining the role of proinflammatory transcription factors in the induction of ERVK expression. Using bioinformatics, we have revealed that the ERVK promoter contains multiple conserved putative binding sites for proinflammatory transcription factors, including nuclear factor kappa B (NF-κB) and interferon response factors (IRFs) [31••]. Specifically, the viral promoter harbors 2 conserved Interferon Stimulated Response Elements (ISREs) (Fig. 1); thus, inflammatory stimuli may modulate ERVK transcription. We have also generated substantial experimental evidence using human neuron and astrocyte in vitro models to support this claim (unpublished results). Thus, ERVK can exploit anti-viral immune responses and perhaps certain disease backgrounds, as select transcription factors can promote ERVK expression.

Additional evidence supports the importance of innate immune signaling in ERVK re-activation, as select antiviral and proinflammatory cytokines can enhance ERVK expression. Cytokines, notably Tumor Necrosis Factor α (TNFα) and Interferon γ (IFNγ), play critical roles in the pathology of many neurodegenerative diseases including ALS [32, 33], SCZ [34], MS [35], and CJD [36]. TNFα and IFNγ are potent activators of NF-κB and IRF1, respectively, and may, thus, enhance ERVK transcription in these neuroinflammatory diseases (Fig. 1). We have recently generated evidence in human neuron and astrocyte in vitro models to support this claim (unpublished results). TNFα has previously been demonstrated to augment ERVK expression in rheumatoid arthritis—another inflammatory disease [37]. TNFα-mediated induction of ERVW env expression, following the binding of NF-κB with the ERVW promoter, has also been documented [35]. In addition, ERVK-18 expression can be enhanced upon IFNα treatment of peripheral blood lymphocytes [15]. Exogenous IFNα drives IRF9 activation and its translocation to the nucleus where it binds to ISREs in target promoters (Fig. 1). These results are consistent with our observation that ISREs in the ERVK LTR serve as key promoter elements. The ERVK *env* may also confer a self-regulating capacity, as an immunosuppressive domain in the transmembrane (TM) protein alters cytokine release through its immunomodulatory effects [38•]. Although recombinant ERVK transmembrane protein and ERVK virions induced substantial IL-10 secretion in peripheral blood mononuclear cells (PBMCs), reproducible inter-individual differences in the IL-10 response were observed [38•]. Moreover, notable enhancement of proinflammatory cytokine expression and impairment of genes involved in innate immunity [38•], further suggests that the ERVK TM

