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SHORT COMMUNICATION

Transcriptional activity of human endogenous retrovirus in Albanian children with autism spectrum disorders

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SUMMARY

Recent studies suggest that autism spectrum disorders (ASD) result from interactions between genetic and environmental factors, whose possible links could be represented by epigenetic mechanisms. Here, we investigated the transcriptional activity of three human endogenous retrovirus (HERV) families, in peripheral blood mononuclear cells (PBMCs) from Albanian ASD children, by quantitative real-time PCR. We aimed to confirm the different expression profile already found in Italian ASD children, and to highlight any social and family health condition emerging from information gathered through a questionnaire, to be included among environmental risk factors. The presence of increased HERV-H transcriptional activity in all autistic patients could be understood as a constant epigenetic imprinting of the disease, potentially useful for early diagnosis and for the development of effective novel therapeutic strategies.

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We wish to dedicate this study to the memory of Carla Arpino, a friend and a teacher.

Autism spectrum disorders (ASD) constitute a group of complex neurodevelopmental disorders characterized by severe impairment in social interaction and communication, often accompanied by restricted, repetitive or stereotyped interests and behaviors (American Psychiatric Association 2013). Therefore, early detection of ASD is crucial to help children improve their language and social skills. The recent progressive increase in ASD (Van Naarden Braun *et al.,* 2015) has stimulated intense research into potential etiopathogenic factors, suggesting that the disease could result from a complex interaction between the individual's genetic profile and the environment that s/he is exposed to (Hunter 2005; Grabrucker 2013). Recent evidence suggests that a possible link between genetic and environmental factors in the pathogenesis of ASD could be the epigenetic mechanisms (Grafodatskaya *et al.,* 2010; Tordjman *et al.,* 2014) that occur in the prenatal period, the most important etiologic window for autism environmental risk factors (Gardener *et al.,* 2009). In fact, epigenetic regulation is essential during development, when somatic and germ cells experience a global epigenetic remodeling that regulates cell differentiation and tissue specification (Gropman and Batshaw 2010).

Human endogenous retrovirus (HERV) sequences, representing more than 8% of the human genome (Lander *et al.,* 2001), were integrated into the genome of mammals millions years ago via exogenous retroviral infections of germ cells (Turner *et al.,* 2001). During evolution, HERVs have been amplified and spread by repeated events of retrotransposition and/or reinfection and their new integrations in the genome could alter the structure and/or function of essential genes (Cordaux and Batzer 2009). Intact HERV sequences share the canonical structure of retroviruses, consisting of an internal region of four essential viral genes (*gag, pro, pol,* and *env)*, flanked by two long terminal repeat (LTR) elements. Neglected by studies on human health and disease, being for a long time considered non-functional genetic elements, HERVs have been involved in a complex network of cellular functions (Kurth and Bannert 2010). Recently we suggested their possible involvement as bridge between genetic predisposition and environmental factors also in the etiology of autistic spectrum disorder (Balestrieri *et al.,* 2012).

Despite the global increase in the incidence of ASD, studies in this field remained substantially overlooked in Albania. To the best of our knowledge, this is the first study analyzing the transcriptional activity of HERVs on young ASD patients and healthy controls from the Albanian population. The present report investigated the transcriptional activity of some HERV sequences in peripheral blood mononuclear cells (PBMCs) from ASD children and healthy matched controls, both born and raised in Albania. The aim was to confirm in an additional group of

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Human endogenous retroviruses, PBMCs, Early diagnosis, Biological marker, Autism spectrum disorders.

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Table 1 - *Demographic information for the autistic and control cohorts.*

		Autistic patients	*Healthy controls*	*p value*
		(n=30)	*(n=30)*	
Gender	Male	25	22	
	Female	5	8	
	Ratio (M/F)	5	2.75	0.765
Median age (range)		5 (3-7)	6 (2.5-8)	0.901

patients the different profile of the same HERV families, already described in our previous study on ASD children from the Italian population (Balestrieri *et al.,* 2012), and also to highlight from the information gathered through the questionnaires, any social and family health condition to be included among environmental risk factors.

