Chapter

Human Endogenous Retroviruses in Autism Spectrum Disorders: Recent Advances and New Perspectives at the Gene-Environment Interface

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Abstract

Human endogenous retroviruses (HERVs) are genetic elements, derived from their exogenous retroviral counterpart by a process of germline infection and proliferation within the human genome, and their integration as proviruses led to the fixation and the vertical transmission, following Mendelian laws. HERVs currently make up ~8% of the genetic material, and some of them have been cooped for physiological functions. Otherwise, their activation in response to environmental factors has been associated with human pathological conditions. In the setting of neurodevelopmental disorders, HERVs have been proposed as contributing factors involved in Autism Spectrum Disorders (ASD), spanning the bridge between genetic susceptibility, environmental risk factors and immune response. We described a distinct expression profile of some HERV families and cytokines in lymphocytes from autistic children and in their mothers suggesting a close mother-child association in ASD. Moreover, *in vitro* treatment with an antiretroviral drug was able to restore the expression level of HERVs and cytokines providing new insights into the potential role of HERVs as biomarkers of ASD and raising the possibility of using HERVs expression as a therapeutic target for a tailored approach to patient care.

Keywords: human endogenous retroviruses; HERVs, biomarker, mother-child association, gene expression, aetiology, antiretrovirals

1. Introduction

In 1943, the child psychiatrist Leo Kanner described children preferring loneliness with repetitive patterns of behaviour. Similar symptoms were reported by Hans Asperger, an Austrian paediatrician, in 1944 mainly in people of high intelligence [1]. Kanner, first spoke about 'childhood or early-onset schizophrenia', and later he called this condition 'infantile autism', and concerning the aetiology of autism, he attributed

autism to a lack of maternal warmth and attachment. Following this hypothesis, Bruno Bettelheim with his book 'The Empty Fortress' popularized the theory of 'refrigerator mother' by stating that 'the infant that misreads the mother's actions or feelings, or correctly assesses her negative feelings, may retreat from her and the world'. This view was widely criticized and nowadays represents an obsolete thought [2]. From this time, many hypotheses and models emerged to explain this complex condition focusing on symptomatology, phenotype and pathogenesis [3, 4]. However, despite many promising hypotheses, the current literature is made up of controversial findings and lacking of definitive proof about the mechanism underlying the complex aethiopathogenesis of Autism Spectrum Disorder (ASD). ASD is currently referred to as a pervasive neurodevelopmental disorder with an impact on emotional and social behaviour that persists throughout life [5]. The clinical presentation is very heterogeneous, and its incidence is continuously increasing [6]. Despite the consolidated evidence that the main contribution to the increase in the incidence of autism comes from the improvement of the diagnostic process, it has also been hypothesized that at the basis of the onset of autism, there is not a single cause but a set of risk factors acting together to produce the phenotype. Decades of studies have indeed shown that autism is a complex pathology influenced by the combination of genetic, environmental and epigenetic factors, mainly acting during prenatal and/or perinatal phases [7–9]. The concordance rate of ASD in monozygotic twins much higher than in dizygotic twins seemed to indicate that genetic factors were more likely to contribute to ASD than environmental factors [10]. More than a thousand ASD-associated genes known to be involved in brain development have been identified to date [11], and many genomic copy number variants have been associated with neurodevelopmental disorders including ASD [12, 13]. Several epidemiological studies indicate that potential risk factors for ASD also include various determinants [14, 15], such as the age of the pregnant woman, advancing paternal age [14, 16, 17] and prematurity [18]. However, an ever increasing important role has been attributed to risk factors related to the early foetal environment, including toxicants, diet, air pollution, smoking or chemicals exposure, which have been suggested to induce a prenatal and/or perinatal brain insult able to contribute to the development of autism in genetically predisposed individuals [19]. These environmental insults share in common the activation of the maternal immune system (MIA), which has therefore been recognized as an additional risk factor for ASD [20]. MIA is an inflammatory response triggered by pathogenic infection and autoimmune diseases in the mother. It is known that several microorganisms, vertically transmitted to the foetus, affect its development resulting in severe complications such as miscarriage and malformations [21]. However, even non-vertically transmitted infections during pregnancy can cause harm to the offspring by producing inflammatory cytokines, which directly damage the foetal brain by crossing the placental and blood-brain barrier [22, 23].

Preclinical studies, using mouse model of MIA induced by prenatal exposure to polyinosinic:polycytidylic acid (Poly I:C), a synthetic double-stranded RNA molecule targeting TLR-3, mimicking viral maternal infection, demonstrated that the exposure to a prenatal insult induced derailed neurodevelopment in offspring. Particularly, in the mothers, the Poly I:C injection leads to the production of interleukin-17 that reaches the foetal brain *via* the placenta inducing cell death and decreasing synaptic density and expression levels of synapse formation-associated proteins and resulting in ASD-like behavioural and morphological brain abnormalities, also described in the pathophysiology of human ASD [24–26]. In line with the hypothesis that maternal immune response could impact on neurodevelopment in the newborn,

epidemiological studies have reported that MIA, caused by autoimmune diseases, also increases the risk of ASD [27]. Altogether these studies suggest that MIA-induced inflammation and cytokines can impair placental function and lead to the disruption of its barrier function, resulting in exposure of the foetus to toxic substances. Accumulated evidence also shows an important role of epigenetic factors, such as DNA methylation, histone modification and noncoding RNA in predisposition to disease development. Epigenetic mechanisms regulate chromatin structure and gene expression without altering the DNA sequence. In consideration of this last characteristic, in the past it was believed to have no role in the growth and development of the individual [28] while the study of their interaction with environmental conditions has highlighted their important role in the development of genes related to brain development. In recent years, there have been rapid advances in the understanding of epigenetic mechanisms that ultimately regulate gene activity and expression during development and differentiation or in response to environmental stimuli. Instead, it is now known that the main function of epigenetic factors is to regulate development through cell differentiation processes, tissue specification and maintenance of cell lineages [29]. Therefore, environmental stimuli can alter the epigenome and consequently gene transcription, changing the phenotype [30].

Within this interplay among genetic susceptibility, MIA, epigenetic and environmental factors are placed the human endogenous retroviruses (HERVs), which we proposed as novel contributing factors involved in ASD.

2. Human endogenous retroviruses and their co-evolution with the host

In contrast to the prevailing early twentieth century conception of genetic material as fixed, in the 1940s, the Nobel Prize Barbara McClintock discovered in maize the 'mutable loci' which were capable to move between chromosomes. This pioneering study paved the way for future research into the role of these 'jumping genes' or transposable elements (TEs) in both health and disease conditions [31]. Indeed, it was later discovered that about 46% of the human genome consists of TEs [32]. They consist of repetitive sequences that are able to insert copies of themselves elsewhere in the genome [33]. They are divided, according to their size and functionally related structures, into short interspersed elements (SINEs), long interspersed elements (LINEs), long terminal repetition retrotransposons (LTR) and DNA transposons [34]. The major subset of LTR retrotransposons is represented by HERVs, which together with their derivative sequences comprise at least 8% of the human genome [32, 35].

These elements have their origin in the numerous environmental events that shaped the human genome during evolution, including the occasional infection of germ cells of our ancestors by exogenous retroviruses and the insertion of their RNA genome as proviruses into the cell's chromosomal DNA [36]. Hence, HERVs are transmitted in a Mendelian manner to all subsequent generations (**Figure 1a**). Retroviral proviruses share the canonical structure of retroviruses consisting of an internal region of four essential viral genes (gag, pro, pol and env), flanked at either side by long terminal repeats sequences (the 5′ and 3′ LTRs) that are identical at the time of integration and contain promoter, enhancer and polyadenylation signals that shape the cellular transcriptome (**Figure 1a**) [37].

A non-coding sequence containing a tRNA-specifc primer-binding site (PBS) is usually present between the end of the 5′ LTR and the first codon of the gag gene,

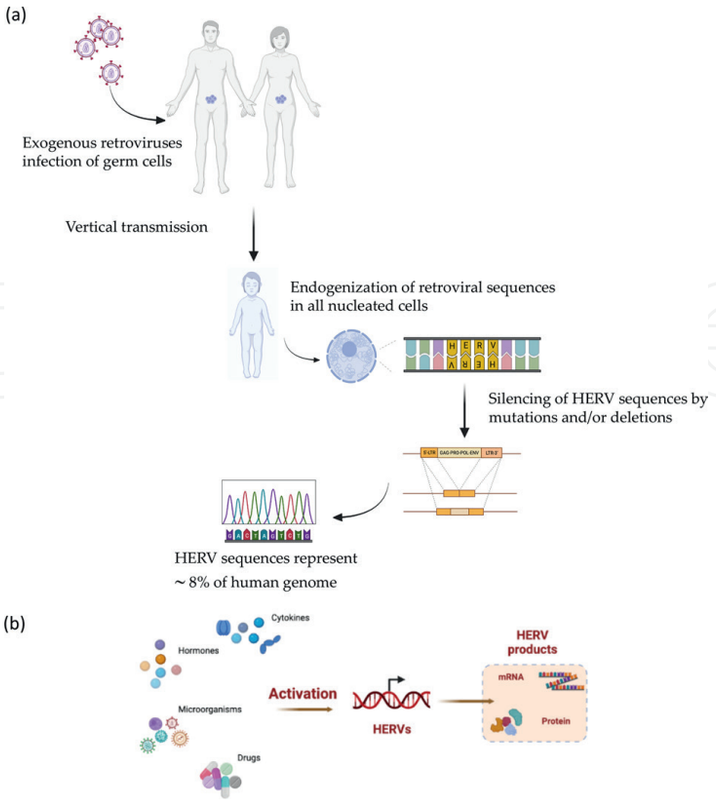

Figure 1.
HERV origin (a) and their activation in response to environmental stimuli (b) (created by BioRender).

and HERVs are classified into families on the basis of the tRNA that binds to the viral primer-binding site to prime reverse transcription.