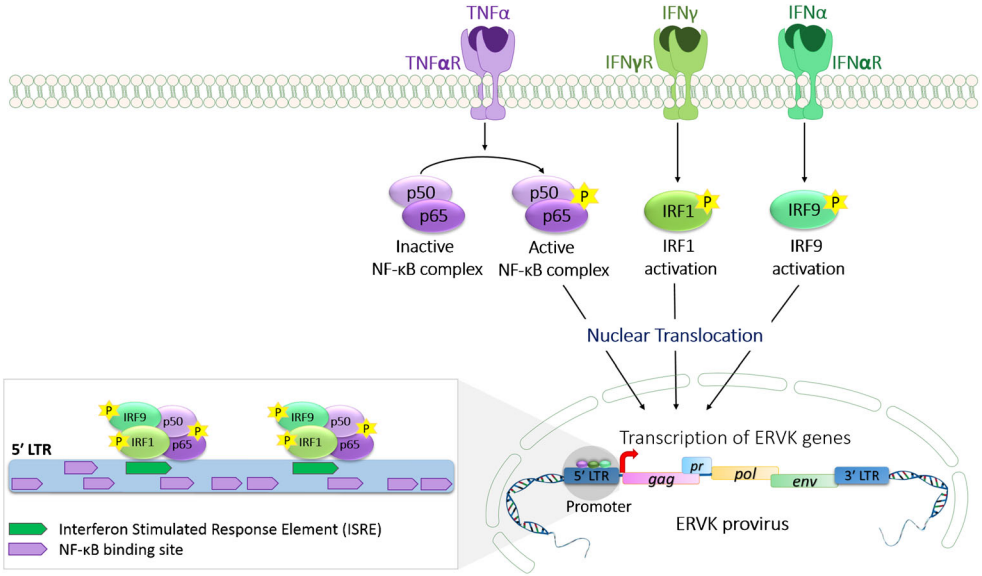

Fig. 1 Proinflammatory signaling cascades and the associated transcription factors that may stimulate ERVK gene expression in multiple neurodegenerative diseases. TNFα, IFNγ and IFNα signaling leads to the phosphorylation (P) and activation of NF-κB (isoforms p50 and p65), IRF1, and IRF9, respectively. These proinflammatory transcription factors then translocate to the nucleus, where they bind their respective sites in the target promoters. The ERVK promoter (5′ LTR) contains multiple conserved putative NF-κB binding sites, as well as 2 interferon stimulated response elements (ISREs) that bind IRFs including IRF1 and IRF9 [31••]. Binding of nuclear NF-κB, IRF1, and/or IRF9 to the ERVK promoter may induce the expression of downstream proviral genes. *Env* envelope, *Gag* group specific antigen, *Pol* polymerase, *Pr* protease

protein will alter the regulation of ERVK, as well as host genes. Additionally, ERVK encoded dUTPase can activate NF-κB and promote proinflammatory cytokine secretion [39]. Additional ERV proteins are suspected to influence protein-protein interactions in humans [40]. Considering that signaling pathways are finely tuned based on the activity of interacting proteins, the genetic background of the host will play a significant role in ERVK expression and immunomodulation. These findings suggest that ongoing signaling cascades in neuroinflammatory disease may trigger ERVK re-activation, thus, promoting the expression of viral RNA and proteins, which may further modulate the pathologic status.

Putative Protective and Pathologic Roles of ERVK in Neurologic Disease

Amyotrophic Lateral Sclerosis (ALS)

Retroviruses, such as human immunodeficiency virus (HIV) and human T-cell leukemia virus (HTLV), have been associated with an increased incidence of ALS-like syndromes [41, 42]. Currently, a single study has demonstrated a direct association between ERVK and ALS [23•], despite evidence for retroviral pathology stemming from the repeated measurement of reverse transcriptase (RT: the retroviral enzyme that transcribes viral RNA into DNA) activity in this disease [43–45]. Elevated levels of ERVK *pol* transcripts (derived from select HML-2 and HML-3 loci) are detectable in postmortem brain tissues of patients with ALS, compared with tissues from Parkinson's disease, systemic disease, and accidental death [23•]. Not only was ERVK RNA expressed in ALS, immunohistological analysis revealed the presence of RT protein in the cortical neurons of patients with ALS [23•]. Clusters of neurons in the prefrontal and motor cortex of patients with ALS exhibited the strongest RT expression, coinciding with the affected brain areas in this disease. An earlier report demonstrated that over half of ALS patients examined showed serum IgG reactivity against ERVK (HML-2) gag protein [46]. Patients with reactive anti-HML-2 gag antibodies exhibited a 10-fold reduction of viral RNA in PBMCs, suggesting an effective and ongoing immune response against ERVK in these patients with ALS [46]. As discussed by Alfahad and Nath [47], these studies open new avenues of investigation into the treatment of ALS.

Schizophrenia (SCZ) and Bipolar Disorder (BD)

Several studies have documented aberrant expression of ERVs in patients with schizophrenia [22, 48–53], and to a lesser extent, in patients with bipolar disorder [22, 48]. ERVW gene expression has been discovered in blood samples [49–51], in cerebrospinal fluid (CSF) [52], and in postmortem brain tissue [52, 53] of patients with SCZ, and has been reviewed extensively elsewhere [54]. Specifically, only ERVK10 (HML-2) RNA was significantly overexpressed in both SCZ and BD compared with healthy postmortem brain tissue [22]. The

ERVK HML-7 clade is also significantly overrepresented in SCZ compared with BD samples (but not in SCZ compared with healthy controls), and under-represented in samples from patients with BD compared with healthy-brain samples [22]. A study by Diem et al further demonstrated that ERVK transcription was not affected by treatment with valproic acid (VPA; a medication used to treat SCZ) or any of the other medications tested, indicating that previous findings of an association between ERVK transcription and SCZ cannot be explained by patient treatment with any of the 4 medications analyzed in this study [55•]. To date, this represents limited and loci-specific alterations in ERVK expression in these neuropsychiatric diseases.