The studied population consisted of 30 Caucasian patients, aged from 3 to 7 years (median age 5 years), of which 25 males and 5 females. All children were taken from a specialized center in Tirana (Albania) and were diagnosed as having ASD, based on the Diagnostic and Statistical Manual of Mental Disorders, 4th edition, Text Revision (DSM-IV-TR criteria). The patient group was compared with a control group (HC) of healthy Caucasian children with normal development, who attended a state school in Tirana. HC included 30 children who were matched to the patients by age and gender, 2.5 to 8 years old (median age 6 years), 22 of whom were males and 8 females. No exclusion criteria were applied to either cases or controls. The samples collection was conducted between 2010 and 2013. Demographic information on ASD patients and controls is summarized in *Table 1*.

Information concerning potential risk factors was obtained using a questionnaire addressed to the parents of the ASD patients. Anonymity and confidentiality of the data were ensured by written informed consent, in accordance with international and Albanian ethical regulations. The questionnaire consisted of three parts:

1) family history;
2) pregnancy history;
3) characteristics of the child during development and autism-related symptoms.

The risk factors investigated in the questionnaire were chosen from those predisposing to ASD (Koufaris and Sismani 2015), and the diseases were chosen from those most likely associated with human endogenous retroviruses (Balada *et al.,* 2010; Christensen 2010; Perron and Lang 2010; Kassiotis 2014).

Transcriptional activity of HERV-H, -K and -W families, selected on the basis of their frequent association with complex human diseases, was evaluated in PBMCs from heparinized blood of patients and controls, stored at -80°C and shipped in dry ice to be analyzed. The transcriptional levels of *env* of HERV families were quantitatively evaluated by real-time PCR by SYBR Green chemistry (Bio-rad, USA), using specific primer pairs as previously described (for methodological details see Balestrieri *et al.,* 2012 and Balestrieri *et al.,* 2015). Data obtained, normalized by housekeeping gene glucoronidase beta (GUSB), were represented by box plots, depicting mild (black dot) and extreme outliers (asterisk) for the two groups. The Mann Whitney U test was used to compare HERV transcriptional activity in patients and controls, the independent samples t-test was used to compare groups characteristics. Statistical analyses were done using the SPSS software (version 17.0). Statistical significant values were considered when $p<0.050$.

HERV-H expression (*Figure 1 left*) was significantly more elevated in ASD children (median value 27.50 Interquartile range, IQR=11.94/82.40) compared to expression levels in the HC group (median value 1.87 IQR=0.32/8.05; $p<0.001$). By contrast, both HERV-K and HERV-W were significantly more expressed in PBMCs from HCs compared to ASD patients. In particular, the median value of HERV-K was 0.091 (IQR=0.02/3.62) for ASDs, while it was 6.69 (IQR=1.92/89.18; $p=0.001$) for HCs (*Figure 1 center*) and the median value of HERV-W was 1.28 (IQR=0.11/2.43) for ASDs, and 12.45 (IQR = 1.44/49.66; $p=0.001$.) for HCs (*Figure 1 right*).

The quantitative determination of HERV-H and HERV-W, carried out in PBMCs from Albanian children confirmed the data reported for the same families in our previous study, performed in PBMCs from Italian individuals. By contrast, a significant difference in HERV-K transcriptional activity between patients and controls, not detected in the Italian study, was highlighted. Moreover, the comparative analysis of expression levels of HERV-H in individual ASD Albanian patients, compared to the Italian population (Balestrieri *et al.,* 2012) shows a greater uniformity in the first group even though it comprises almost the same number of patients (data not shown). The cause, perhaps attributable to the different ethnicity, could support a predominant contribution of genetic *versus* environmental factors in the pathogenesis of the disease.

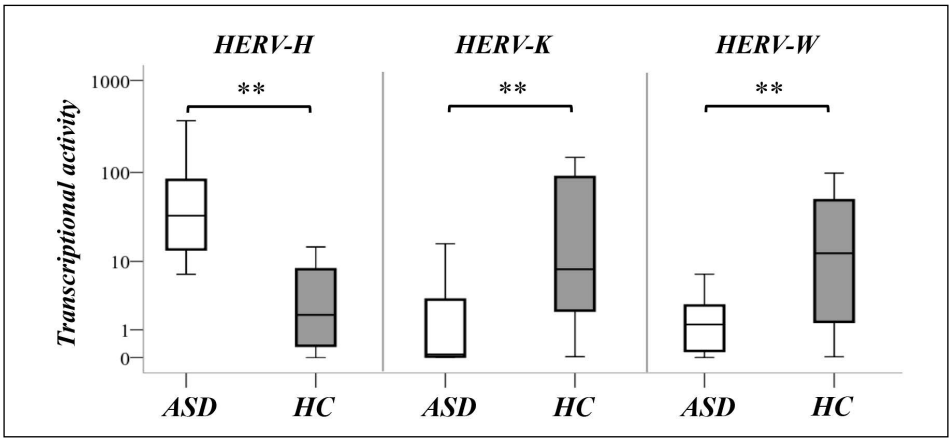

Figure 1 - Transcriptional profile of three HERV families in PBMCs from autistic patients (ASD) and healthy controls (HC). *ASD patients showed an alteration in the transcriptional activity of all HERV families in comparison with HC. In particular, the transcriptional level, analysed by real-time RT-PCR, was higher for HERV-H and lower for HERV-K and HERV-W. The differences in the transcriptional activity were always statistically significant (Mann Witney test).*

Overall, the results obtained further support our hypothesis that HERV-H plays a causative role in the genesis of autism (Balestrieri *et al.,* 2012), in the wake of other works implicating HERV in schizophrenia (Suntsova *et al.,* 2013) and multiple sclerosis (Nexo *et al.,* 2011).

The parents of young patient questionnaire answers were analyzed to highlight the possible correlations between family characteristics and medical data with ASD disorder. The information show that both parental age (fathers: median 33 years, range 24-43; mothers: median 28 years, range 19-38) at the time of the offspring's birth was below that considered a possible risk factor for ASD (Shelton *et al.,* 2010; Parner *et al.,* 2012). In addition, among the parents of children enrolled in the study, none declared consanguinity, a further documented risk factor for the disease (Mamidala *et al.,* 2015). In this respect, despite the statements by the parents, it should be recalled that consanguinity is relatively common in Albania, as documented by isonymy, although it is a weak marker for inbreeding (Mikerezi *et al.,* 2013).

Analysis of the data showed that only three mothers (10%) and one father (3.33%) were suffering from autoimmune diseases; four mothers (13.33%) had developed infectious diseases outside of the period of pregnancy and 20% of mothers and 6.67% of fathers were allergic. The number of parents who claimed to be suffering from dysmetabolic diseases such as diabetes, was negligible.

Interestingly, the information provided by the questionnaire revealed that 6.67% of fathers and 13.33% of mothers presented neuropsychiatric disorders, and that including their relatives the percentage of ASD children who had mental disorders in the family reached 40%. The most common disorders were mental retardation and language disorders and in two cases, autism.

While no mother claimed to have been exposed to potentially toxic substances or to have consumed alcohol, drugs or tobacco during pregnancy, a high percentage of them (30%) recalled having had fever, infections and inflammatory processes in that period.

A matter of particular relevance is that 40% of mothers had had multiple miscarriages before giving birth to the autistic child, in agreement with the consideration that autism is associated with a marked reduction in birth rates (Uher, 2009). Because epidemiological data for the period 2010-2012 indicate that in the Albanian population, abortions per 1000 women aged 15-49 years were estimated to be between 50.5 and 67.0 (http://www.johnstonsarchive.net/policy/abortion) and the pregnancy loss was generally related to the exposure to environmental toxic substances (Kumar 2011), not detected in this specific population, we assume the infections and inflammatory processes as the possible cause both of miscarriage and HERV reactivation, as extensively demonstrated (Sutkowski *et al.,* 2001; Frank *et al.,* 2006; Nellaker *et al.,* 2006; Ruprecht *et al.,* 2006)

During development recurrent infections and fever were present only in a small number of young patients, and autism-related symptoms, such as head and trunk control, was shown in 40% and 70% of them, respectively, while independent ambulation was noted in almost all children observed (90%). Communication development milestones such as babbling were present in 15/30 patients, first word age was normal in 11/30 and indicative gestures in 16/30.

Of the 30 patients analyzed 8 showed symptoms such as constipation or diarrhea, 3 allergic reactions, 4 anemia and 2 epilepsy. None had food intolerance or other dysimmune diseases.

With respect to the data summarized, between 15 and 36 months, 19 of the 30 children included in the study showed developmental regression, represented by the loss of any skill in social communication and/or relationship and the manifestation of repetitive behaviors. Finally, the young patients enrolled in the study included two monozygotic twins and two brothers.

We are aware that our study has some limitations: first, the lack of information on families of children used as controls and second, the quite small number of individuals in whom the HERV expression was assessed.

However, the information provided indicates that the high percentage of the families of ASD children (40%) included in our study manifest autistic features as well as a variety of other neuropsychiatric conditions such as schizophrenia and bipolar disorders. All these pathologies carry strong evidence of genetic transmission (Constantino *et al.,* 2013) and consequently a familial risk for ASD (Sandin *et al.,* 2014) associated with a common etiologic factor (Sullivan *et al.,* 2012) and recently with the involvement of HERVs (Perron *et al.,* 2012; Balestrieri *et al.,* 2012).

Moreover, the high percentage of mothers developing infections, inflammation and fever during pregnancy is in accordance with epidemiological data suggesting their association with an increased risk of ASD (Mazina *et al.,* 2015). HERVs are responsive to a variety of stressing stimuli (Cho *et al.,* 2008; Miousse *et al.,* 2015), a characteristic that could also explain their altered expression profiles in response to pathological conditions (van der Kuyl 2012; Mameli *et al.,* 2013).

The presence of ASD identical twins and brothers in our population is further justified in the inheritance of the disease and in the consideration that siblings of children with ASD are 10-20 times more likely to receive a diagnosis of ASD themselves (Robinson *et al.,* 2014).

The hypothesis that the onset of ASD may be attributed to the interactions between genetic and environmental factors together with the growing evidence showing the involvement of epigenetic dysregulation (Tordjman *et al.,* 2014; Grayson and Guidotti 2015) supports the idea that epigenetic mechanisms are the interface between these two components. Therefore, the increase in HERV-H transcriptional activity detected in autistic Albanian patients, already highlighted in autistic Italian patients, can be understood as a constant epigenetic imprinting of this pathology. Our data are also in agreement with the theory that autism has a strong genetic basis, supported by evidence that in the families of patients analyzed there was an abundance of mental illnesses and neurodevelopmental disorders. In addition, the environmental influence, specifically, infections and inflammation developed during pregnancy, may have provided etiologically stimulating factors for the development of the disease among which, the most significant is the increased expression of HERV-H. From this perspective, it might also be reasonable to suggest that the altered expression of HERV plays a causative role in the very high percentage of miscarriage.

While it is still unclear how environmental factors might influence epigenetic changes and exert their effects at a cellular level, it is known how infections and inflammatory processes can cause abortion (Giakoumelou *et al.,* 2015). It is however conceivable that no single environmental factor is sufficient to influence the ASD, but it is rather the combination of several factors with a genetic and/or epigenetic predisposition to ASD that are likely to have a significant impact (Chaste and Leboyer, 2012; Tordjman *et al.,* 2014).

Overall, the data obtained in the present study lead us to further support the hypothesis that HERV transcriptional activity is influenced by all the factors mentioned above. Additional work is required to determine if HERV-H expression could be proposed as a biological marker, useful for early detection of children at high risk for ASD, before the appearance of clinical symptoms and for the development of effective new therapeutic strategies. To this end, an in-depth characterization of the potential role of HERV-H in ASD is the major objective of a study currently in progress in murine models. Currently, up to 2% of children worldwide are estimated to be diagnosed with an ASD (Pedersen *et al.,* 2014) and the consistent increment in the prevalence of ASD is considered a pressing challenge for the global public health system. Because children represent more than a third of the Albanian population (Albanian Institute of Statistics 2011) autism is a serious socio-economic problem and its early diagnosis could represent a significant improvement in the treatment of the disease. In fact, if the autistic condition is diagnosed early, a growing repertoire of evidence-based therapies can be applied to give children the best possible chance of life.

References

Albanian Institute of Statistics. Albania in figures 2010. Tirana 2011.

American Psychiatric Association (APA) (2013). Diagnostic and statistical manual of mental disorders. 5th ed. Washington DC.

Balada E., Vilardell-Tarrés M., Ordi-Ros J. (2010). Implication of human endogenous retroviruses in the development of autoimmune diseases. *Int Rev Immunol.* **29**, 351-370.

Balestrieri E., Arpino C., Matteucci C., Sorrentino R., Pica F., Alessandrelli R., et al. (2012). HERVs expression in Autism Spectrum Disorders. *PLoS One.* **7**, e48831.

Balestrieri E., Pica F., Matteucci C., Zenobi R., Sorrentino R., Argaw-Denboba A., et al. (2015). Transcriptional activity of human endogenous retroviruses in human peripheral blood mononuclear cells. Biomed Res Int. 2015.

Chaste P., Leboyer M. (2012). Autism risk factors: genes, environment, and gene-environment interactions. *Dialogues Clin Neurosci.* **14**, 281-292.

Cho K., Lee Y.K., Greenhalgh D.G. (2008). Endogenous retroviruses in systemic response to stress signals. *Shock.* **30**, 105-116.

Christensen T. (2010). HERVs in neuropathogenesis. J Neuroimmune Pharmacol. 5, 326-335.

Constantino J.N., Todorov A., Hilton C., Law P., Zhang Y., Molloy E., et al. (2013). Autism recurrence in half siblings: strong support for genetic mechanisms of transmission in ASD. *Mol Psychiatry*. **18**, 137-138.

Cordaux R., Batzer M.A. (2009). The impact of retrotransposons on human genome evolution. *Nat Rev Genet*. **10**, 691-703.

Frank O., Jones-Brando L., Leib-Mösch C., Yolken R., Seifarth W. (2006). Altered transcriptional activity of human endogenous retroviruses in neuroepithelial cells after infection with Toxoplasma gondii. *J Infect Dis*. **194**, 1447-1449.

Gardener H., Spiegelman D., Buka S.L. (2009). Prenatal risk factors for autism: comprehensive meta-analysis. *Br J Psychiatry.* **195**, 7-14.

Giakoumelou S., Wheelhouse N., Cuschieri K,. Entrican G., Howie S.E., Horne A.W. (2015). The role of infection in miscarriage. *Hum Reprod Update.* **0**, 1-18.

Grabrucker A.M. (2013). Environmental factors in autism. *Front Psychiatry.* **3**, 118.

Grafodatskaya D., Chung B., Szatmari P., Weksberg R. (2010). Autism spectrum disorders and epigenetics. *J Am Acad Child Adolesc Psychiatry*. **49**, 794-809.

Grayson D.R., Guidotti A. (2015). Merging data from genetic and epigenetic approaches to better understand autistic spectrum disorder. *Epigenomics*. 2015 Nov 9 (Epub ahead of print).

Gropman A.L., Batshaw M.L. (2010). Epigenetics, copy number variation, and other molecular mechanisms underlying neurodevelopmental disabilities: new insights and diagnostic approaches *J Dev Behav Pediatr*. 31, 582-591.

http://www.johnstonsarchive.net/policy/abortion

Hunter D.J. (2005). Gene-environment interactions in human diseases. *Nat Rev Genet.* **6**, 287-298.

Kassiotis G. (2014). Endogenous retroviruses and the development of cancer. *J Immunol.* **192**, 1343-1349.

Koufaris C., Sismani C. (2015). Modulation of the Genome and Epigenome of Individuals Susceptible to Autism by Environmental Risk Factors. *Int J Mol Sci.* **16**, 8699-8718.

Kumar S. (2011). Occupational, environmental and lifestyle factors associated with spontaneous abortion. *Reprod Sci.* **18**, 915-930.

Kurth R, Bannert N. (2010). Beneficial and detrimental effects of human endogenous retroviruses. *Int J Cancer*. **126**, 306-314.

Lander E.S., Linton L.M., Birren B., Nusbaum C., Zody M.C., Baldwin J., et al. (2001). Initial sequencing and analysis of the human genome. *Nature.* **409**, 860-921.

Mameli G., Madeddu G., Mei A., Uleri E., Poddighe L., Delogu L.G., et al. (2013). Activation of MSRV-type endogenous retroviruses during infectious mononucleosis and Epstein-Barr virus latency: the missing link with multiple sclerosis? *PLoS One.* **8**, e78474.

Mamidala M.P., Kalikiri M.K., Praveen Kumar P.T., Rajesh N., Vallamkonda O.R., Rajesh V. (2015). Author information Consanguinity in India and its association with autism spectrum disorder. *Autism Res.* **8**, 224-228.

Mazina V., Gerdts J., Trinh S., Ankenman K., Ward T., Dennis M.Y., et al. (2015). Epigenetics of autism-related impairment: copy number variation and maternal infection. *J Dev Behav Pediatr.* **36**, 61-67.

Mikerezi I., Xhina E., Scapoli C., Barbujani G., Mamolini E., Sandri M., et al. (2013). