

First evidence of HERV-H transcriptional activity reduction after methylphenidate treatment in a young boy with ADHD

Elisa D'Agati¹, Mariabernarda Pitzianti¹, Emanuela Balestrieri², Claudia Matteucci², Paola Sinibaldi Vallebona², Augusto Pasini¹

¹Department of Systems Medicine, Unit of Child Neurology and Psychiatry, "Tor Vergata" University of Rome, Italy;

²Department of Experimental Medicine and Surgery, "Tor Vergata" University of Rome, Italy

SUMMARY

Human endogenous retroviruses (HERVs) have been associated with many complex diseases including neuropsychiatric diseases, such as attention deficit hyperactivity disorder (ADHD). In ADHD an over-expression of HERV-H family in peripheral blood mononuclear cells has been documented. It has been hypothesized that HERVs may represent the link between genetic and environmental risk factors, contributing to the clinical onset and/or to the progression of the neurodevelopmental disease. The effect of pharmacological treatment on HERV transcriptional activity in psychiatric disorders has been attracting attention.

Using a real-time RT-PCR we investigated the influence of methylphenidate on HERV transcription in peripheral blood mononuclear cells of a young patient with ADHD.

In this clinical case we describe for the first time the reduction of HERV-H expression and the significant improvement of ADHD symptoms after 6 months of methylphenidate treatment

Received October 5, 2015

Accepted July 17, 2016

INTRODUCTION

Several lines of evidence suggest that human endogenous retroviruses (HERVs) are associated with many complex diseases with multifactorial etiology and genetic basis, including neurological and psychiatric diseases. Up-regulation of transcriptional activity of some HERV sequences was found in schizophrenia, bipolar disorder (Perron *et al.*, 2012), autism spectrum disorders (Balestrieri *et al.*, 2012) and attention deficit hyperactivity disorder (ADHD) (Balestrieri *et al.*, 2014). ADHD is one of the most common neurodevelopmental disorders, with onset in early childhood, strong heritability and documented brain abnormalities, whose etiology is probably the result of a complex interaction of genetic, biological and environmental factors (Curatolo *et al.*, 2009; Pluess *et al.*, 2005). Recently, we described for the first time an over-expression of the HERV-H family in peripheral blood mononuclear cells (PBMCs) from 30 drug-naïve children with ADHD (Balestrieri *et al.*, 2014) compared with healthy children. HERVs represent about 8% of the human genome (IHGC, 2001) and are part of the superfamily of repeated and transposable elements endowed with the ability to integrate into any location of the genome, altering the structure and/or function of other genes (Bannert and Kurth, 2001; Rowe and Trono, 2011).

Elevated levels of HERV transcripts have been detected in brain tissues, cerebrospinal fluid and blood from patients with psychiatric disorders such as schizophrenia (Perron *et al.*, 2012). These patients are treated with drugs known to influence gene expression by inducing epigenetic modifications. The analysis of the HERV transcription pattern of human brain cell lines treated with various concentrations of valproic acid and neuroleptic drugs suggests that these medications may contribute to modify the expression of distinct HERV families (Diem *et al.*, 2012). In our previous study, ADHD patients were all drug-naïve, and therefore we can exclude the influence of pharmacological treatment on HERV expression. MPH is the most common drug treatment for children with ADHD because of its effect (0.8-1.0) in reducing ADHD symptoms (Banaschewski *et al.*, 2006). MPH inhibits the reuptake of dopamine and noradrenaline into the presynaptic neuron, increasing their concentrations in the extra-neuronal space enhancing neurotransmission (Anderson *et al.*, 2006). This effect may be responsible for the improvement in ADHD symptoms observed after MPH treatment. To date there are no available studies on the effect of methylphenidate (MPH) on HERV families' expression. This is the first clinical report describing the effect of MPH on HERV-H expression in a young drug-naïve patient with ADHD.