It has been postulated that it may not be mutations in genes associated with SCZ that result in a disease state, but rather mutations in the regulatory regions of these genes [56]. ERV LTRs are known to have promoter, enhancer, and regulatory functions [57]. Approximately 50 % of all human-specific ERVK (hsERVK–HML-2) elements show promoter activity in human tissues [58]. Epigenetic silencing of ERVs by DNA methylation is a known phenomenon and is thought to be a part of the antiretroviral defense system [25]. Therefore, the silencing or downregulation of genes with ERV sequences in their regulatory regions may be the consequence of the host's attempts to stop the expression of these endogenous viruses.

Recently, a full-length almost intact ERVK (HML-2) sequence that displays strong enhancer activity, was identified near the *PRODH* gene [59•]. Mutations in *PRODH*, which encodes a mitochondrial enzyme, have been found to be associated with neuropsychiatric disorders, including SCZ [60]. Given this link between *PRODH* and schizophrenia, Suntsova et al attempted to characterize this ERVK locus (referred to as hsERV$_{PRODH}$) and its potential enhancer activity for *PRODH* [59•]. They showed that the enhancer activity of hsERV$_{PRODH}$ is regulated by methylation, and it acts synergistically with the *PRODH* internal CpG island to activate the *PRODH* promoter. Transcriptional analysis showed that *PRODH* displays the highest expression level in the hippocampus, where hsERV$_{PRODH}$ is hypo-methylated [59•]. The hippocampus is known to be one of the structures of the brain that is most affected in SCZ [61]; if hyper-methylation of hsERV$_{PRODH}$ occurred, aberrant expression of *PRODH* in the hippocampus would likely result.

Similarly, an ERVW LTR is located in the regulatory region of the GABA receptor B1 gene (GABBR1) [56], a gene located in region associated with risk for SCZ [62]. It is speculated that hyper-methylation of this ERVW LTR may downregulate GABBR1 in brains of patients with SCZ [56], thus, accounting for its altered expression pattern [63, 64]. As a result, Hegyi et al propose that the overexpression of ERVs at the onset of disease leads to their subsequent silencing by hypermethylation, which may pathologically contribute to diseases such as SCZ [56]. This hypothesis also offers an explanation as to why ERVW transcripts are readily found in the CSF of patients with recent-onset SCZ, but rarely in chronic patients [49, 52, 65]. It could be that the activation of ERVs occurs early in the etiopathology of schizophrenia or during highly symptomatic periods of disease, resulting in the upregulation of some genes for which ERV elements act as promoters or enhancers. This may be followed by hypermethylation of ERV sequences as a defense mechanism, leading to downregulation of ERV-regulated genes.

Multiple Sclerosis (MS)

Among ERVs associated with MS, ERVW has been the most extensively studied. Many studies have reported significant upregulation of ERVW RNA in brain samples from MS patients [35, 66]. ERVW env protein is highly expressed within astrocytes and microglia in MS plaques, and correlates with the extent of inflammation and active demyelination [35, 66]. Augmented ERVW expression has also been observed in the CSF and blood of MS patients [67, 68]. A recent study has also shown enhanced ERVW DNA copy number in the PBMCs of women with MS; this phenomenon correlated with disease severity scores [68]. In contrast, other studies depict a lack of association between enhanced ERVW expression and MS. Using high-throughput amplicon sequencing, Schmitt et al reported a lack of significant difference in ERVW transcripts between MS and control brain tissue samples, despite clear evidence of inter-individual variability [69]. Similarly, enhanced ERVW expression in the CSF and blood of MS patients could not be detected in several studies [70, 71]. Thus, a definitive association between ERVW activation and MS neuropathology remains to be established.