During human evolution, HERVs invaded the human genome undergoing amplification, retrotransposition and/or reinfection events, and resulting in the presence of multiple copies fixed in the DNA of all nucleated cells [36]. While a majority of these sequences have accumulated mutations and/or deletions and are mostly defective, several HERVs preserve the properties of the ancient viruses and, still being transcriptionally active and competent to produce some retroviral proteins, have been co-opted for physiological functions [36, 38, 39] (**Figure 1a**). In the light of the current knowledge, what Weiss stated in 2016 'If Charles Darwin reappeared today, he might be surprised to learn that humans are descended from viruses as well as from apes' [40] is still current.

3. The role of human endogenous retroviruses in physiological conditions

For a long time, HERVs have been considered as junk DNA with no impact on the host. However, during the last decades, the great efforts of the scientific community highlighted that some 'well preserved' ERV sequences influence different physiological properties being involved in a variety of biological pathways [41, 42]. The most intriguingly example of the co-option of HERVs during the host evolution comes from the well-studied properties of the syncytins, the products of the Envelope (Env) genes of HERV-W-1 and HERV-FRD. Specifically, Syncytin-1, encoded by the HERV-W-1

gene, is the first retroviral protein found to have a defined physiological function mediating the cell-cell fusion as the terminal differentiation of the trophoblast lineage [43]. As emerging from different reports, the decrease of syncytin expression and the consequential fusion deficiency could contribute to placental anomalies including pre-eclampsia disorder [44]. Moreover, other studies suggested that syncytin-1 also possesses non-fusogenic activities, as the regulation of trophoblast proliferation and apoptosis and indicated a widely expression in different cell types such as granulocytes, T lymphocytes, monocytes, glial cell of the brain and cancer cells [45, 46]. In human, syncytin-1 interacts with the type D mammalian retrovirus receptor ASCT-1/ASCT-2 (sodium-dependent neutral amino acid transporter type 1/2) on cell membranes. Syncytin-2, encoded by the HERV-FRD gene, is also expressed in trophoblasts and able of mediating cell fusion by interacting with a different receptor known as MFSD2 (major facilitator superfamily domain containing 2) [47, 48]. Moreover, Syncytin-2 is involved in maternal immune tolerance towards the semi-allogenic foetus [49]. A similar functional domestication emerged for ERV-encoded GAG gene, involved in the memory consolidation in the mammalian brain, including long-term potentiation and depression [50]. Given the abundance of HERVs in the human genome, they represent an important source of genomic variability, also providing potential coding and regulatory elements for the acquisition of new cellular functions [51, 52]. In line, growing evidence has been obtained regarding the general expression of HERVs in normal tissues [53, 54], and in this context, we demonstrated an age-related transcriptional activity of HERV-H, HERV-K and HERV-W in peripheral blood mononuclear cells (PBMCs) from a large cohort of healthy human subjects aged between 1 and 80 years, reinforcing the hypothesis of a physiological correlation between HERV activity and the different stages of life in human [55]. Among the proposed mechanisms by which HERVs could contribute to the human physiology, it is recognized that various sequences, concentrated in the LTRs, are involved in the regulation of the expression of neighbouring genes acting as promoters, enhancers, polyadenylation signals, regulators of chromatin folding and binding sites for transcriptional factors [56, 57]. In the genome, most HERVs reside as solo-LTRs, resulting from homologous recombination between the LTRs of a full-length HERV [58] able to act as alternative tissue-specific promoters to drive the expression of host genes [59–61]. During embryo development, some HERV sequences are also engaged by the host for the regulation of gene expression [62]. In particular, non-coding RNA expressed by the HERV-H group and the recruitment of specific cellular transcriptional factors on HERV-H LTRs seem to be involved in the conservation of stem cell identity [57, 63]. Of note, the HERV-H loci seem to be more preserved in a full-length state than other HERV families, suggesting that the full-length elements rather than solo-LTRs are useful to the host and that the internal regions of HERV-H may be involved in the process of exaptation [64]. Similarly, an ancestral env gene named HEMO [human endogenous MER34 (medium reiteration-frequency-family-34) ORF] has been found highly expressed in embryos, already in the early stages of development and in all subsequent differentiation periods as well as in the placenta and in the blood of pregnant women [65]. Finally, in the regulation of stem cell function, HERV-K ENV was highly expressed in the cell membrane of pluripotent stem cells and signals *via* direct binding to CD98HC, leading to activation of signalling pathways that regulate stem cell function [66]. Moreover, the expression of HERVs has a direct key role for the maintenance of human embryonic stem cells and induced pluripotent stem cells (iPSCs), and their activation could be considered a marker of pluripotency [67].

To conclude, the more profound insight into the mechanisms explaining the roles of HERVs in various biological/physiological contexts will help to clarify the contribution of HERVs in pathological conditions.

4. The activation of human endogenous retroviruses

In addition to their physiological role, HERVs have been also proposed as possible cofactors in the aetiology of several human diseases. Proviruses are known to remain dormant for long periods of time within the host, but they may occasionally be triggered by factors present in the environment. Indeed, one of the peculiar features of HERVs is their intrinsic responsiveness to microenvironmental stress and various stimuli likely *via* epigenetic mechanisms [68, 69]. Epigenetics represents a fine mechanism to control HERV activity to ensure genomic stability and integrity and, on the other side, it represents one of the mechanisms by which HERVs could modulate the gene expression. HERV sequences are epigenetically silenced by DNA methylation and histone modifications in addition to being located in chromosomal regions with heterochromatic chromatin architecture leading to a low transcriptional degree in most cell types [70, 71]. It is known that epigenetics is a driver of the embryonic development, contributing to global remodeling, cell commitment and tissue specification [72] and that ERVs are highly active during early embryogenesis and germline development [62, 73]. Indeed, they are involved in the pre-implantation transcription network although the mechanisms are still unclear [62, 74]. Thus, during a sensitive phase, as the embryogenesis, any environmental insults could have an impact on the development, and epigenetic modifications could directly link the environmental stimuli and the molecular regulatory pathways, explaining some aspects of complex pathologies including HERV activation. A multitude of environmental factors and xenobiotics can activate HERV expression causing DNA rearrangements, HERV reinsertions and HERV copy number variation, resulting in abnormal HERV activity that could, in turn, potentially affect crucial pathway such as inflammation [69, 75, 76]. Microorganisms, cytokines, hormones, vitamins, nutrients and drugs could represent triggers leading to HERV transactivation (**Figure 1b**). Of particular relevance is the role played by the interaction with microbes, including viruses, exogenous retroviruses, intestinal microbiota and protozoan. Among viruses, the herpesviruses, Hepatitis B virus, the human immunodeficiency virus-1 (HIV-1), Influenza A virus and more recently SARS-CoV-2 have been found able to deregulate HERV activity and although so far, an unequivocal pathogenic cause-effect relationship has not been established, their contribution to the development of viral diseases, including virus-associated tumors, has been suggested [77–83]. Moreover, several *in vitro* studies demonstrated that cells express HERVs at high levels in response to stimulation by using lipopolysaccharide or interferon-γ (IFN-γ), cytokines as Interleukine-1β (IL-1β) and tumor necrosis factor-α (TNF-α) or mitogens, such as phytohemagglutinin [84–86]. These observations could be due to the fact that HERVs showed various regulatory sequences that have been linked to the transcriptional modulation systems [87]. More recently, the hormonal regulation of HERVs has been investigated, and specifically, cross talks among the female sex hormones and HERVs in contributing to breast cancer tumorigenesis and proliferation have been elucidated providing useful knowledge for the development of novel cancer therapies. Specifically, the effect of progesterone on HERV-K expression is at least partly mediated by OCT4 known to be involved in

embryogenesis and expressed in diverse cancer types as well [88]. These findings were in line with the peculiar expression of HERVs in peripheral leukocytes during the menstrual cycle suggesting a well-coordinated hormonal regulation of HERV activity [89]. Also, drugs are able to modulate HERV expression, both *in vitro* and *in vivo* with different proposed mechanisms, mainly linked to the epigenetic one. In particular, neuroleptics and antidepressants influence HERV activity in human brain cell lines and in post-mortem brain samples of patients with mental disorders in therapy during their lifetime [90].

Thus, HERVs have been found particularly responsive to environmental stimuli that can determine their dysregulation at transcriptional levels and/or encoded protein expression that could influence the onset of complex diseases.

5. The contribution of human endogenous retroviruses in disease development

HERV expression is tightly controlled in normal adult tissues but is reported to be aberrantly expressed in cancer [68], inflammatory and autoimmune diseases [91], aging [92], type 1 diabetes [93] neurological disorders [94] and recently also viral disease [83, 95, 96]. Most of the diseases in which HERVs play a role as cofactor are characterized by a multifactorial aetiology and an inflammatory landscape. As such, HERVs have been proposed as spanning the bridge between environment and genetic background and as shapers of the immune system.

5.1 Human endogenous retroviruses as shapers of the immune system

HERVs can modulate the human immune response by different mechanism. In fact, being integrated as proviruses within the genome and physiologically expressed, HERV antigens can be recognized by the innate immune system as 'self-determinants' but also as potential pathogens. Probably due to their similarity with exogenous viral proteins they are able to activate pathogen recognition receptors (PRRs) by evoking the production of pro-inflammatory mediators (such as IFN, cytokines and chemokines), which in turn can activate and trigger the adaptive immune response [42]. Moreover, the involvement of HERVs in the host antiviral immune system seems to be linked to the Interferon pathway by acting as enhancer elements to directly affect the expression of adjacent interferon-stimulated genes [53, 54, 97]. Since HERVs are an integral part of host immunity, they protect the host from exogenous retroviral infections by PRRs, of which a major class are the Toll-like receptors (TLRs), the first line of defence in detecting a wide variety of pathogens. TLRs' engagements with viral components lead to the activation of MAP kinase and NF-kB resulting in the production of pro-inflammatory cytokines, involved in the infection control. It is also known that HERV RNAs are able to activate the immune system, thus stimulating the production of pro-inflammatory cytokines, which in turn can activate and prime the adaptive immune response [42]. This mechanism could be explained by the presence of transcriptional regulatory elements within retroviral long terminal repeats LTRs [98, 99]. This feedback loop made by HERV upregulation, inflammatory mediators and epigenetic dysregulation could be one way in which HERVs could have pathogenic potential leading to chronic stimulation of the immune system that could sustain the development and/or the progression of several human diseases.

5.2 The role of human endogenous retroviruses in neuroinflammatory and neuropsychiatric disorders

Outdated scientific evidence has reported the possibility of different viruses such as herpesviruses, HIV and Ebola virus to reach the central nervous system (CNS) contributing to the development of diseases. As such, despite the inaccessibility of CNS and the immunological structure that makes it a 'privileged district', the viral replication can occur by exceeding controls and can also happen multiple times during an individual's lifetime increasing the risk of developing neuropathologies [100]. In the last few decades, many researchers demonstrated that the activation of endogenous viral sequences in response to exogenous stimuli, especially viruses, can contribute to a variety of neuroinflammatory and neuropsychiatric disorders, including multiple sclerosis (MS), amyotrophic lateral sclerosis (ALS), schizophrenia (SCZ) and bipolar disorder (BD). The pathways in which retroelements are involved in derailment of the nervous system are diverse: HERVs mainly interact with innate and adaptive immunity [52, 101], LINE activity is linked to neurogenesis, in particular neuronal differentiation and cognitive processes, both in adult brain and in progenitor cells [102, 103], and Alu elements, the most common member of SINEs, are involved in neurogenesis, brain development and cognition pathways [102, 104]. The first paper describing the involvement of HERVs in neurological disorders, especially in MS, dates back to the late 1900s when Perron and colleagues discovered retroviral elements in the leptomeningeal cells of MS patients [105]. From this initial work, several studies succeeded, both *in vitro* and *in vivo*, culminating in the identification of an aetiopathogenetic model in which HERV-W was further revealed to play functional roles in inflammatory processes. Specifically, pro-inflammatory cytokine expression was shown to be induced in both human and murine monocytes upon *in vitro* stimulation with HERV-W recombinant Env protein by a process that required TLR-4 receptor activation [69]. In line with these intriguingly findings, several research groups have contributed to the topic, focusing on different aspects concerning the role of HERVs of the aetiology and/or progression of the disease. MS is a neurodegenerative and neuroinflammatory disease affecting CNS in which it causes multifocal demyelinating lesions leading to physical and cognitive impairments and despite the plethora of studies, definitive proof regarding the aetiology being still lacking. In this setting, HERV-W Env protein has been extensively studied and to date has been recognized as a contributor factor in the MS pathogenesis. As such, the expression level of HERV-W in the brain of MS patients positively correlates with the severity of the clinical signs [106]. Moreover, the env transcripts and proteins of HERV-W are overexpressed in the brain [107] and in peripheral blood and serum of MS patients [108, 109] as a constant imprinting of the disease. Another important aspect characterizing the role of HERVs in MS is the interplay of HERVs with the immune system. Indeed, HERVs can stimulate both the production of pro-inflammatory mediators and innate and adaptive immune cells, which in turn could affect endothelial cells of the blood-brain barrier as well as oligodendroglial precursor and microglial cells [94]. Another pathological condition in which HERV activity has been investigated is ALS, a progressive nervous system disease that affects nerve cells in the brain and spinal cord, causing loss of muscle control. ALS is also characterizing by an imbalance of the immunological mediators with a marked production of inflammatory cytokines. The first demonstration of the involvement of a HERV was the discovery of the activation of HERV-K (subtype HML-2) in the brains of individuals with ALS [110, 111]. Subsequently, the reverse transcriptase activity of

HERV-K was identified in brain, cerebrospinal fluid (CSF) and blood of ALS patients [112, 113], and the expression of HML-2 Env transcripts and protein was found in cortical and anterior horn cells of the spinal cord samples [114]. Although there is consistent evidence linking HML-2 to ALS, very little is known about the mechanisms by which it may cause observed neurotoxicity. Recently, the neurotoxicity of HML-2 Env was clarified in transgenic animals that express the envelope protein developing an ALS-like syndrome. Interestingly, these observations provide the possibility to use HERV-K env-specific antibody in preclinical models to prevent Env toxicity and pave the way for new treatment strategies in sporadic ALS [115]. Schizophrenia, a major neuropsychiatric disorder, is a chronic brain disorder, and when active, symptoms include delusions, hallucinations, trouble with thinking and concentration and lack of motivation [5]. While disease onset typically occurs in late adolescence or early adulthood, several lines of evidence suggest that SCZ results from aberrations occurring in foetal development [116]. Furthermore, growing evidence demonstrates the increased risk of SCZ following early-life exposure to infectious agents or inflammatory stimuli, suggesting the involvement of the immune system in the aetiopathogenesis of the disease [117]. The strongest evidence for an association between HERV and SCZ comes from studies of HERV transcripts in the brain, cerebrospinal fluid and blood samples from affected individuals in which elevated levels of HERV-H, HERV-K and HERV-W were detected [72, 118, 119]. HERV-W Env protein expression in hippocampus was recently shown to alter the N-methyl-d-aspartate receptor-mediated synaptic organization and plasticity leading to defective glutamate synapse maturation, behavioural impairments and psychosis [120]. In addition, the epigenetic status of HERV-K sequences, particularly lower methylation levels in blood samples, has been indicated as marker of the early stages of SCZ [119]. Similarly, HERV-W transcripts and proteins were found to be elevated in the blood, CSF and brains of BD patients [118, 121, 122]. BD is a group of brain disorders that cause extreme fluctuation in a person's mood, energy and ability to function [5]. The precise aetiopathology of BD is unclear, and several reports indicate the involvement of the innate and adaptive immune system including inflammation [123]. Notably, an association between HERV-W Env protein, an increased level of IL-1β and an earlier disease onset was described in BD patients with respect to patients who were negative for HERV-W Env protein, suggesting HERV-W as marker able to define a specific group of patients in bipolar condition [122].

6. Human endogenous retroviruses in neurodevelopmental disorders

The first article pioneering the hypothesis of the possible involvement of HERVs in the aetiopathogenesis of neurodevelopmental disorders was published by us 10 years ago. Specifically, we hypothesized a link between HERV activity and ASD highlighting a distinct expression profile in Italian autistic children in which HERV-H was highly expressed in peripheral lymphocytes, when compared with controls, with higher levels in younger children, supporting the hypothesis that HERV-H overexpression might be regarded as a potential early biomarker of ASD. This view was even more supported by the fact that ASD children with more severe impairments in Communication and Motor Psychoeducational Profile-3 showed the highest expression levels of HERV-H [124]. The analysis of HERV expression profile was then replicated in a cohort of Albanian ASD children who showed HERV-H high expression level in peripheral lymphocytes as

already found in Italian ASD children. This allowed us to conclude that HERV-H could be considered as a molecular signature of the disease unrelated to ethnicity [125]. These findings opened a challenging scenario to extend the study to a cohort of attention deficit hyperactivity disorder (ADHD) children in order to verify whether the peculiar HERV expression profile could also be identified in a 'twin disorder' of ASD. Indeed, the two disorders are highly correlated [126], and ADHD often occurs in conjunction with ASD [127]. ADHD children showed the highest expression levels of HERV-H in peripheral lymphocytes correlated with inattention and hyperactivity symptoms, suggesting HERV-H a molecular biomarker also for ADHD [128]. All the evidence emerged from these initial papers was always obtained by studying drug-naïve children considering that, as indicated at the time by other research groups, HERV activity could be strongly influenced by drugs exposure [90]. And it is from all of this that the subsequent work arose, in which the intention was to evaluate HERV-H as a potential biomarker of response to treatment in ADHD patients undergoing methylphenidate (MPH) therapy. As such, the reduction of HERV-H expression levels H in peripheral lymphocytes from ADHD children was found after only 1 week of MPH therapy with a further decrease at 24 weeks of treatments in parallel with improvement in symptoms. These findings suggested HERV-H as a predictive marker of the response to MPH therapy despite the awareness that the absence of a non-responsive patient group is a major limitation of this research preventing definitive conclusions [129]. The validation of early results about HERV expression profile in ASD children and new evidence came from a paper we published in 2019 in which we also included the parents of children in order to investigate the parent-of-origin effects in ASD in terms of HERVs and immune deregulation. ASD children and their mothers shared common expression levels of HERV-H and HEMO and of the cytokines such as TNF-α, IFN-γ and IL-10 in peripheral lymphocytes. Therefore, the abnormal expression of HERVs and cytokines was not an exclusive trait of autistic patients but also of their mothers, suggesting a close mother-child association within the ASD families. Taken together, these findings support the potential use of selected HERVs and cytokines in a set of biomarkers that accounts for the multifaceted nature of the disorder and can complement existing clinical methods [130]. A subgroup of this cohort was also used to conduct a proof of concept study in which lymphocytes from ASD children and their parents were exposed to stimulating factors (Interleukin-2/Phytohaemagglutinin) or drugs, such as the antiepileptic drug valproic acid (VPA) and the antiretroviral drug efavirenz (EFV) with the intent to investigate whether the expression level of HERVs and cytokines could be modulated. Lymphocytes from ASD children and their mothers share intrinsic responsiveness to stimulating factors and VPA in expressing HERVs and cytokines. EFV specifically restored the HERV activity with a concomitant modulation of cytokines, in particular lowering the pro-inflammatory while maintaining high regulatory ones. This evidence provided new insights into the potential role of HERVs as biomarkers of ASD and raising the possibility of using HERV expression as a therapeutic target for a tailored approach to patient care [86].

With the intent of deciphering other factors linked to HERV activity that could contribute to ASD pathogenesis, the expression of epigenetic effectors known to regulate HERV expression and brain functions has been evaluated in ASD children. The authors found a correlation among the overexpression of these

elements and several HERVs suggesting their involvement in pathogenetic mechanisms leading to ASD [131]. All the studies above described were conducted using peripheral lymphocytes from ASD cohorts in agreement with the findings of various research studies that there is a shared gene expression profile between whole blood and brain tissues, suggesting that the cautious and thoughtful use of peripheral gene expression may be a useful surrogate for analysis in the brain. Of course, the brain remains the district of choice for studying neurodevelopmental alterations and to circumvent the issue, different preclinical models of ASD have been developed enabling studies on the aetiology, pathogenesis and possible prevention and treatment modalities of ASD. The main categories of models comprise genetic animal models, idiopathic strain, models of infection/inflammation and chemically induced animal models displaying robust and well-replicated social deficits and repetitive behaviours [132]. The idiopathic strain BTBR T+tf/J (BTBR) and the prenatally CD-1 VPA-induced models of ASD have been extensively studied to characterize the expression profile of ERV and immune mediators. As such, whole embryos at about half of gestation, brain and blood tissues at different postnatal ages have been analysed. Both ASD models showed higher expression levels of ERVs beginning from intrauterine life and up to adulthood (**Figure 2**). Moreover, the aberrant expression of some ERV families correlated with expression levels of pro-inflammatory cytokines and TLR-3 and TLR-4 in embryos and brain tissues, supporting the interplay between ERVs and neuroinflammation as contributing factors in the appearance of ASD-like phenotype [133]. Subsequently, we conceived a study to evaluate the multigenerational impact of prenatal VPA exposure, demonstrating transgenerational changes in both behaviour and ERV expression that last, with fading of epigenetic memories across generations, till the third one that lacks a direct exposure

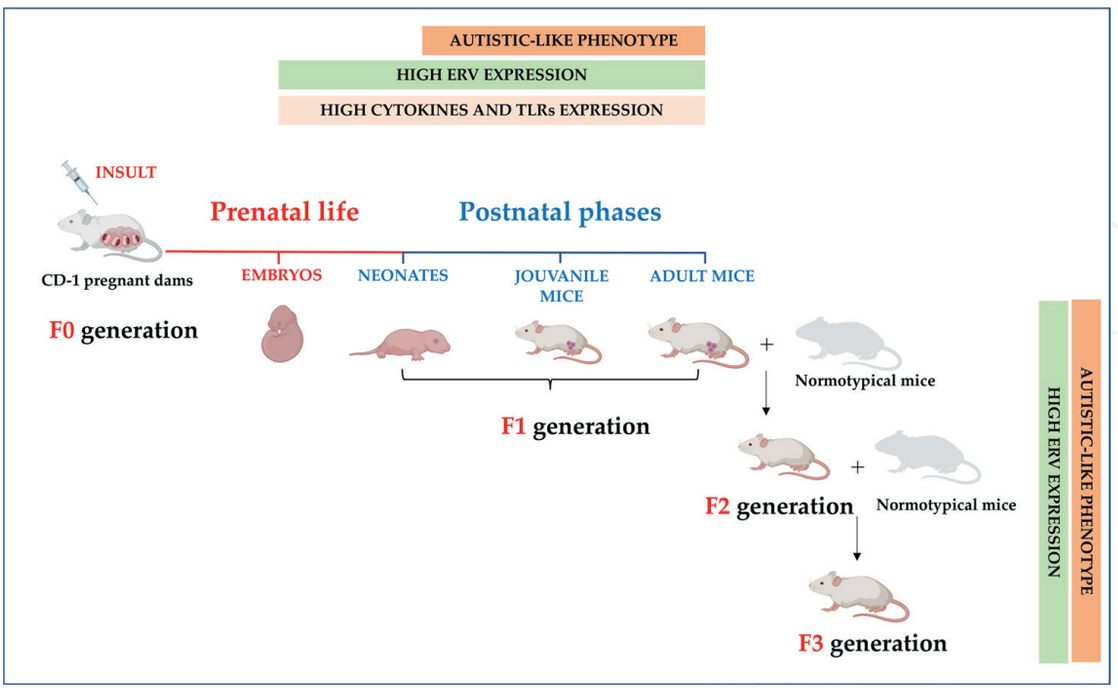

Figure 2.
The abnormal ERV, cytokines and TLRs expression in mice with autistic-like phenotype prenatally exposed to valproic acid (created by BioRender).

to the drug (**Figure 2**). Of note, offspring from maternal lineages showed more marked transcriptional effects compared with paternal lineages both in the second and in third generations suggesting the hypothesis of maternal imprinting as a contributing factor in increasing susceptibility to ASD [134]. Moreover, these findings also suggest ERV blood transcriptional levels as a stable peripheral biomarker, even at early life stages, of derailed brain development [135]. In our last paper, we demonstrated that MIA induced abnormal expression of ERVs and immune mediators in mouse off-spring in a sex-dependent fashion. Specifically, we demonstrated that the prenatal exposure to Poly I:C in C57BL6/J mice induced a tissue-specific expression of several ERVs, ERV-related genes and inflammatory mediators with ERVs as the main carriers of the peculiar profile found in brain areas from Poly I:C mice. In addition, Poly I:C induced larger effects on the expression of some retroviral elements only in pre-frontal cortex from female offspring reinforcing the view on sex bias as a possible risk factor for ASD [136].

Taken together, these findings candidate ERV activation as common feature shared by several risk factors of appearance of ASD and suggest ERVs as main carriers of changes occurring in brain from autistic mice, primarily in female offspring. These papers could represent the starting point to set up a new preclinical experimental design to verify the chance to inhibit ERV activity to rescue ERV activation, immune dysregulation and ASD-like phenotype observed in offspring trying to figure out cause and effect in this complex interplay. Moreover, a deep characterization of the molecular mechanisms by which gender differences could affect the neurodevelopment will help in identifying gender-specific diagnosis and personalized treatment strategies.

7. Human endogenous retroviruses: from aetiological to therapeutic implications in neurological diseases

Given the now well-established knowledge regarding the implication of HERVs in different pathological conditions, new avenues for the development of targeted therapies directed against HERV products have been opened. In this direction, a humanized monoclonal antibody (mAb) directed against HERV-W ENV, called GNbAC1 or temelimab, has been developed. The drug targets a linear non-glycosylated epitope of the surface unit domain of the HERV-W Env, blocking its interaction with the TLR-4 receptor and thus, the release of pro-inflammatory mediators and the inhibition of the myelin repair process. Different *in vitro* and preclinical studies offered promising results that culminated in clinical trials in which temelimab has been proposed as novel drug for MS treatment first to test pharmacokinetics, safety and efficacy providing encouraging results about its neuroprotective and regenerative effects in parallel with antiretroviral effects, which does not impair the immune system [137, 138]. Starting from the observations that also in type 1 diabetes (T1D) patients, HERV-W Env has been detected in blood and pancreatic acinar cells and after different reports concerning the activity of temelimab in *in vivo* and *in vitro* models of T1D [139], this drug was offered to patients as a part of a clinical trial to test the safety and its effect on the autoimmune process. Also in the case, the drug was well tolerated and reduced the events of hypoglycaemia and the levels of anti-insulin autoantibodies after the first period of treatment [139, 140]. More recently, the same research group

proposed an anti-HERV-K Env mAb for the treatment of ALS. In addition to the antibody-based immunotherapy targeting HERV ENV, also the use of antiretroviral drugs has been evaluated in different setting, ranging from the *in vitro* studies to clinical trials, opening new prospects for exploring novel treatments of diseases such as MS [141]. The underlying rationale is that patients with HIV treated with antiretroviral drugs have a lower risk of developing MS than non-infected, healthy population thus suggesting that the antiretroviral treatment may reduce the risk of evolving MS also acting on HERV expression [141, 142]. Also in the treatment of patients with ALS the use of antiretroviral drugs has been proposed. As such, a recent clinical trial including a combination of antiretroviral drug has been conducted showing a decrease in HERV-K expression as well as of disease markers when administered to patients. Remarkably, a high percentage of patients were classified as 'responsive' to treatment reinforcing even more the role of HERV-K in the clinical course of the disease [143, 144].

Taken together, these findings provide the background for hypothesizing other therapeutic approaches targeting HERVs in different clinical setting towards a personalized medicine.

8. Conclusions and new future perspectives

The physiological roles of HERVs in pregnancy and embryogenesis, their intrinsic responsiveness to external stimuli and the interaction with the immune

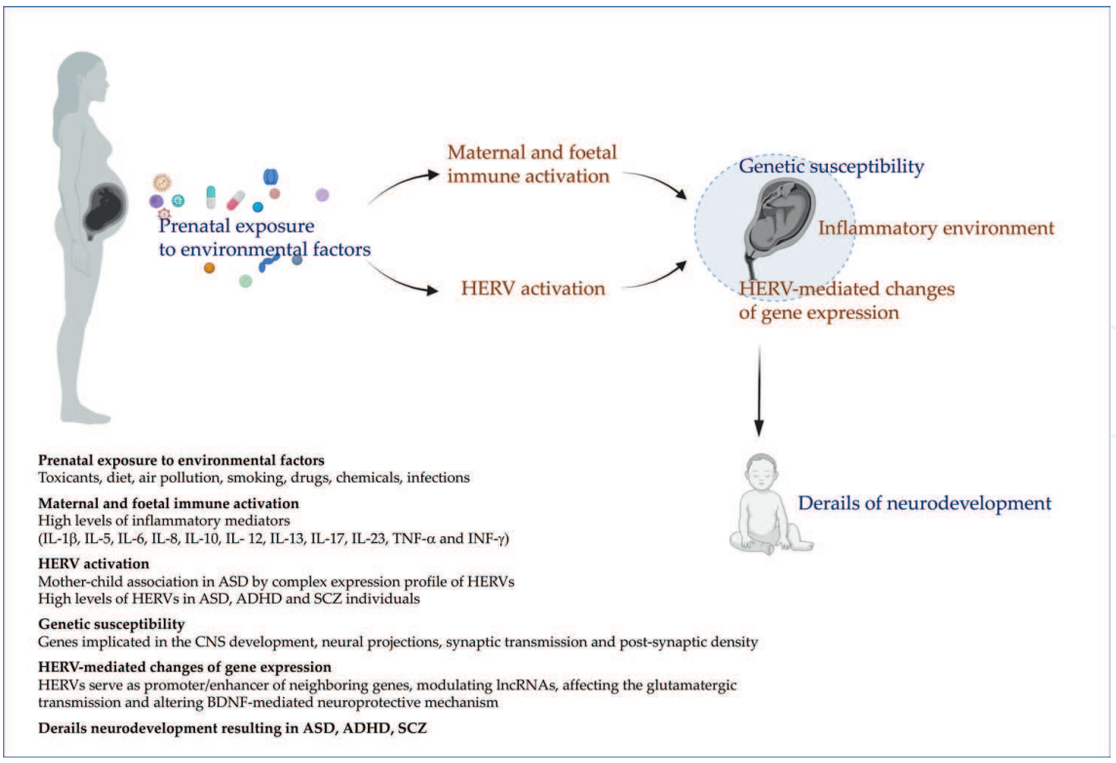

Figure 3.
The potential involvement of human endogenous retroviruses (HERVs) in the interaction among genetic susceptibility, environmental risk factors and immune activation in complex neurodevelopmental disorders (created by BioRender).

system support the hypothesis that their deregulation affects the neurodevelopmental process (**Figure 3**). Nevertheless, it is still debated if HERVs are cofactors or epiphenomenon in neurodevelopmental disorders, and more efforts are needed to investigate the potentially detrimental effect of HERV products in the aetiopathogenic processes. In this complex landscape, the use of animal models could offer countless advantages to deeply investigate the embryonic phase that is certainly crucial for the onset of ASD, and brain district, mostly inaccessible in human studies. Since the brain remains the district of choice for the study of neurodevelopmental alterations, preclinical models of ASD allow to explore and characterize this anatomical district in depth, helping clinical research to make further progress towards the identification of new biomarkers, potentially useful for diagnosis and pharmacological intervention. Moreover, HERVs seem to be a reliable biomarker for ASD, readily detectable in peripheral blood, representing a potential efficient diagnostic tool to complement the current clinical behavioural diagnosis. Furthermore, a biomarker detectable before the onset of symptoms could facilitate early screening and timely initiation of treatment by improving the long-term prognosis of the mental health of affected individuals. Further studies are needed and could represent a new approach to unravel the aethiopathogenesis of ASD, bearing in mind that the retroelements cannot be appropriately understood only through a virologic or genetic approach, since their complex roles in physiology as well in diseases. Both preclinical models and human studies indicate that the abnormal expression of ERVs could represent a molecular signature of neurodevelopmental disorders.

Conflict of interest

The authors declare no conflict of interest

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References

[1] Simon BC. Leo Kanner, Hans Asperger and the discovery of autism. The Lancet. 2015;**386**(10001):1329-1330

[2] Klin A. Autismo e síndrome de Asperger: uma visão geral [Autism and Asperger syndrome: An overview]. Braz J Psychiatry. 2006;**28**(1):S3-S11

[3] Betancur C. Etiological heterogeneity in autism spectrum disorders: More than 100 genetic and genomic disorders and still counting. Brain Research. 2011;**1380**:42-77

[4] Inui T, Kumagaya S, Myowa-Yamakoshi M. Neurodevelopmental Hypothesis about the Etiology of Autism Spectrum Disorders. Frontiers in Human Neuroscience. 2017;**11**:354

[5] American Psychiatric Association [APA]. Neurodevelopmental disorders. In: Diagnostic and Statistical Manual of Mental Disorders. Washington, DC: APA; 2013. pp. 31-86

[6] Maenner MJ, Shaw KA, Bakian AV, et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 Sites, United States, 2018. MMWR Surveillance Summary. 2021;**70**(11):1-16

[7] Abrahams BS, Geschwind DH. Advances in autism genetics: On the threshold of a new neurobiology. Nature Review Genetics. 2008;**9**(5):341-355

[8] Landrigan PJ, Lambertini L, Birnbaum LS. A research strategy to discover the environmental causes of autism and neurodevelopmental disabilities. Environmental Health Perspectives. 2012;**120**(7):a258-a260

[9] Lyall K, Croen L, Daniels J, Fallin MD, Ladd-Acosta C, Lee BK, et al. The changing epidemiology of autism spectrum disorders. Annual Review of Public Health. 2017;**38**:81-102

[10] Bailey A, Le Couteur A, Gottesman I, Bolton P, Simonoff E, Yuzda E, et al. Autism as a strongly genetic disorder: Evidence from a British twin study. Psychological Medicine. 1995;**25**(1):63-77

[11] Yoon SH, Choi J, Lee WJ, Do JT. Genetic and epigenetic etiology underlying autism spectrum disorder. Journal of Clinical Medicine. 2020;**9**(4):966

[12] Chawner SJRA, Doherty JL, Anney RJL, et al. A genetics-first approach to dissecting the heterogeneity of autism: Phenotypic comparison of autism risk copy number variants. American Journal of Psychiatry. 2021;**178**(1):77

[13] Douard E, Zeribi A, Schramm C, Tamer P, Loum MA, Nowak S, et al. Effect sizes of deletions and duplications on autism risk across the genome. The American Journal of Psychiatry. 2021;**178**(1):87-98

[14] Durkin MS, Maenner MJ, Newschaffer CJ, Lee LC, Cunniff CM, Daniels JL, et al. Advanced parental age and the risk of autism spectrum disorder. American Journal of Epidemiology. 2008;**168**(11):1268-1276

[15] Atladóttir HO, Thorsen P, Østergaard L, Schendel DE, Lemcke S, Abdallah M, et al. Maternal infection requiring hospitalization during pregnancy and autism spectrum disorders. Journal of Autism

and Developmental Disorders. 2010;**40**(12):1423-1430

[16] Croen LA, Najjar DV, Fireman B, Grether JK. Maternal and paternal age and risk of autism spectrum disorders. Archives of Pediatrics & Adolescent Medicine. 2007;**161**(4):334-340

[17] Sandin S, Hultman CM, Kolevzon A, Gross R, MacCabe JH, Reichenberg A. Advancing maternal age is associated with increasing risk for autism: A review and meta-analysis. Journal of American Academy Child Adolescence Psychiatry. 2012;**51**(5):477-486

[18] Allen L, Leon-Attia O, Shaham M, Shefer S, Gabis LV. Autism risk linked to prematurity is more accentuated in girls. PLoS One. 2020;**15**(8):e0236994

[19] Doi M, Usui N, Shimada S. Prenatal environment and neurodevelopmental disorders. Frontiers in Endocrinology. 2022;**12**:860110

[20] Estes ML, McAllister AK. Maternal immune activation: Implications for neuropsychiatric disorders. Science. 2016;**353**(6301):772-777

[21] Meyer U. Neurodevelopmental resilience and susceptibility to maternal immune activation. Trends in Neurosciences. 2019;**42**(11):793-806

[22] Knuesel I, Chicha L, Britschgi M, Schobel SA, Bodmer M, Hellings JA, et al. Maternal immune activation and abnormal brain development across CNS disorders. Nature Reviews. Neurology. 2014;**10**(11):643-660

[23] Massarali A, Adhya D, Srivastava DP, Baron-Cohen S, Kotter MR. Virus-induced maternal immune activation as an environmental factor in the etiology of autism and schizophrenia. Frontiers in Neuroscience. 2022;**16**:834058

[24] Choi GB, Yim YS, Wong H, Kim S, Kim H, Kim SV, et al. The maternal interleukin-17a pathway in mice promotes autism-like phenotypes in offspring. Science. 2016;**351**(6276):933-939

[25] Shin Yim Y, Park A, Berrios J, Lafourcade M, Pascual LM, Soares N, et al. Reversing behavioural abnormalities in mice exposed to maternal inflammation. Nature. 2017;**549**(7673):482-487

[26] Pendyala G, Chou S, Jung Y, Coiro P, Spartz E, Padmashri R, et al. Maternal immune activation causes behavioral impairments and altered cerebellar cytokine and synaptic protein expression. Neuropsychopharmacology. 2017;**42**(7):1435-1446

[27] Jain S, Baer RJ, McCulloch CE, Rogers E, Rand L, Jelliffe-Pawlowski L, et al. Association of maternal immune activation during pregnancy and neurologic outcomes in offspring. Journal of Pediatrics. 2021;**238**:87-93

[28] Goldberg AD, Allis CD, Bernstein E. Epigenetics: A landscape takes shape. Cell. 2007;**128**(4):635-638

[29] LaSalle JM, Powell WT, Yasui DH. Epigenetic layers and players underlying neurodevelopment. Trends in Neurosciences. 2013;**36**(8):460-470

[30] Toraño EG, García MG, Fernández-Morera JL, Niño-García P, Fernández AF. The impact of external factors on the epigenome: In utero and over lifetime. BioMed Research International. 2016;**2016**:2568635

[31] Mcclintock B. The origin and behavior of mutable loci in maize. Proceedings of the National Academy of Sciences of the United States of America. 1950;**36**(6):344-355

[32] Lander ES, Linton LM, Birren B, Nusbaum C, Zody MC, et al. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature. 2001;**409**(6822):860-921

[33] Lanciano S, Cristofari G. Measuring and interpreting transposable element expression. Nature Reviews. Genetics. 2020;**21**(12):721-736

[34] Deininger PL, Moran JV, Batzer MA, Kazazian HH Jr. Mobile elements and mammalian genome evolution. Current Opinion in Genetics & Development. 2003;**13**(6):651-658

[35] Medstrand P, van de Lagemaat LN, Dunn CA, Landry JR, Svenback D, Mager DL. Impact of transposable elements on the evolution of mammalian gene regulation. Cytogenetic and Genome Research. 2005;**110**(1-4):342-352

[36] Bannert N, Kurth R. The evolutionary dynamics of human endogenous retroviral families. Annual Review of Genomics and Human Genetics. 2006;**7**:149-173

[37] Coffin JM, Hughes SH, Varmus HE. The interactions of retroviruses and their hosts. In: Coffin JM, Hughes SH, Varmus HE, editors. Retroviruses. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press; 1997. pp. 335-341

[38] Löwer R, Löwer J, Kurth R. The viruses in all of us: Characteristics and biological significance of human endogenous retrovirus sequences. Proceedings of the National Academy of Sciences of the United States of America. 1996;**93**(11):5177-5184

[39] Bannert N, Kurth R. Retroelements and the human genome: New perspectives on an old relation. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(2):14572-14579

[40] Weiss RA. Human endogenous retroviruses: Friend or foe? APMIS. 2016;**124**(1-2):4-10

[41] Greenwood AD, Ishida Y, O'Brien SP, Roca AL, Eiden MV. Transmission, evolution, and endogenization: Lessons learned from recent retroviral invasions. Microbiology and Molecular Biology Reviews. 2017;**82**(1):e00044-e00017

[42] Grandi N, Tramontano E. Human endogenous retroviruses are ancient acquired elements still shaping innate immune responses. Frontiers in Immunology. 2018;**9**:2039

[43] Blond JL, Lavillette D, Cheynet V, Bouton O, Oriol G, Chapel-Fernandes S, et al. An envelope glycoprotein of the human endogenous retrovirus HERV-W is expressed in the human placenta and fuses cells expressing the type D mammalian retrovirus receptor. Journal of Virology. 2000;**74**(7):3321-3329

[44] Cui L, Wang H, Lu X, Wang R, Zheng R, Li Y, et al. Effects of individually silenced N-glycosylation sites and non-synonymous single-nucleotide polymorphisms on the fusogenic function of human syncytin-2. Cell Adhesion & Migration. 2016;**10**(1-2):39-55

[45] Huang Q, Li J, Wang F, Oliver MT, Tipton T, Gao Y, et al. Syncytin-1 modulates placental trophoblast cell proliferation by promoting G1/S transition. Cellular Signalling. 2013;**25**(4):1027-1035

[46] Huang Q, Chen H, Wang F, Brost BC, Li J, Gao Y, et al. Reduced syncytin-1 expression in choriocarcinoma BeWo

cells activates the calpain1-AIF-mediated apoptosis, implication for preeclampsia. Cellular and Molecular Life Sciences. 2014;**71**(16):3151-3164

[47] Marin M, Lavillette D, Kelly SM, Kabat D. N-linked glycosylation and sequence changes in a critical negative control region of the ASCT1 and ASCT2 neutral amino acid transporters determine their retroviral receptor functions. Journal of Virology. 2003;**77**(5):2936-2945

[48] Cheynet V, Oriol G, Mallet F. Identification of the hASCT2-binding domain of the Env ERVWE1/syncytin-1 fusogenic glycoprotein. Retrovirology. 2006;**3**:41

[49] Lokossou AG, Toudic C, Nguyen PT, Elisseeff X, Vargas A, Rassart É, et al. Endogenous retrovirus-encoded Syncytin-2 contributes to exosome-mediated immunosuppression of T cells. Biology of Reproduction. 2020;**102**(1):185-198

[50] Pastuzyn ED, Day CE, Kearns RB, Kyrke-Smith M, Taibi AV, McCormick J, et al. The neuronal gene arc encodes a repurposed retrotransposon gag protein that mediates intercellular RNA transfer. Cell. 2018;**172**(1-2):275-288

[51] Jern P, Coffin JM. Effects of retroviruses on host genome function. Annual Review of Genetics. 2008;**42**:709-732

[52] Chuong EB, Elde NC, Feschotte C. Regulatory activities of transposable elements: From conflicts to benefits. Nature Reviews. Genetics. 2017;**18**(2):71-86

[53] Ehlhardt S, Seifert M, Schneider J, Ojak A, Zang KD, Mehraein Y. Human endogenous retrovirus HERV-K(HML-2) Rec expression and transcriptional activities in normal and rheumatoid arthritis synovia. The Journal of Rheumatology. 2006;**33**(1):16-23

[54] Schmitt K, Heyne K, Roemer K, Meese E, Mayer J. HERV-K(HML-2) rec and np9 transcripts not restricted to disease but present in many normal human tissues. Mobile DNA. 2015;**6**:4

[55] Balestrieri E, Pica F, Matteucci C, Zenobi R, Sorrentino R, Argaw-Denboba A, et al. Transcriptional activity of human endogenous retroviruses in human peripheral blood mononuclear cells. BioMed Research International. 2015;**2015**:164529

[56] Buzdin A, Kovalskaya-Alexandrova E, Gogvadze E, Sverdlov E. At least 50% of human-specific HERV-K (HML-2) long terminal repeats serve in vivo as active promoters for host nonrepetitive DNA transcription. Journal of Virology. 2006;**80**(21):10752-10762

[57] Wang J, Xie G, Singh M, Ghanbarian AT, Raskó T, Szvetnik A, et al. Primate-specific endogenous retrovirus-driven transcription defines naive-like stem cells. Nature. 2014;**516**(7531):405-409

[58] Hughes JF, Coffin JM. Human endogenous retrovirus K solo-LTR formation and insertional polymorphisms: Implications for human and viral evolution. Proceedings of the National Academy of Sciences of the United States of America. 2004;**101**(6):1668-1672

[59] Ting CN, Rosenberg MP, Snow CM, Samuelson LC, Meisler MH. Endogenous retroviral sequences are required for tissue-specific expression of a human salivary amylase gene. Genes & Development. 1992;**6**(8):1457-1465

[60] Bièche I, Laurent A, Laurendeau I, Duret L, Giovangrandi Y, Frendo JL, et al.

Placenta-specific INSL4 expression is mediated by a human endogenous retrovirus element. Biology of Reproduction. 2003;**68**(4):1422-1429

[61] Dunn CA, Medstrand P, Mager DL. An endogenous retroviral long terminal repeat is the dominant promoter for human beta1,3-galactosyltransferase 5 in the colon. Proceedings of the National Academy of Sciences of the United States of America. 2003;**100**(22):12841-12846

[62] Grow EJ, Flynn RA, Chavez SL, Bayless NL, Wossidlo M, Wesche DJ, et al. Intrinsic retroviral reactivation in human preimplantation embryos and pluripotent cells. Nature. 2015;**522**(7555):221-225

[63] Glinsky GV. Transposable elements and DNA methylation create in embryonic stem cells human-specific regulatory sequences associated with distal enhancers and noncoding RNAs. Genome Biology and Evolution. 2015;**7**(6):1432-1454

[64] Gemmell P, Hein J, Katzourakis A. Phylogenetic analysis reveals that ERVs "Die Young" but HERV-H is unusually conserved. PLoS Computational Biology. 2016;**12**(6):e1004964

[65] Heidmann O, Béguin A, Paternina J, Berthier R, Deloger M, Bawa O, et al. HEMO, an ancestral endogenous retroviral envelope protein shed in the blood of pregnant women and expressed in pluripotent stem cells and tumors. Proceedings of the National Academy of Sciences of the United States of America. 2017;**114**(32):E6642-E6651

[66] Wang T, Medynets M, Johnson KR, Doucet-O'Hare TT, DiSanza B, Li W, et al. Regulation of stem cell function and neuronal differentiation by HERV-K via mTOR pathway. Proceedings of the National Academy of Sciences of the United States of America. 2020;**117**(30):17842-17853

[67] Göke J, Ng HH. CTRL+INSERT: Retrotransposons and their contribution to regulation and innovation of the transcriptome. EMBO Reports. 2016;**17**(8):1131-1144

[68] Matteucci C, Balestrieri E, Argaw-Denboba A, Sinibaldi-Vallebona P. Human endogenous retroviruses role in cancer cell stemness. Seminars in Cancer Biology. 2018;**53**:17-30

[69] Perron H, Lang A. The human endogenous retrovirus link between genes and environment in multiple sclerosis and in multifactorial diseases associating neuroinflammation. Clinical Reviews in Allergy and Immunology. 2010;**39**(1):51-61

[70] Schulz WA, Steinhoff C, Florl AR. Methylation of endogenous human retroelements in health and disease. Current Topics in Microbiology and Immunology. 2006;**310**:211-250

[71] Turelli P, Castro-Diaz N, Marzetta F, Kapopoulou A, Raclot C, Duc J, et al. Interplay of TRIM28 and DNA methylation in controlling human endogenous retroelements. Genome Research. 2014;**24**(8):1260-1270

[72] Li F, Sabunciyan S, Yolken RH, Lee D, Kim S, Karlsson H. Transcription of human endogenous retroviruses in human brain by RNA-seq analysis. PLoS One. 2019;**14**(1):e0207353

[73] Göke J, Lu X, Chan YS, Ng HH, Ly LH, Sachs F, et al. Dynamic transcription of distinct classes of endogenous retroviral elements marks specific populations of early human embryonic cells. Cell Stem Cell. 2015;**16**(2):135-141

[74] Fu B, Ma H, Liu D. Endogenous retroviruses function as gene expression regulatory elements during mammalian pre-implantation embryo development. International Journal of Molecular Sciences. 2019;**20**(3):790

[75] Perron H, Dougier-Reynaud HL, Lomparski C, Popa I, Firouzi R, Bertrand JB, et al. Human endogenous retrovirus protein activates innate immunity and promotes experimental allergic encephalomyelitis in mice. PLoS One. 2013;**8**(12):e80128

[76] Zhang M, Liang JQ, Zheng S. Expressional activation and functional roles of human endogenous retroviruses in cancers. Reviews in Medical Virology. 2019;**29**(2):e2025

[77] Chen J, Foroozesh M, Qin Z. Transactivation of human endogenous retroviruses by tumor viruses and their functions in virus-associated malignancies. Oncogene. 2019;**8**(1):6

[78] Ruprecht K, Obojes K, Wengel V, Gronen F, Kim KS, Perron H, et al. Regulation of human endogenous retrovirus W protein expression by herpes simplex virus type 1: Implications for multiple sclerosis. Journal of Neurovirology. 2006;**12**(1):65-71

[79] Leung A, Trac C, Kato H, Costello KR, Chen Z, Natarajan R, et al. LTRs activated by Epstein-Barr virus-induced transformation of B cells alter the transcriptome. Genome Research. 2018;**28**(12):1791-1798

[80] Charvet B, Reynaud JM, Gourru-Lesimple G, Perron H, Marche PN, Horvat B. Induction of proinflammatory multiple sclerosis-associated retrovirus envelope protein by human herpesvirus-6A and CD46 receptor engagement. Frontiers in Immunology. 2018;**9**:2803

[81] Young GR, Terry SN, Manganaro L, Cuesta-Dominguez A, Deikus G, Bernal-Rubio D, et al. HIV-1 infection of primary CD4+ T cells regulates the expression of specific human endogenous retrovirus HERV-K (HML-2) elements. Journal of Virology. 2017;**92**(1):e01507-e01517

[82] Li F, Nellåker C, Sabunciyan S, Yolken RH, Jones-Brando L, Johansson AS, et al. Transcriptional derepression of the ERVWE1 locus following influenza A virus infection. Journal of Virology. 2014;**88**(8):4328-4337

[83] Balestrieri E, Minutolo A, Petrone V, Fanelli M, Iannetta M, Malagnino V, et al. Evidence of the pathogenic HERV-W envelope expression in T lymphocytes in association with the respiratory outcome of COVID-19 patients. eBioMedicine. 2021;**66**:103341

[84] Johnston JB, Silva C, Holden J, Warren KG, Clark AW, Power C. Monocyte activation and differentiation augment human endogenous retrovirus expression: Implications for inflammatory brain diseases. Annals of Neurology. 2001;**50**(4):434-442

[85] Mommert M, Tabone O, Oriol G, Cerrato E, Guichard A, Naville M, et al. LTR-retrotransposon transcriptome modulation in response to endotoxin-induced stress in PBMCs. BMC Genomics. 2018;**19**(1):522

[86] Cipriani C, Giudice M, Petrone V, Fanelli M, et al. Modulation of human endogenous retroviruses and cytokines expression in peripheral blood mononuclear cells from autistic children and their parents. Retrovirology. 2022. DOI: 10.1186/s12977-022-00603-6

[87] Ito J, Sugimoto R, Nakaoka H, Yamada S, Kimura T, Hayano T, et al. Systematic identification and characterization of

regulatory elements derived from human endogenous retroviruses. PLoS Genetics. 2017;**13**(7):e1006883

[88] Nguyen TD, Davis J, Eugenio RA, Liu Y. Female sex hormones activate human endogenous retrovirus Type K through the OCT4 transcription factor in T47D breast cancer cells. AIDS Research and Human Retroviruses. 2019;**35**(3):348-356

[89] Mueller O, Moore DW, Giovannucci J, Etter AR, Peterson EM, Mudge A, et al. Expression of human endogenous retroviruses in peripheral leukocytes during the menstrual cycle suggests coordinated hormonal regulation. AIDS Research and Human Retroviruses. 2018;**34**(11):909-911

[90] Diem O, Schäffner M, Seifarth W, Leib-Mösch C. Influence of antipsychotic drugs on human endogenous retrovirus (HERV) transcription in brain cells. PLoS One. 2012;**7**(1):e30054

[91] Greenig M. HERVs, immunity, and autoimmunity: Understanding the connection. PeerJ. 2019;**7**:e6711

[92] Gorbunova V, Seluanov A, Mita P, McKerrow W, Fenyö D, Boeke JD, et al. The role of retrotransposable elements in ageing and age-associated diseases. Nature. 2021;**596**(7870):43-53

[93] Levet S, Charvet B, Bertin A, Deschaumes A, Perron H, Hober D. Human endogenous retroviruses and type 1 diabetes. Current Diabetes Reports. 2019;**19**(12):141

[94] Küry P, Nath A, Créange A, Dolei A, Marche P, Gold J, et al. Human endogenous retroviruses in neurological diseases. Trends in Molecular Medicine. 2018;**24**(4):379-394

[95] Römer C. Viruses and endogenous retroviruses as roots for neuroinflammation and neurodegenerative diseases. Frontiers in Neuroscience. 2021;**15**:648629

[96] van der Kuyl AC. HIV infection and HERV expression: A review. Retrovirology. 2012;**9**:6

[97] Wang M, Wang L, Liu H, Chen J, Liu D. Transcriptome analyses implicate endogenous retroviruses involved in the host antiviral immune system through the interferon pathway. Virologica Sinica. 2021;**36**(6):1315-1326

[98] Manghera M, Douville RN. Endogenous retrovirus-K promoter: A landing strip for inflammatory transcription factors? Retrovirology. 2013;**10**:16

[99] Hurst TP, Magiorkinis G. Activation of the innate immune response by endogenous retroviruses. The Journal of General Virology. 2015;**96**(Pt 6):1207-1218

[100] Louveau A, Harris TH, Kipnis J. Revisiting the mechanisms of CNS immune privilege. Trends in Immunology. 2015;**36**(10):569-577

[101] Chuong EB, Elde NC, Feschotte C. Regulatory evolution of innate immunity through co-option of endogenous retroviruses. Science. 2016;**351**(6277):1083-1087

[102] Baillie JK, Barnett MW, Upton KR, Gerhardt DJ, Richmond TA, De Sapio F, et al. Somatic retrotransposition alters the genetic landscape of the human brain. Nature. 2011;**479**(7374):534-537

[103] Coufal NG, Garcia-Perez JL, Peng GE, Yeo GW, Mu Y, Lovci MT, et al. L1 retrotransposition in human neural progenitor cells. Nature. 2009;**460**(7259):1127-1131

[104] Mehler MF, Mattick JS. Noncoding RNAs and RNA editing in brain development, functional diversification, and neurological disease. Physiological Reviews. 2007;**87**(3):799-823

[105] Perron H, Geny C, Laurent A, Mouriquand C, Pellat J, Perret J, et al. Leptomeningeal cell line from multiple sclerosis with reverse transcriptase activity and viral particles. Research in Virology. 1989;**140**(6):551-561

[106] Sotgiu S, Mameli G, Serra C, Zarbo IR, Arru G, Dolei A. Multiple sclerosis-associated retrovirus and progressive disability of multiple sclerosis. Multiple Sclerosis. 2010;**16**(10):1248-1251

[107] Perron H, Garson JA, Bedin F, Beseme F, Paranhos-Baccala G, Komurian-Pradel F, et al. Molecular identification of a novel retrovirus repeatedly isolated from patients with multiple sclerosis. The Collaborative Research Group on Multiple Sclerosis. Proceedings of the National Academy of Sciences of the United States of America. 1997;**94**(14):7583-7588

[108] Garson JA, Tuke PW, Giraud P, Paranhos-Baccala G, Perron H. Detection of virion-associated MSRV-RNA in serum of patients with multiple sclerosis. Lancet. 1998;**351**(9095):33

[109] Perron H, Germi R, Bernard C, et al. Human endogenous retrovirus type W envelope expression in blood and brain cells provides new insights into multiple sclerosis disease. Multi Sclerosis. 2012;**18**(12):1721

[110] Viola MV, Frazier M, White L, Brody J, Spiegelman S. RNA-instructed DNA polymerase activity in a cytoplasmic particulate fraction in brains from Guamanian patients. The Journal of Experimental Medicine. 1975;**142**(2):483-494

[111] Li Y, Chen Y, Zhang N, Fan D. Human endogenous retrovirus K (HERV-K) env in neuronal extracellular vesicles: A new biomarker of motor neuron disease. Amyotroph Lateral Scler Frontotemporal Degener. 2022;**23**(1-2):100-107

[112] Alfahad T, Nath A. Retroviruses and amyotrophic lateral sclerosis. Antiviral Research. 2013;**99**(2):180-187

[113] Douville R, Liu J, Rothstein J, Nath A. Identification of active loci of a human endogenous retrovirus in neurons of patients with amyotrophic lateral sclerosis. Annals of Neurology. 2011;**69**(1):141-151

[114] Li W, Lee MH, Henderson L, Tyagi R, Bachani M, Steiner J, et al. Human endogenous retrovirus-K contributes to motor neuron disease. Science Translational Medicine. 2015;**7**(307):307ra153

[115] Steiner JP, Bachani M, Malik N, DeMarino C, Li W, Sampson K, et al. Human endogenous retrovirus K envelope in spinal fluid of amyotrophic lateral sclerosis is toxic. Annals of Neurology. 2022;**92**(4):545-561

[116] Cannon TD, van Erp TG, Bearden CE, Loewy R, Thompson P, Toga AW, et al. Early and late neurodevelopmental influences in the prodrome to schizophrenia: Contributions of genes, environment, and their interactions. Schizophrenia Bulletin. 2003;**29**(4):653-669

[117] Brown AS, Meyer U. Maternal immune activation and neuropsychiatric illness: A translational research perspective. The American Journal of Psychiatry. 2018;**175**(11):1073-1083

[118] Perron H, Hamdani N, Faucard R, Lajnef M, Jamain S, Daban-Huard C, et al. Molecular characteristics of Human Endogenous Retrovirus type-W in schizophrenia and bipolar disorder. Translational Psychiatry. 2012;**2**(12):e201

[119] Mak M, Samochowiec J, Frydecka D, Pełka-Wysiecka J, Szmida E, Karpiński P, et al. First-episode schizophrenia is associated with a reduction of HERV-K methylation in peripheral blood. Psychiatry Research. 2019;**271**:459-463

[120] Johansson EM, Bouchet D, Tamouza R, Ellul P, Morr AS, Avignone E, et al. Human endogenous retroviral protein triggers deficit in glutamate synapse maturation and behaviors associated with psychosis. Science Advances. 2020;**6**:eabc0708

[121] Li W, Lin L, Malhotra R, Yang L, Acharya R, Poss M. A computational framework to assess genome-wide distribution of polymorphic human endogenous retrovirus-K In human populations. PLoS Computational Biology. 2019;**15**(3):e1006564

[122] Tamouza R, Meyer U, Foiselle M, Richard JR, Wu CL, Boukouaci W, et al. Identification of inflammatory subgroups of schizophrenia and bipolar disorder patients with HERV-W ENV antigenemia by unsupervised cluster analysis. Translational Psychiatry. 2021;**11**(1):377

[123] Harrison PJ, Geddes JR, Tunbridge EM. The emerging neurobiology of bipolar disorder. Focus (Am Psychiatr Publ). 2019;**17**(3):284-293

[124] Balestrieri E, Arpino C, Matteucci C, Sorrentino R, Pica F, Alessandrelli R, et al. HERVs expression in autism spectrum disorders. PLoS One. 2012;**7**(11):e48831

[125] Balestrieri E, Cipriani C, Matteucci C, Capodicasa N, Pilika A, Korca I, et al. Transcriptional activity of human endogenous retrovirus in Albanian children with autism spectrum disorders. The New Microbiologica. 2016;**39**(3):228-231

[126] Cross-Disorder Group of the Psychiatric Genomics Consortium. Cross-disorder group of the psychiatric genomics consortium. genomic relationships, novel loci, and pleiotropic mechanisms across eight psychiatric disorders. Cell. 2019;**179**(7):1469-1482

[127] Mellahn OJ, Knott R, Tiego J, Kallady K, Williams K, Bellgrove MA, et al. Understanding the diversity of pharmacotherapeutic management of ADHD with co-occurring autism: An Australian Cross-Sectional Survey. Frontiers in Psychiatry. 2022;**13**:914668

[128] Balestrieri E, Pitzianti M, Matteucci C, D'Agati E, Sorrentino R, Baratta A, et al. Human endogenous retroviruses and ADHD. The World Journal of Biological Psychiatry. 2014;**15**(6):499-504

[129] Cipriani C, Pitzianti MB, Matteucci C, D'Agati E, Miele MT, Rapaccini V, et al. The decrease in human endogenous retrovirus-H activity runs in parallel with improvement in ADHD symptoms in patients undergoing methylphenidate therapy. International Journal of Molecular Sciences. 2018;**19**(11):3286

[130] Balestrieri E, Cipriani C, Matteucci C, Benvenuto A, Coniglio A, Argaw-Denboba A, et al. Children with autism spectrum disorder and their mothers share abnormal expression of selected endogenous retroviruses families and cytokines. Frontiers in Immunology. 2019;**10**:2244

[131] Tovo PA, Davico C, Marcotulli D, Vitiello B, Daprà V, Calvi C, et al. Enhanced expression of human endogenous retroviruses, TRIM28 and SETDB1 in autism spectrum disorder. International Journal of Molecular Sciences. 2022;**23**(11):5964

[132] Ornoy A, Weinstein-Fudim L, Ergaz Z. Prevention or amelioration of autism-like symptoms in animal models: Will it bring us closer to treating human ASD? International Journal of Molecular Sciences. 2019;**20**(5):1074

[133] Cipriani C, Ricceri L, Matteucci C, De Felice A, Tartaglione AM, Argaw-Denboba A, et al. High expression of Endogenous Retroviruses from intrauterine life to adulthood in two mouse models of Autism Spectrum Disorders. Scientific Reports. 2018;**8**(1):629

[134] Tartaglione AM, Cipriani C, Chiarotti F, Perrone B, Balestrieri E, Matteucci C, et al. Early behavioral alterations and increased expression of endogenous retroviruses are inherited across generations in mice prenatally exposed to valproic acid. Molecular Neurobiology. 2019;**56**(5): 3736-3750

[135] Balestrieri E, Matteucci C, Cipriani C, Grelli S, Ricceri L, Calamandrei G, et al. Endogenous retroviruses activity as a molecular signature of neurodevelopmental disorders. International Journal of Molecular Sciences. 2019;**20**(23):6050

[136] Cipriani C, Tartaglione AM, Giudice M, D'Avorio E, Petrone V, Toschi N, et al. Differential expression of endogenous retroviruses and inflammatory mediators in female and male offspring in a mouse model of maternal immune activation. International Journal of Molecular Sciences. 2022;**23**:13930

[137] Curtin F, Perron H, Kromminga A, Porchet H, Lang AB. Preclinical and early clinical development of GNbAC1, a humanized IgG4 monoclonal antibody targeting endogenous retroviral MSRV-Env protein. MAbs. 2015;**7**(1):265-275

[138] Curtin F, Vidal V, Bernard C, Kromminga A, Lang AB, Porchet H. Serum pharmacokinetics and cerebrospinal fluid concentration analysis of the new IgG4 monoclonal antibody GNbAC1 to treat multiple sclerosis: A Phase 1 study. MAbs. 2016;**8**(5):854-860

[139] Curtin F, Bernard C, Levet S, Perron H, Porchet H, Médina J, et al. Simpson R; RAINBOW-T1D investigators. A new therapeutic approach for type 1 diabetes: Rationale for GNbAC1, an anti-HERV-W-Env monoclonal antibody. Diabetes, Obesity & Metabolism. 2018;**20**(9):2075-2084

[140] Curtin F, Champion B, Davoren P, Duke S, Ekinci EI, Gilfillan C, et al. A safety and pharmacodynamics study of temelimab, an antipathogenic human endogenous retrovirus type W envelope monoclonal antibody, in patients with type 1 diabetes. Diabetes, Obesity & Metabolism. 2020;**22**(7):1111-1121

[141] Morandi E, Tanasescu R, Tarlinton RE, Constantin-Teodosiu D, Gran B. Do antiretroviral drugs protect from multiple sclerosis by inhibiting expression of MS-associated retrovirus? Frontiers in Immunology. 2019;**9**:3092

[142] Gold J, Goldacre R, Maruszak H, Giovannoni G, Yeates D, Goldacre M. HIV and lower risk of multiple sclerosis: Beginning to unravel a mystery using a record-linked database study. Journal of Neurology, Neurosurgery, and Psychiatry. 2015;**86**(1):9-12

[143] Gold J, Rowe DB, Kiernan MC, Vucic S, Mathers S, van Eijk RPA, et al. Safety and tolerability of Triumeq in amyotrophic lateral sclerosis: The Lighthouse trial. Amyotrophic Lateral Sclerosis Frontotemporal Degeneration. 2019;**20**(7-8):595-604

[144] Garcia-Montojo M, Fathi S, Norato G, Smith BR, Rowe DB, Kiernan MC, et al. Inhibition of HERV-K (HML-2) in amyotrophic lateral sclerosis patients on antiretroviral therapy. Journal of the Neurological Sciences. 2021;**423**:117358