Key words:

ADHD, Human endogenous retroviruses, Methylphenidate.

Corresponding author:

Emanuela Balestrieri

E-mail: balestrieri@med.uniroma2.it

CASE REPORT

The patient is a 16 year-old male. He is the second child of a non-consanguineous healthy couple. His mother had an uneventful pregnancy and a spontaneous, uncomplicated delivery. The family history is unremarkable. The patient,

Table 1 - CPRS-T scores (Cognitive symptoms/Inattention, Hyperactivity, ADHD index and Oppositional) and CGI-I and CGS-S scores before and after 6 months of MPH treatment. CPRS, Conners' Parents Rating Scales (Score ranges from a low-T score of 61 mildly atypical to above 70 markedly atypical); CGI-S, Clinical Global Impressions - Severity (1-normal; 2-borderline; 3-mildly ill; 4-moderately ill; 5-strongly ill; 6-seriously ill; 7-extremely ill); CGI-I, Clinical Global Impressions - Improvement (1-greatly improved; 2-much improved; 3-improved a little; 4-no improvement; 5-worsened a little; 6-much worse; 7-extremely deteriorated).

	Before MPH treatment	After 6 months MPH treatment
Cognitive symptoms/ Inattention	80	65
Hyperactivity	90	67
ADHD index	95	65
Oppositional	84	67
CGI-S	6	2
CGI-I	4	2

with a diagnosis of ADHD combined type, according to DSM-IV-TR criteria, has been treated with cognitive behavioral therapy since he was 12 years old. The patient and his parents consulted child psychiatrists to discuss a possible drug prescription because he continued to be highly distractible and exhibited a significant impairment in social and school functioning. The diagnostic assessment included medical history, IQ evaluation, and the K-SADS clinical interview to exclude other psychiatric disorders. The Conners' Parents Rating Scales (CPRS) were used to assess ADHD clinical symptoms. Symptom severity and clinical improvement of the patient were assessed with the Clinical Global Impression-Severity (CGI-S) and Clinical Global Impression-Improvement (CGI-I) scales. Clinical response to MPH was evaluated using the CPRS and the CGI. The clinical evaluation using DSM-5 criteria (APA, 2013), confirmed a severe form of ADHD (CGI-S=6; seriously ill). The patient was drug-naïve. The ECG, EEG, haemogram, renal, liver and thyroid functions tests were normal. Findings on physical examination were normal. The clinicians confirmed the diagnosis of ADHD and decided

to prescribe MPH treatment. The patient and his parents gave their written informed consent for pharmacological treatment, and signed a written informed consent form for the study of HERV-H expression before and after 6 months of MPH treatment. MPH-immediate release was started at an initial daily dose of 10 mg in the morning and was rapidly increased to 20 mg daily in two doses, with a marked improvement of ADHD core symptoms. The T scores of the CPRS decreased significantly between the baseline and after six months treatment. CGI-S and CGI-I scores indicated that MPH led to a significant control of symptoms (Table 1). At baseline and after six months, the patient had a BMI of 19.

PBMCs from the patient's heparinized blood sample were analyzed immediately after collection, before and after 6 months of MPH treatment (Figure 1). The expression levels of the env sequence from the HERV-H family were quantitatively assessed by real-time RT-PCR. Briefly, 250 µg of DNase-treated RNA from PBMCs were reverse-transcribed and amplified using primers specific for the HERV-H family using SYBR Green chemistry. The housekeeping gene GUSB was used to normalize the results (Balestrieri *et al.*, 2012). Each experiment was completed with a melting curve analysis to confirm the specificity of amplification and the lack of non-specific comparative method.

The results obtained are shown in Figure 1 as the relative expression calculated using the threshold cycle (Ct) comparative method and, as calibrator, the mean of 5 samples from HC, matched for age and sex. The transcriptional level of HERV-H was drastically reduced after the MPH treatment from 119.98 to 0.67, while the transcriptional level of HC was 1.057±0.53.

DISCUSSION

HERV activity seems to be implicated in the pathogenesis of many complex diseases characterized by multifactorial etiology and genetic basis, such as ADHD (Balestrieri *et al.*, 2014). It has been hypothesized that HERVs may represent the link between genetic and environmental risk factors, contributing to the clinical onset and/or to the progression of neurodevelopmental diseases. In children with ADHD we demonstrated that HERV-H expression correlates with the core symptoms of the dis-

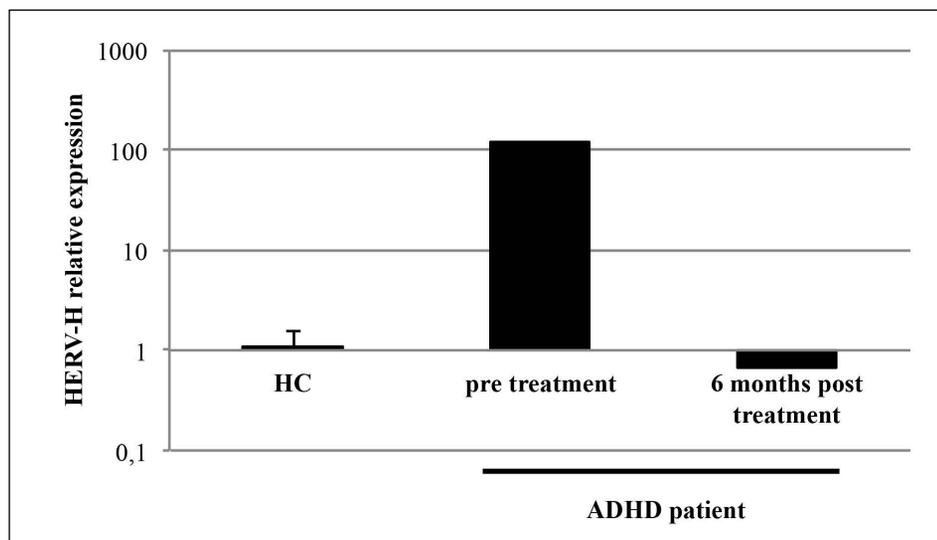


Figure 1 - HERV-H relative expression before and after MPH treatment.

The relative expression of HERV-H in an ADHD patient before and after methylphenidate treatment, compared with healthy controls was evaluated by real-time RT-PCR.

order (Balestrieri *et al.*, 2014). Recently, scientific studies demonstrated that pharmacological treatments may influence HERV expression, but no data are yet available on MPH treatment.

This case report describes for the first time the reduction of HERV-H expression in a young patient with ADHD after 6 months of MPH treatment, which determined a significant improvement of ADHD symptoms. This finding suggests that transcriptional activation of certain retroviral elements might be associated with the disease. HERVs have dynamic effects on gene function, and there are several potential mechanisms through which they can cause human disease (Diem *et al.*, 2012). A recent study demonstrated that the expression of HERV proteins increases the promoter activation of some genes, such as the dopamine receptor DRD3, and increases the expression of this gene in the neuroglia cells of patients with early onset schizophrenia (Huang *et al.*, 2011). Furthermore, more than 700 genes were upregulated in the striatum of MPH-treated rats. These genes are involved in the migration of immature neural/glial cells and/or growth of novel axons, in active axonal myelination, and in the upregulation of mature processes. Most of these genes are involved in a more enduring enhancement of neurobehavioral plasticity (Adriani *et al.*, 2006), through DNA methylation (Wu *et al.*, 2015).

Our finding suggests the possible role of HERVs in the pathophysiology of ADHD. The delineation of a role for retroviruses in disease pathogenesis might lead to new methods for the diagnosis and treatment of neurodevelopmental disorders. Future studies might investigate the expression of HERV families in patients with ADHD and correlate these findings with pharmacological treatment in a wider sample of ADHD patients.

References

- Adriani W., Leo D., Guarino M., Natoli A., Di Consiglio E., De Angelis G. (2006). Short-term effects of adolescent methylphenidate exposure

- on brain striatal gene expression and sexual/endocrine parameters in male rats. *Ann N Y Acad Sci.* **1074**, 52-73.
- American Psychiatric Association. (2013). Diagnostic and Statistical Manual of Mental Disorders. Fifth Edition. Edited by American Psychiatric Association; Washington, DC.
- Anderson V.R., Keating G.M. (2006). Methylphenidate controlled delivery capsules (EquasymXL, Metadate CD): a review of its use in the treatment of children and adolescents with attention deficit hyperactivity disorder. *Paediatric Drugs.* **8**, 319-333.
- Balestrieri E., Arpino C., Matteucci C., Sorrentino R., Pica F., Alessandrelli R., Coniglio A., Curatolo P., Rezza G., Macchiardi F., Garaci E., Gaudi S., Sinibaldi-Vallebona P. (2012). HERVs expression in Autism Spectrum Disorders. *PLoS One.* **7**, e48831.
- Balestrieri E., Pitzianti M., Matteucci C., D'Agati E., Sorrentino R., Baratta A., Caterina R., Zenobi R., Curatolo P., Garaci E., Sinibaldi-Vallebona P., Pasini A. (2014). Human endogenous retroviruses and ADHD. *World J Biol Psychiatry.* **15**, 499-504.
- Banaschewski T., Coghill D., Santosh P., Zuddas A., Asherson P., Buitelaar J., Danckaerts M., Döpfner M., Faraone S.V., Rothenberger A., Sergeant J., Steinhausen H.C., Sonuga-Barke E.J., Taylor E. (2006). Long-acting medications for the hyperkinetic disorders. A systematic review and European treatment guideline. *Eur Child Adolesc Psychiatry.* **15**, 476-495.
- Bannert N., Kurth R. (2006). The evolutionary dynamic of human endogenous retroviral families. *Annual Review of Genomics and Human Genetics.* **7**, 149-173.
- Curatolo P., Paloscia C., D'Agati E., Moavero R., Pasini A. (2009). The neurobiology of attention deficit/hyperactivity disorder. *Eur J Paediatr Neurol.* **13**, 299-304.
- Diem O., Schäffner M., Seifarth W. and Leib-Mösch C. (2012). Influence of antipsychotic drugs on human endogenous retrovirus (HERV) transcription in brain cells. *PLoS One.* **7**, e30054.
- Huang W., Li S., Hu Y., Yu H., Luo F., Zhang Q. et al. (2011). Implication of the env gene of the human endogenous retrovirus W family in the expression of BDNF and DRD3 and development of recent-onset schizophrenia. *Schizophr Bull.* **37**, 988-1000.
- International Human Genome Consortium. (2001). Initial sequencing and analysis of the human genome. *Nature.* **409**, 860-921.
- Perron H., Hamdani N., Faucard R., Lajnef M., Jamain S., Daban-Huard C. et al. (2012). Molecular characteristics of Human Endogenous Retrovirus type-W in schizophrenia and bipolar disorder. *Transl Psychiatry.* **4**, 2, e201.
- Pluess M., Belsky J., Neuman R.J. (2009). Prenatal smoking and attention-deficit/hyperactivity disorder: DRD4-7R as a plasticity gene. *Biol Psychiatry.* **66**, 5-6.
- Rowe H.M., Trono D. (2011). Dynamic control of endogenous retroviruses during development. *Virology.* **411**, 273-287.
- Wu T., Chen C., Yang L., Zhang M., Zhang X., Jia J., Wang J. (2015). Distinct lncRNA expression profiles in the prefrontal cortex of SD rats after exposure to methylphenidate. *Biomed Pharmacother.* **70**, 239-247.

