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Reduced expression of human endogenous retrovirus (HERV)-W GAG protein in the cingulate gyrus and hippocampus in schizophrenia, bipolar disorder, and depression

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Summary The human endogenous retrovirus (HERV)-W multicopy family was identified in human DNA from the previously characterized multiple sclerosis associated retroviral element (MSRV). Upregulation of the HERV-W POL has been reported in cerebrospinal fluid of patients with schizophrenia. The expression of capsid (GAG) protein of HERV-W was studied by immunohistochemistry and western blotting in postmortem brain tissue of the anterior cingulate cortex and hippocampal formation of normal controls and of patients with schizophrenia, bipolar disorder and major depression. A physiological expression of GAG protein was detected in neurons as well as astroglial cells in normal brain both in the anterior cingulate cortex and in the hippocampal formation. There was a statistically significant reduction of this expression in neurons and astroglial cells in brains from individuals with schizophrenia, major depression, and bipolar disorder. The results from the present study confirm that GAG protein encoded by the HERV-W multicopy gene family is expressed in cells of the central nervous system under normal conditions. Our findings of a cell type-, brain region- and disease-specific reduced expression in schizophrenia, major depression, and bipolar disorder are compatible with a pathophysiological role of HERVs in human brain disorders. The causes and biological consequences of this differential regulation will be the subject of further investigations.

Keywords: Human endogenous retrovirus-W (HERV-W), neuron, glia, schizophrenia, bipolar disorder, major depression

Introduction

Schizophrenia, bipolar disorder, and major depression are complex multigenetic, multifactorial diseases, leading to disruptive psychopathologies involving thought, perception, emotion, movement, behavior, cognition, and mood. The possible pathogenetic mechanisms at work are still not clearly understood. The morphological changes seen in the brains of patients constitute the platform upon which various pathogenetic factors may act at different points in time. The morphological changes encompass, among others, reductions in the volume of different brain regions, focally disturbed cytoarchitecture of various cortical areas, changes in synaptic numbers and proteins, loss of myelin proteins, and a not yet fully understood reaction pattern of astrocytes and microglia (for review see Harrison and Roberts, 2000; Soares and Gershon, 2000). Recent large-scale microarray studies indicate that a multitude of genes representing various pathways are up- or down-regulated in schizophrenia and bipolar disorder. The etiology of these mutlifaceted changes remains enigmatic.

Retroelements make up a large portion of the human genome. One class of retroelements, the human endogenous retroviruses (HERVs), is composed of remnants of exogenous retroviruses that have accessed the germ line at different times during the course of human evolution (Löwer et al., 1996). In human DNA, the HERV-W family is a multicopy gene family, most copies of which are truncated or lack open reading frames (ORF), with only a few retaining potential orfs for retroviral proteins. HERV-W proteins may be produced as complete or truncated proteins from various chromosomal copies in a cell-, tissue- or temporally-restricted manner (Löwer et al., 1993, 1996).

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Although, most proviruses underwent extensive deletions and mutations, HERV-derived transcripts and proteins are detected in healthy and diseased human tissues (Bannert and Kurth, 2004).

Both the physiological and pathological functions of HERVs remain enigmatic, and much more research has to be directed towards its elucidation. Potential roles of HERVs include the contribution to genomic plasticity through reverse transcription, the regulation of adjacent genes and the involvement in physiological and pathological processes relevant to autoimmunity and reproduction (Löwer et al., 1996; Taruscio and Mantovani, 1998; Obermayer-Straub and Manns, 2001). HERV-encoded ENV proteins may play a role in the formation of the syncytiotrophoblast layer of the placenta (Blond et al., 2000; Mi et al., 2000; Blaise et al., 2003). Another role of HERV-encoded ENV proteins might be their involvement in suppressing possible maternal immunological reactions against the fetus (Larsson and Anderson, 1998; Villareal, 1997; Wilkinson et al., 1994).

Endogenous retroviruses have been proposed as contributory mechanisms to the pathophysiology of complex human disorders. Previous studies on RNA associated with viral particles produced in choroid plexus or B-lymphocyte cultures from patients with multiple sclerosis (MS) evidenced sequences corresponding to overlapping regions of a retroviral genome (Komurian-Pradel et al., 1999; Perron et al., 1997), which was named MSRV (multiple sclerosis associated retrovirus element) and was found to have genetically homologous elements in human DNA defining a novel family, the human endogenous retrovirus type W (HERV-W) (Blond et al., 1999; Peron et al., 1997). A complete HERV-W provirus is present on chromosome 7, HERV-W7q (Perron et al., 2000), in a region associated with genetic susceptibility to MS (Charmley et al., 1991; Wei et al., 1995). Although HERV-W7q provirus is not fully functional, it encodes an envelope (ENV) protein that is strongly expressed in placenta (Blond et al., 1999), called ''syncytin'' (Mi et al., 2000), and involved in the physiological process of syncytiotrophoblast fusion (Blond et al., 2000). An association of virion-associated MSRV RNA with the occurrence and the prognosis of MS was recently reported (Dolei et al., 2002; Sotgiu et al., 2002). Differential MSRV/HERV-W RNA levels between MS and controls were also reported in lymphoïd cells (Nowak et al., 2003).

Recently, a differential RNA expression of various GAG, POL and ENV HERV-W copies from different chromosomes has been described in normal and tumour tissues (Yi et al., 2004), which suggests that several HERV-W genes in addition to syncytin may be transcribed in human cells.

Previous studies have shown evidence of endogenous retrovirus activity in the brain and CSF of schizophrenic patients (Yolken et al., 2000). Furthermore, an upregulation of HERV-W POL in the cerebrospinal fluid of patients with schizophrenia was reported (Karlsson et al., 2001). Therefore, the aim of the present study was to characterize the expression of HERV-W GAG protein in normal human brain and in schizophrenia, bipolar disorder, and major depression by using antibodies specific for GAG proteins encoded by the HERV-W retroviral family. The anterior cingulate gyrus and the hippocampus were selected for investigation, since they represent major relay stations in the pathophysiological circuitry of these disorders. The present study shows that HERV-W proteins are physiologically expressed in the human brain and that this expression is altered in schizophrenia, major depression, and bipolar disorder.

Methods

Material

In the present study, the anterior cingulate gyrus and the hippocampus were investigated in 15 brains of each group consisting of controls, patients with schizophrenia, bipolar disorder, and major depression. These brains are part of the Stanley Neuropathology Consortium Collection, details about which were reported elsewhere (Torrey et al., 2000).

Generation of anti-HERV-W antibodies

Details about the generation of the antibodies were published elsewhere (Perron et al., 2005).

Recombinant proteins for immunodepletion

His-tag containing recombinant HERV-W GAG CL2 (AF123881) protein were expressed in *E. coli* by Amplicon Express Inc. (Washington DC, USA).

In addition, the longest open reading frame of the HERV-W GAG gene (AF123881) was cloned into the BAC to BAC baculovirus expression system from Invitrogen creating a fusion protein containing an N-terminal histidine tag. The HERV-W GAG protein was then expressed in roller bottles by infecting HiFive cells purchased from Invitrogen. The expressed protein was purified from cell lysates via Ni-NTA resin using standard procedures. The purified GAG protein was used for the described antibody adsorption assays during immunohistochemistry.

Immunohistochemistry

Immunohistochemistry was performed on formalin-fixed and paraffin-embedded 5 µm-thick sections on Superfrost Plus slides (M6146-Plus, Allegiance, McGraw Park, IL, USA). Deparaffinized, rehydrated sections underwent antigen retrieval (DakoCytomation, Carpinteria, CA, USA, NO: S1700; equivalent to a 10 mmol/L citrate buffer, pH 6.0) for 20 min in a water bath at 95–100°C. All subsequent steps were carried out using the DAKO Autostainer Immunostaining System (DAKO S3400) and the EnVison^{TM} + kit (code K4011, DakoCytomation, Carpinteria, CA, USA). Endogenous peroxidase activity was blocked with 3% H_2O_2 for 5 min followed by protein block (DAKO code X0909) for 5 min.

Table 1. *P-values of the immunohistochemical staining for HERV-W GAG. P-values were adjusted for fixation, heavy alcohol abuse and heavy street drug abuse in case of HERV-W GAG*

Region	Cell type	IH-Variable	HERV-W GAG		
			Co/SZ	Co/BP	Co/DP
CA – Alveus	Glia	intensity	0.02	0.68	0.62
	Glia	density	0.02	0.96	0.87
CA – Stratum lacunosum	Glia	intensity	0.01	0.69	0.83
	Glia	compound	0.03	0.96	0.87
CA – Stratum lucidum	Axon	density	0.02	0.82	0.93
	Glia	intensity	0.000	0.87	0.13
	Glia	density	0.000	0.03	0.43
	Glia	compound	0.000	0.32	0.19
	Region-total	region	0.02	0.76	0.56
CA – Stratum moleculare	Glia	density	0.02	0.41	0.84
	Glia	compound	0.02	0.55	0.77
	Interneuron	intensity	0.01	0.99	0.78
	Interneuron	density	0.04	0.17	0.57
	Interneuron	compound	0.000	0.72	0.36
	Region-total	region	0.01	0.89	0.86
CA – Stratum oriens	Glia	intensity	0.01	0.91	0.64
	Glia	compound	0.04	0.91	0.66
	Region-total	region	0.05	0.6	0.93
CA – Stratum radiatum	Axon-Dendrite	intensity	0.05	0.65	0.02
	Axon-Dendrite	compound	0.15	0.17	0.03
	Glia	compound	0.73	0.21	0.04
	Region-total	region	0.23	0.15	0.01
CA1 – Pyramidal layer	Basal dendrite	density	0.03	0.04	0.08
	Basal dendrite	compound	0.03	0.93	0.58
	Interneuron	compound	0.04	0.59	0.22
CA2 – Pyramidal layer	Glia	intensity	0.03	0.24	0.93
	Glia	density	0.04	0.34	0.14
	Glia	compound	0.02	0.54	0.41
	Interneuron	intensity	0.42	0.21	0.03
	Interneuron	density	0.63	0.03	0.46
CA3 – Pyramidal layer	Glia	intensity	0.000	0.98	0.6
	Glia	density	0.000	0.96	0.38
	Glia	compound	0.02	0.57	0.54
CA4 – Pyramidal layer	Basal dendrite	density	0.000	0.48	0.79
	Glia	density	0.000	0.24	0.86
DG – Polymorphic layer	Glia	intensity	0.000	0.95	0.72
	Glia	density	0.000	0.17	0.56
	Glia	compound	0.000	0.75	0.53
	Region-total	region	0.03	0.42	0.34
DG – Stratum granulare	Axon	compound	0.04	0.08	0.65
	Basal dendrite	compound	0.05	0.05	0.66
	Glia	intensity	0.01	0.56	0.44
	Glia	density	0.01	0.48	0.16
	Glia	compound	0.01	0.49	0.39
	Neuron	density	0.02	0.91	0.59
	Region-total	region	0.03	0.12	0.52
DG – Stratum moleculare	Glia	density	0.000	0.16	0.98
	Glia	compound	0.02	0.83	0.4
	Interneuron	density	0.05	0.14	0.49
	Interneuron	compound	0.04	0.33	0.58
	Region-total	region	0.02	0.42	0.47
Cingulate gyrus – GM	Neuron	intensity	0.03	0.65	0.41
	Neuron	compound	0.03	1	0.16
	Glia	compound	0.24	0.61	0.02
	Glia	region	0.21	0.88	0.04

Co Controls, *SZ* schizophrenia, *BP* bipolar disorder, *DP* major depression, *CA* cornu ammonis, *DG* dentate gyrus, *GM* gray matter.

The primary monoclonal antibody directed against HERV-W GAG (3H1H6) was used at a concentration of 1:100 for 60 min, respectively. Sections were then incubated with the secondary anti-mouse antibody for 30 min. The reaction product was visualized using 3,3′diaminobenzidine chromogen (liquid DAB+, K3468, DakoCytomation, Carpinteria, CA, USA) for 5 min. Then, the sections were counterstained with Gill 2 hematoxylin (Richard–Allan Scientific, Kalamazoo, MI, USA).

As a negative control, the same immunohistochemical protocol was carried out, except that the primary antibody was omitted and replaced by normal mouse serum (code X0903, DakoCytomation, Carpinteria, CA, USA). Furthermore, the primary antibody for GAG was preadsorbed with the antigen expressed in HiFive cells (see above). Sections from placental tissue (normal placenta and choriocarcinoma), multiple sclerosis lesions as well as of various tumors [carcinomas of breast (invasive ductal), colon, kidney (clear cell), stomach, lung as well as one teratoma] were used as a positive controls.

Evaluation of the immunohistochemical stains

For the *anterior cingulate gyrus*, on each immunohistochemically-stained section, neurons, glial cells and nerve fibers were analyzed, separately, for the gray and white matter. The following *hippocampal regions* with their specific cell types/cell parts were investigated separately: (1) stratum oriens with its interneurons, axons and basal dendrites of stratum pyramidale neurons, and glia, (2) alveus with its axons (of hippocampal and subicular pyramidal neurons), and glia, (3) pyramidal layer of CA1 with its pyramidal neurons, their apical and basal dendrites, interneurons, and glia, (4) pyramidal layer of CA2 with its pyramidal neurons, their apical and basal dendrites, interneurons, and glia, (5) pyramidal layer of CA3 with its pyramidal neurons, their apical and basal dendrites, interneurons, and glia, (6) pyramidal layer of CA4 with its pyramidal neurons, their apical and basal dendrites, interneurons, and glia, (7) stratum radiatum with its axons and dendrites (from pyramidal neurons, Schaffer collaterals, fibers from septal nuclei and commissure), and glia, (8) stratum lacunosum with its axons and dendrites (from perforant fibers and Schaffer collaterals), and glia, (9) stratum moleculare of the cornu ammonis with its interneurons, axons and dendrites, and glia, (10) stratum moleculare of the dentate gyrus with its interneurons and glia, (11) stratum granulosum of the dentate gyrus with its neurons, their basal dendrites and axons, and glia, (12) polymorphic layer of the dentate gyrus with its interneurons, axons of granular neurons, and glia, and (13) stratum lucidum with its axons.

For each cell type or structure, the staining intensity was score-rated as follows: (0) no staining, (1) weak staining, (2) moderate staining, and (3) strong staining. The density (% of positive cells/structures relative to the density of the total population of that specific cell type/structure) of stained cells or structures was rated as follows: (0) no cell/structure stained, (1) low density (<25%), (2) moderate density (25–50%), and (3) high density (>50%). The histological analysis was performed by an experienced pathologist blind to the diagnosis. For each single analyzed component (i.e. neurons and glia), compound values were calculated by multiplying the staining intensity score with the density score. Finally, for the anterior cingulate, sums were calculated for the gray matter by adding the compound values of neurons, glia and nerve fibers, and for the white matter by adding the compound values of glia and nerve fibers. For each hippocampal region, sums were calculated by adding the compound values of each constituting structure/component.

Statistical analysis

Differences between the control and the diagnostic groups were calculated using the non-parametric Kolmogorov–Smirnov test for overall differences and Mann–Whitney-*U*-test for post-hoc testing between the various groups; the resulting *p*-values of the significant differences are given in Table 1.

Confounding variables were used to assess their influence on the dependent variables and included age, sex, side of the examined hemisphere, suicide status, smoking at time of death, postmortem interval, brain pH, cerebellar granular cell layer necrosis, rate of death, fixation time of brain tissue, lifetime antipsychotic intake (in fluphenazine mg. equivalents), data on intake of 1st and 2nd generation antipsychotic generic drugs, mood stabilizer generic drugs, lithium, antidepressant generic drugs, and anticholinergic generic drugs.

At first, we assessed if there was a significant difference for the confounding variables between the various diagnostic groups either by ANOVA or logistic regression. As a second step, to determine if the effect of a confounding variable could account for the differences we obtained in our immunohistochemistry, significant differences between disease groups and measured dependent variables as well as confounders and measured dependent variables were compared. In case that a confounding variable was significant in step one, it was checked if this variable had also a significant change in step two. Only confounding variables that significantly influenced all measured variables, were added to the ANOVA/regression model to remove the confounding effect on the disease/outcome relationship. For example: if fixation time was different between the studied groups, fixation time would only be added to the ANOVA/regression model, if we observed a significant effect on staining density and intensity of neurons, glial cells, and fibers. In other words, we did not include confounding variables in our ANOVA/regression model which didn't contribute to the observed differences. A critical value of $\alpha = 0.05$ was used for all of the analyses.

Serological assays

IgG class antibodies to *Toxoplasma gondii*, Herpes simplex virus 1 and 2 (HSV1, HSV2), and Cytomegalovirus (CMV) were measured by solid phase immunoassay as previously described (Dickerson et al., 2003). For each antibody, seropositivity and seronegativity were defined by comparison of reactivity with that of defined standard samples.

Western blotting

Lysates (20% homogenate) of brains of patients and controls were prepared in extraction buffer [1% (v/v) Triton X-100, 0.10% (w/v) SDS, 0.100 M NaCl, 50.0 mM Tris pH 8.0, 5.00 mM EDTA] electrophoresed and blotted using standard procedures. The blots were blocked overnight, then incubated with 1:1000 dilution of primary antibody for 1 hour at room temperature., and finally washed with TBS-T and incubated with anti-mouse IgG at 1:10,000 dilution. The signal was detected using the Amersham ECL reagents.

Results

In general, neurons (Fig. 1A, C, E, F) and glial cells (Fig. 1B, H) stained with the antibody. Compared to baculovirus-infected HiFive cells (Fig. 1G), positive control tissues from the placenta (Fig. 1K), multiple sclerosis (Fig. 1I, J), and tumors (choricarcinoma) (Fig. 1L), the expression of HERV-W GAG was weaker in the human brain of both controls and psychiatric patients. Based on morphological criteria, most of the HERV-W-GAG-immunopositive glial cells were identified as astrocytes.

The western blot showed for HERV-W GAG four bands observed between 45 and 30 kDa where the 45 kDa band provided the strongest signal (Fig. 1H).

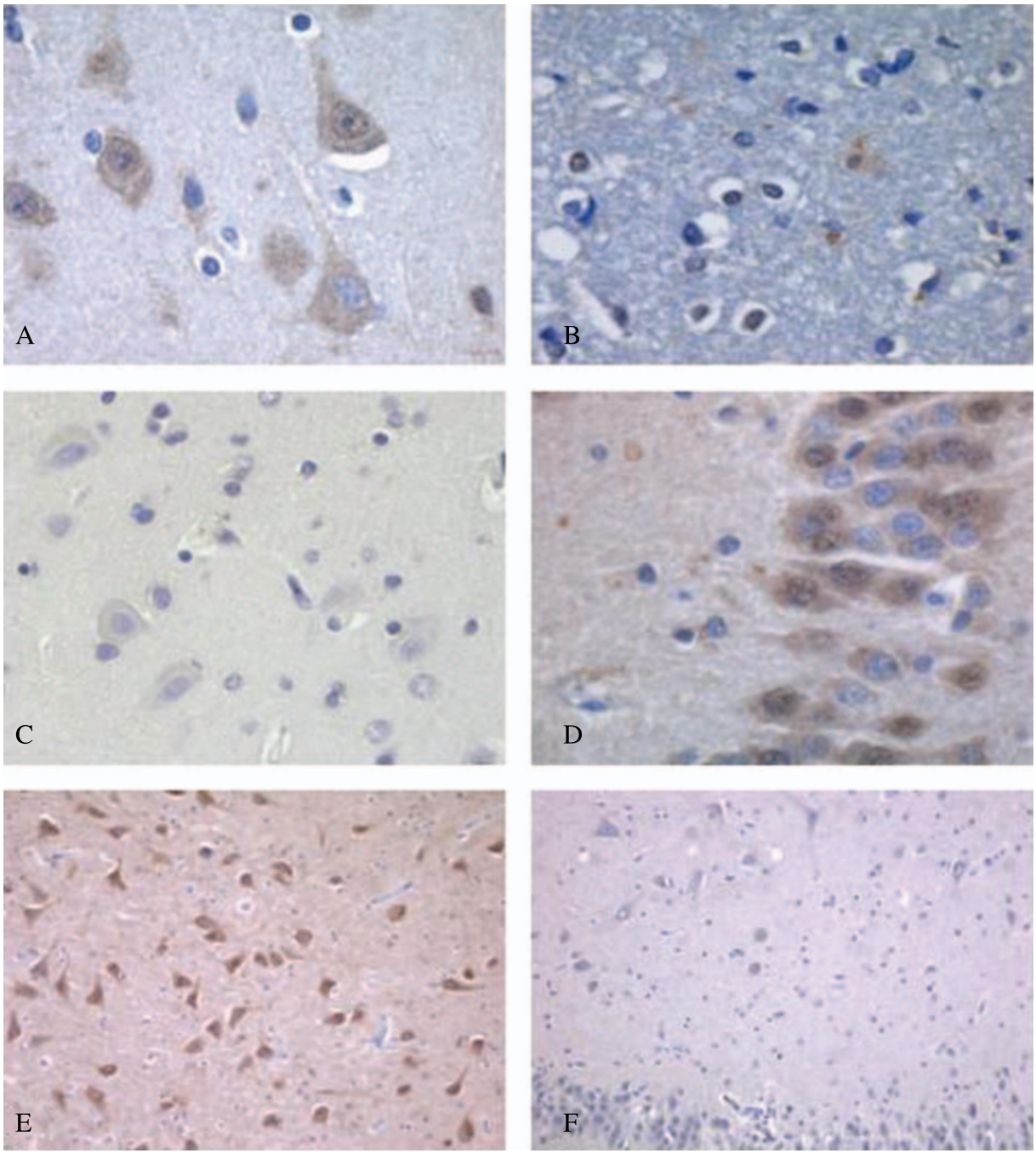

Fig. 1. Immunohistochemical demonstration of HERV-W GAG in various tissues. **A** Immunopositive neurons and astroglial cells (arrow) in the gray matter of the anterior cingulate cortex (original magnification: 40×). **B** Immunopositive astroglial cells (arrow) in the white matter of the anterior cingulate cortex (original magnification: 40×). **C** No immunreactivities are seen in the cells (neurons and glial cells) after immunabsorbance with the antigen produced by the HiFive cells infected with baculovirus expressing HERV-W GAG in the anterior cingulate cortex (original magnification: 40×). **D** Immunopositive interneuron in the stratum oriens of the hippocampus (original magnification: 40×). **E** Immunopositive neurons in the CA4 layer of the hippocampus in a normal control (original magnification: 10×). **F** Immunopositive neurons in the CA4 layer of the hippocampus in a patient with schizophrenia (original magnification: 10×). **G** HiFive cells infected with baculovirus expressing HERV-W GAG (original magnification: 40×). **H** Western blot for HERV-W GAG. **I** Case of multiple sclerosis with area of demyelination (delimited by arrows) with HERV-W GAG – immunopositive astroglial and microglial cells (original magnification: 4×). **J** Case of multiple sclerosis with area adjacent to a focus of demyelination with HERV-W GAG – immunopositive astroglial and microglial cells (arrows) (original magnification: 20×). **K** Normal term placenta with HERV-W GAG – immunopositive cells (original magnification: 20×). **L** Choriocarcinoma of the placenta HERV-W GAG (original magnification: 10×)

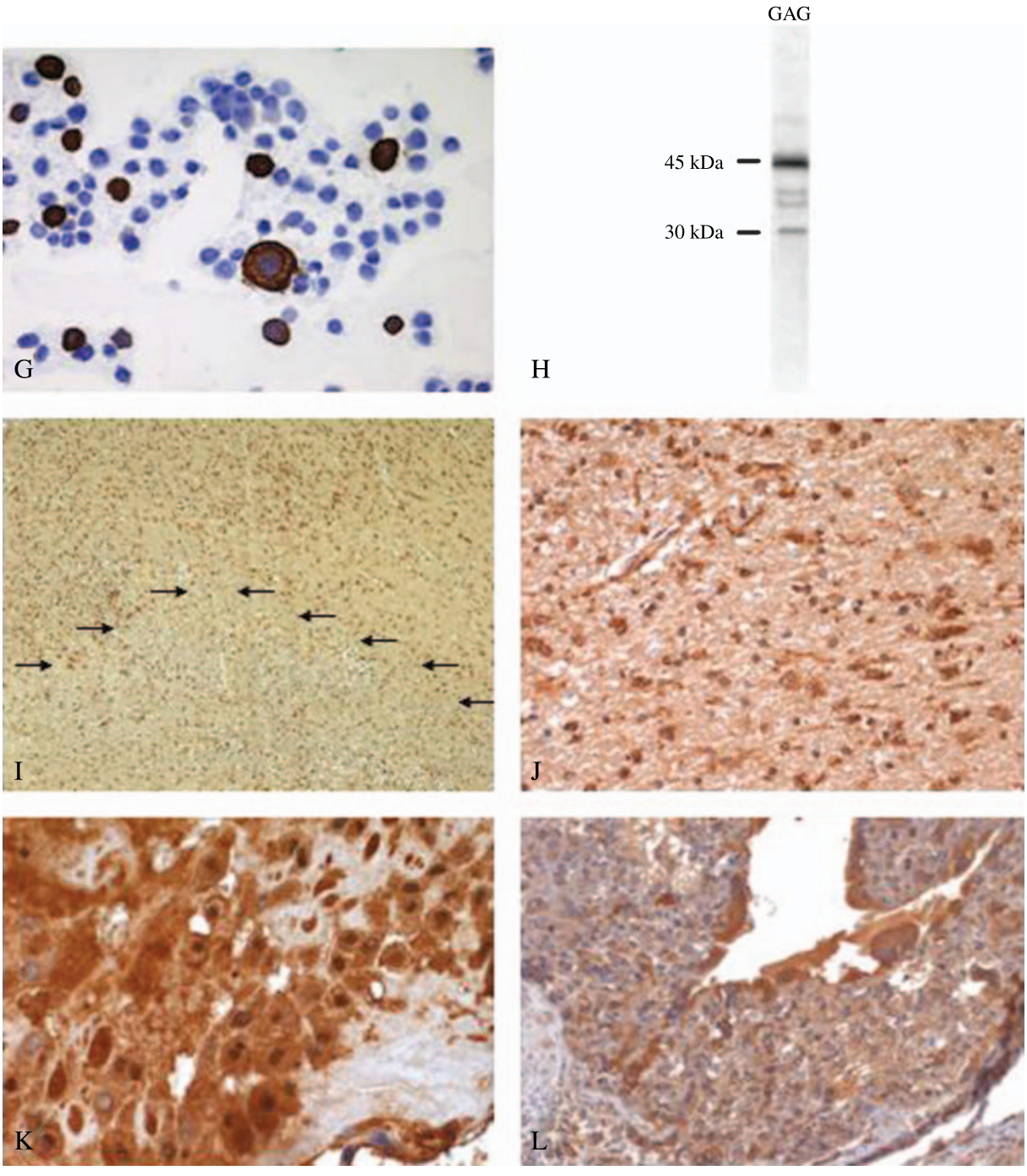

Fig. 1 (*continued*)

Confounding variables

The following confounding variables differed significantly compared to controls: postmortem time in schizophrenia; time between death and body refrigeration, fixation time, suicide, and heavy drug abuse in schizophrenia, bipolar disorder and depression; rate of death, being on drugs of abuse, cocaine abuse, and heavy alcohol abuse in bipolar disorder.

The confounding variables that most influenced the estimated parameters were: fixation time, alcohol and drug abuse as well as heavy alcohol and drug abuse.

It should be noted that a reduced density of immunoreactive cells does not imply that these cells are lost through apoptotic/necrotic death mechanisms, but rather it means that these cells which still exist have lost their immunoreactivity. Only significant results will be described subsequently.

Controls versus schizophrenia

In the *anterior cingulate gyrus*, the intensity score and compound value of neurons was significantly decreased compared to controls. In the *hippocampus*, the following structures/variables were significantly reduced: density of neurons in the granular layer of the dentate gyrus; the interneurons of the CA1, stratum moleculare of the cornu ammonis and of the dentate gyrus; axons in the granular layer of the dentate gyrus and in the stratum lucidum; basal dendrites of pyramidal neurons of CA1, CA4 and the granular layer of the dentate gyrus; glial cells in the Alveus, CA2, CA3, CA4, and strata moleculare, lacunosum, and lucidum of the CA, as well as the polymorphic, molecular, and granular layers of the dentate gyrus.

Controls versus bipolar disorder

In the *anterior cingulate gyrus*, no changes were observed for the neurons, neuropil or glial cells. In the *hippocampus*, the following structures/variables were significantly reduced: density of interneurons in CA1; density of basal dendrite of CA1 pyramidal neurons and compound value of stratum granulare of the dentate gyrus; density of glial cells in the stratum lucidum of the CA.

Controls versus major depression

In the *anterior cingulate gyrus*, the compound value of gray matter glial cells was significantly reduced. In the *hippocampus*, the intensity of CA2 interneurons was significantly reduced. A significant reduction in intensity and compound value of axon-dendrites of CA stratum radiatum neurons was observed. The compound value of the glia in the stratum radiatum was significantly reduced.

Effects of medication

The effect of medication was studied within each group separately (data not shown in tabular form). Thus, the use of second generation antipsychotics reduced the intensity, density and compound value of HERV-W- GAG interneurons in the alveus of patients with schizophrenia. The use of lithium or mood stabilizers did not have a significant effect on the expression of HERV-W GAG in patients with bipolar disorder.

Effects of seropositivities for toxoplasma gondii, herpes simplex virus 1 and 2, and cytomegalovirus

Within each diagnostic group, differences in the expression of HERV-W GAG between individuals who were seronegative or seropositive for *Toxoplasma gondii*, HSV1, HSV2, and CMV were studied. No significant differences in HERV-W GAG expression were noted between seronegative and seropositive individuals for any of the four infectious agents studied (data not shown in tabular form).

Discussion

In the present study, we showed, for the first time, significant differences in the expression of the human endogenous retroviral protein HERV-W GAG in the brains of patients suffering from major psychiatric disorders, i.e. schizophrenia, bipolar disorder, and major depression. Neurons and astroglial cells stained positively with the antibody used. In decreasing order, most of the changes were seen in schizophrenia followed by major depression and bipolar disorder. Also, cell- and brain-region-type specific changes for the HERV-W GAG protein expression was observed.

Immunohistochemical studies for HERV-W GAG and ENV expression in the brain are rare and were carried out only once, previously, using control brains and brains from patients with multiple sclerosis (Perron et al., 2005). Perron et al. (2005) described a physiological expression in the human brain of HERV-W ENV in microglia of both gray and white matter and in certain blood vessel endothelial cells, whereas expression of HERV-W GAG antigens was observed in neurons (cell body, axons, dendrites). In the present study, we could identify neurons and astroglial cells as being immunoreactive with HERV-W GAG. De facto, a physiological role for this HERV-W GAG antigen in human neurons, as previously evoked, becomes highly probable and comparable to that of HERV-W ENV7q in placenta (Mi et al., 2000). The most likely explanation for such an expression pattern is that HERV-W GAG is synthesized in low to moderate amounts within normal nerve cells and is axonally transported to the terminals, similar to that described for the amyloid precursor protein. Such neuronal proteins accumulate at sites of impaired axonal transport in demyelinated lesions (Kornek et al., 2000). Perron et al. (2005) concluded that the results from their study, have provided evidence that: (1) a physiological expression of an HERV-W GAG antigen exists in certain human brain cells, but most prominently in neurons, (2) a physiological expression of HERV-W ENV antigens is also detected in human brain, but mainly associated with infiltrating lymphoid cells or brain macrophages, (3) ''MS-specific'' GAG and ENV patterns were detected in MS lesions, essentially at the level of endothelial and microglial cells, and (4) in demyelinating diseases, an HERV-W GAG antigen accumulates in dystrophic axons within lesions.

The HERV-W GAG antibodies described in this study yield multiple bands in western blots performed on human brain tissue. This finding is not surprising given that numerous open reading frames exist for HERV-W GAG proteins in the genome (Voisset et al., 2000). Hence, it is likely that more than one HERV-W GAG open reading frame is translated into protein. However, we can not rule in the possibility that the multiple bands observed are a result of either degradation or post-translational modifications. Since staining can be completely blocked by pre-incubating the antibodies with the appropriate proteins (GAG), we conclude that the immunohistochemistry protocol developed in this report specifically targets HERV-W GAG proteins in the human brain.

Recently, Antony et al. (2004) reported that HERV-W encoded glycoprotein syncytin is up-regulated in glial cells within acute demyelinating lesions of multiple sclerosis patients. They also suggested that syncytin expression in astrocytes induces the release of redox reactants which are cytotoxic to oligondendrocytes. Tissue from a chronic multiple sclerosis lesion in one brain used as a positive control in our study showed not only HERV-W GAG protein expression in the demyelinating region but also highly upregulated expression in the neighbouring normal-appearing brain tissue of the gray and white matter (Fig. 1J). In addition to the astroglial cells, we also observed staining in a large number of microglial cells as well as neurons.

HERVs and schizophrenia

Evidence of endogenous retrovirus activity was first shown by Yolken et al. (2000) as they identified viral sequences in the brain and CSF of schizophrenic patients. Also, the authors showed increased activity of virally-encoded reverse transcriptase. Karlsson et al. (2001) found sequences homologous to retroviral POL genes in the cell-free cerebrospinal fluids of 29% of individuals with recent onset schizophrenia or schizoaffective disorder. Retroviral sequences were identified in the CSF of 1 of 20 individuals with chronic schizophrenia, but none were found in the CSFs from patients with non-inflammatory neurological diseases or from persons without evidence of neurological or psychiatric diseases. The identified sequences were related to those of the human endogenous retroviral HERV-W family of endogenous retroviruses and to other retroviruses and the murine leukemia virus genus. They also found these members to be differentially upregulated in the frontal cortex of brains obtained post-mortem from individuals with schizophrenia as compared to controls. They speculated that the findings might indicate that the expression of HERV-W RNA present in particles detectable in both CSF and plasma from patients with schizophrenia may be secondary to primary infectious events eliciting an inflammatory response in the host.

Our studies at the protein expression level are partly corroborated by microarray data published recently by the group of Seifarth et al. (2005) and Frank et al. (2005). Seifarth et al. (2005) used an oligonucleotide-based microarray allowing the detection and identification of most known retroviral reverse transcriptase related nucleic acids and analyzed members of 20 HERV families in 19 different normal tissues. With regard to the expression in brain tissue, the results showed a clear signal for HERV-W. Using the same microarray approach, Frank et al. (2005) suggested that HERV POL transcription in the brain is weakly correlated with schizophrenia and related diseases, but may be influenced by individual genetic background, brain-infiltrating immune cells, or medical treatment. The authors also reported that the ENV genes of HERV-W, HERV-FRD and HML-2 are transcriptionally active, but no significant differences in transcription levels between healthy controls and schizophrenic patient could be observed. They also concluded that POL and ENV gene transcripts are likely to be independently regulated. The results of our immuno-

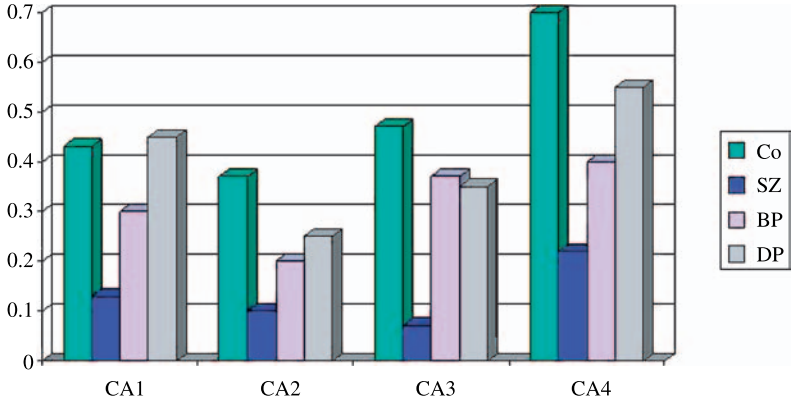

Fig. 2. Histogram showing the rating scores of HERV-W GAG for the CA1 to CA4 pyramidal layers in the four groups studied

histochemical studies show that HERV-W GAG proteins are expressed in all analyzed brain regions in both neuronal and astroglial cells.

In the study of Frank et al. (2005), it was evident that the inter-individual differences in HERV expression were more evident than the brain region specific variation observed in each single individual. Our data confirm that the inter-individual variability within each group is quite high; nevertheless, the statistical analysis showed a significant difference among the diagnostic groups. This inter-individual variability may reflect the individual genetic background (Seifarth et al., 2005) but might also reflect the number of copies present in the genome of an individual (Zawada et al., 2003). It was also suggested by Frank et al. (2005) that the results might reflect a differing stage of brain/infiltrating immune cells. Our on-going study on histopathological changes in the brains of patients with schizophrenia, bipolar disorder and depression did not show significant lymphocytic infiltration. Thus, this aspect cannot account for the presence or for the varying up- or down-regulation of HERV transcripts. There were also no differences in the expression of HERV-W GAG between subjects seronegative or seropositive for *Toxoplasma gondii*, HSV-1, HSV-2 and CMV. Medical treatment was also proposed as a possible factor influencing the expression of HERV transcripts. Our results showed minimal effect of medication on the expression of HERV-W GAG.

The fact that the abuse of alcohol and street drugs is confounding is intriguing but needs further follow-up and clarification. Until now, no paper was published addressing this aspect of abuse. It is likely that the down-regulation observed in our study is a by-product of overall abnormal gene regulation associated with psychiatric disease. It is not unreasonable to assume that processes disturbing the normal gene expression profile of endogenous human brain proteins may also alter the expression levels of HERV products.

Another possible explanation for the down-regulation of HERV antigens in the brain may result from well known defective/interfering genetic mechanisms that may occur in certain circumstances during HERV transcription at the RNA level (Mack et al., 2004; Mura et al., 2004; Schneider et al., 2001; Svobod et al., 2004). Indeed, if wide and potent activation of numerous HERV copies occurs at a time in a given cell, it could be due to the activation by a replicating virus member of the same family or due to triggering co-factors like selectively activating promoters from this HERV family, certain stress factors or cytokines, etc. As a result, the majority of RNAs will be defective, unable to encode any peptide fragment (stop codon, no stabilizing 3′UTR sequence etc.) and, in certain instance, may represent antisense RNA when inverted retro-insertions of HERV members have occurred. Thus, a potent stimulation and over-expression of HERV RNA could absolutely result in a paradoxical decrease of physiological antigen production from normally expressed members. This mechanism could explain the observed discrepancies between over-expression at the RNA level (Karlsson et al., 2001; Yolken et al., 2000) and down-regulation at the protein level (present study).

In conclusion, our study documents the widespread expression of HERV-W GAG in neurons and glial cells in normal brains. The importance of this physiological expression has yet to be elucidated. Based on current data, it is not clear if these proteins perform any purpose in the brain nor if their presence interferes with any cell function. In the brains of patients with schizophrenia and bipolar disorder, significantly reduced expression at the protein level for both HERV-W GAG could be seen both in neurons and in glial cells. The etiopathogenetic mechanism and importance of this finding are likewise not yet known. Contributing factors influencing the expression of HERV transcripts such as genetic background of individuals, or the infiltration with immune cells into the brain tissue, as well as medication effects could be partly excluded in the present study. These results illustrate the multifaceted aspects of human endogenous retroviruses with regard to physiology, pathology, and genetics. Further investigations will be directed towards estimating the copy number of HERV transcripts present in each brain, elucidating if HERV-W transcripts are up- or down-regulated in parallel to other genes, and investigating in more detail the role of these transcripts and that of their proteins in disease pathogenesis.

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