Nonetheless, other human endogenous retroviruses, including ERVK, have been reported to be upregulated in MS. Elevated levels of ERVK RNA have been found in the brain tissue from MS patients [72]. As mentioned above, the ERVK-18.3 *env* allele has been determined to be a risk factor for MS. Interestingly, ERVK-18 env superantigen can be transactivated by Epstein Barr Virus (EBV) latent membrane protein LMP-2A [73, 74], and EBV infection is considered to be one of the major risk factors for MS [75]. Similarly, ERVW env protein also displays superantigenic properties, and can be transactivated by EBV infection of astrocytes in vitro [76]. Together, these superantigens may promote the nonspecific activation of T lymphocytes in the CNS, leading to extensive demyelination and neuronal injury [77]. Thus, ERV-derived superantigens may contribute to MS immunopathogenesis, particularly in the context of EBV infection.

Activation of the host immune system has been implicated as the ultimate effector in MS pathogenesis. Re-activation of human endogenous retroviruses in the CNS may play an important role in this process, as the immune system may mount an antiviral response against ERV elements in order

to eliminate ERV-expressing cells. Antiretroviral defense mechanisms can be mediated by a variety of innate immune sensors including Pattern Recognition Receptors (PRRs) that detect retroviral RNA and proteins. PRRs, including Tripartite motif containing 5 (TRIM5) and Toll like receptor 4 (TLR4), are known to recognize retroviral capsid and envelope proteins, respectively; engagement of these sensors with their viral ligands activates signaling cascades that stimulate innate immunity [78•, 79]. The role of TRIM5 in detection of gag proteins encoded by MS-associated ERVs has not yet been studied. Nonetheless, single nucleotide polymorphisms (SNPs) in *TRIM5* gene (as well as SNPs in other viral restriction factors) have been associated with the risk of MS [80]. However, the functional outcomes of these SNPs remain unclear.

Another mechanism by which ERV proteins may trigger MS immunopathology is through molecular mimicry. Recently, ERVW env proteins were predicted to share several T and B cell epitope regions with myelin oligodendrocyte glycoprotein (MOG) and myelin basic protein (MBP) [81]. This suggests that ERVW env overexpression in the CNS may break tolerance toward host MOG and MBP, generating an autoimmune response against these myelin proteins, which can explain extensive demyelination typically observed in MS. However, the cross-reactivity between ERVW env and myelin protein epitopes, and the resulting autoimmune reaction, needs to be validated experimentally. In addition, whether antigen mimicry is also employed by other MS-associated ERVs, such as ERVK, remains to be explored.

HIV Infection

ERVK activity is well-documented in HIV infection [6, 82] (reviewed in [4]), including the nervous system (Douville and Nath, unpublished) [83••]. Recently, Bhat et al have provided evidence that enhanced ERVK (HML-2) env protein expression in the brains of HIV infected individuals may confer neuroprotective effects [83••]. This is based on the observation that neuroblastoma cells transfected with an ERVK *env* expressing construct were protected from injury by staurosporine and the HIV-1 Vpr protein, compared with the control vector alone. Moreover, the protection from HIV-1 Vpr toxicity was recapitulated in vpr/RAG1$^{-/-}$ mice which were adoptively transferred with neural stem cells expressing ERV-K *env* into the striatum; these animals exhibited a significant reduction in TNFα expression compared with controls. Exaptation of ERVK *env* may provide neurons a degree of protection in the context of chronic neurodegenerative diseases.

As well, cellular cytotoxic responses and antibodies produced against ERVK can prove to be detrimental to HIV-infected cells [84, 85•]. During HIV infection, ERVK env peptides can be a target for cytotoxic T cells [84]. NK cells may also destroy HIV-infected cells via an antibody-dependent cytotoxic mechanism, based on in vitro assays [85•]. Additionally, it was observed that either the HIV strain or the host were important factors in determining the extent of ERVK *env* induction in HIV-infected cells [85•, 86], and thus, may alter the degree of CNS tissue injury in HIV-associated neurocognitive disorder (HAND).

In addition, other ERVK proteins may promote changes in dendritic spine morphology in pyramidal neurons. The ERVK regulatory protein Rec has been shown to interact with the mRNA binding protein Staufen-1, causing its accumulation in the nucleus [5]. This interaction may alter Staufen-1-mediated mRNA trafficking and turnover, functions that are essential for regulation of neuronal synapses during long-term plasticity in learning and memory [87]. The interaction with Staufen-1 also favored Rec-dependent viral RNA transport [5], and thus may enhance ERVK protein expression. Moreover, ERVK and HIV gag proteins can both independently interact with Staufen-1 to enhance their respective production of virions [5, 88], as well as ERVK *env* expression within HIV-1 virions [89]. Together, these studies suggest that ERVK expression in the CNS may have both protective and pathologic consequences.

Prion Disease

Prion diseases, such as Creutzfeldt-Jakob disease (CJD) in humans, are a group of rare but fatal neurodegenerative disorders. The causative agent of these diseases is believed to be an infectious misfolded cellular protein called a prion protein (PrP^{SC}), which is resistant to proteinase degradation and accumulates inside neurons, leading to neuronal toxicity and death [24, 90]. The disease propagates upon transmission of PrP^{SC} to new cells, which further catalyzes the conversion of the normal cellular prion protein (Prp^{C}) into its abnormal form; however, the mechanisms behind this conversion have not been clearly elucidated.

Recently, augmented expression of several ERVs has been observed in the CSF of CJD patients [24]. Although the frequency of ERVK transcripts was higher in CJD CSF samples compared with the controls, this result did not reach statistical significance. Nonetheless, the increased expression of ERVs in CJD suggests that endogenous retroviruses may contribute to the pathogenesis of this prion disease. For instance, ERV viral RNA molecules may elicit the transformation of PrP^{C} to PrP^{SC} [90]. In support of this hypothesis, small highly structured RNAs have been shown to interact with human recombinant PrP^{C} and stimulate its conversion to a proteinase resistant isoform [91]. Interestingly, RNA molecules derived from ERVK elements have extremely conserved complex secondary structures resembling that of the small highly structured RNAs used in these studies [91] (Carr and Douville, unpublished). Highly structured RNAs derived from

HIV-1 have also been shown to interact with the human recombinant PrP^{C} and impart proteinase resistance to it in vitro [92]. Thus, increased levels of ERVK RNA in the CSF of CJD patients have the potential to drive the transformation of the normal human prion protein to its infectious misfolded isoform.

In addition, ERVs may facilitate the spread of pathologic prion agents intercellularly by recruiting prion proteins to virions as ERVs replicate. In fact, it was recently demonstrated that murine PrP^{SC} associates with gag and env proteins on Moloney Murine Leukemia Virus (MMLV) particles, and infection with MMLV strongly enhances the extracellular release of murine PrP^{SC}, thus, augmenting the infectivity of this prion protein [90, 93]. Similarly, human PrP^{SC} has also been shown to be recruited by HIV-1 virions [93]. Human endogenous retroviruses, ERVW and ERVK, which are capable of producing virions [7, 21], may also be able to recruit PrP^{SC} either through interactions with surface gag and env proteins or with viral RNA, thereby transmitting prion proteins to new cells and facilitating the progression of human prion diseases.

Moreover, CJD is neuroinflammatory and marked by augmented levels of proinflammatory cytokines, including TNFα [36, 94]. Mice models of CJD also exhibit increased TNFα, as well as NF-κB activity [95]. Recently, the toxic domain of human prion protein has been shown to activate NF-κB and lead to TNFα production in a macrophage cell line [96]. Based on our prediction of NF-κB responsive elements in the ERVK promoter [31••] (Fig. 1), it is possible that PrP^{SC}-induced TNFα production and NF-κB activation may enhance ERVK transcription in CJD brains. This may culminate in a positive feedback loop favoring further neuroinflammation, ERVK re-activation, and prion infection.

Conclusions

Although ERVK has not been shown to be a causative agent of nervous system disease, its expression can clearly influence both protective and pathologic aspects of motor neuron, neuropsychiatric, and neurodegenerative diseases (Table 1). A common thread among ERVK-associated disease appears to be the presence of inflammatory signals; but how this retrovirus fits into the complex interplay between infection, immunity, autoimmunity, and environmental exposures is yet to be fully elucidated. Activation of multiple ERVs may cooperatively stimulate a multitude of host antiretroviral immune responses against ERV-expressing cells; and, ERVs may exploit this response, culminating in a positive feedback loop favoring further viral gene expression, excessive neuroinflammation, and subsequent neuronal injury and loss. Moreover, it is important to consider that specific ERVK loci can confer select pathologic contributions. Bulk measurement of ERVs (without consideration of the individual integrations and their genomic context) may be an insufficient methodology to address their role in distinct neurologic diseases. Examining an individual's unique complement of ERVs may prove to be a better predictor of disease risk, once further inroads are made in understanding the protective and pathologic roles of each integrated provirus. It will be important for future studies to expand how we measure ERVK activity in the CNS; improved screening for ERVK expression in specific cell types, CNS regions and disease stages, as well as an expansion toward single-loci ERVK measurements will broaden our current knowledge in this area. Current studies are limited by the availability of commercial ERV-specific reagents for molecular biology and the expense of high-throughput screening techniques – a possible solution for our field would be the

Table 1 Summary of potential mechanisms of ERVK protection and pathogenesis in neurodegenerative diseases

Neurological disease	Putative mechanisms of protection or pathogenesis
Amyotrophic Lateral Sclerosis (ALS)	ERVK RNA and proteins may stimulate a proinflammatory immune response against ERVK-expressing neurons [23•], leading to neuronal injury and loss. ERVK may also exploit this inflammatory response [31••], thus establishing a cycle of ERVK reactivation and excessive inflammation.
Schizophrenia (SCZ) and Bipolar Disorder (BD)	ERVK LTR sequences and env protein may act as regulatory elements for genes associated with SCZ and BD [56, 59•]. Upon detection of ERVK over-expression (brought on by infection or inflammation), methylation of ERVK sequences may decrease the subsequent expression of SCZ and BD associated genes (necessary for normal neurological function), leading to a diseased state.
Multiple Sclerosis (MS)	ERVK-encoded superantigens may exacerbate neuroinflammation [73, 77], and thus lead to demyelination. Recognition of ERVK RNA and proteins by innate immune sensors may generate an antiretroviral response against ERVK-expressing cells in the CNS [78•, 79], thus causing neuronal injury and loss. ERVK env may mimic myelin proteins, which may produce an autoimmune response and contribute to demyelination [81].
HIV infection	ERVK env protein may confer protection against HIV-1 Vpr-induced toxicity [83••]. Humoral and cytotoxic immune responses targeted at ERVK antigens may promote the killing of HIV-infected cells [84, 85•]. ERVK Rec protein may promote changes in neuronal dendritic spine morphology by interacting with Staufen-1 [5].
Creutzfeldt-Jakob Disease (CJD)	ERVK RNA may stimulate conversion of normal proteins to pathogenic prion proteins [91]. ERVK virions may recruit and facilitate intercellular transmission of prion agents [92]. ERVK RNA and proteins may exacerbate neuroinflammation.

development of an ERV resource bank, as has been accomplished with the NIH AIDS Reagent Program.

Another benefit of ERV research in the context of nervous system disease is the possibility of improved therapeutics. For example, patients with schizophrenia and bipolar disorder are treated with a range of chemotherapeutics including antipsychotics and lithium. Lithium is protective against HIV neurotoxicity, and HIV patients treated with this medication show cognitive improvements [97]. Clozapine is an antipsychotic drug, which actually inhibits HIV replication in vitro [98]. Since both of these drugs interact with exogenous retroviruses, it is possible that they may have some effect on ERVs as well. In support of this notion, there is epidemiologic evidence that incidence of ALS is extremely rare among individuals with SCZ (much lower than predicted for the general population) [99]. Common medications for SCZ may convey prophylactic neuroprotection, inhibiting the development of ALS [99]. There is some evidence that medications routinely prescribed to schizophrenics may stop inflammation and support neuronal survival [100]. Abating inflammation may also decrease the expression of ERVK, which is upregulated by inflammatory transcription factors [31••]. With improved biomarkers for neurologic disease risk, the use of currently vetted SCZ medications may be repurposed for the prevention or delay of ERVK-associated nervous system diseases.

Compliance with Ethics Guidelines

Conflict of Interest Mamneet Manghera and Jennifer Ferguson declare that they have no conflict of interest. Renée Douville has received establishment and operating grants from the Manitoba Health Research Council, an operating grant from the Manitoba Medical Services Foundation, a starter grant from the ALS Association, and major and discretionary awards from the University of Winnipeg.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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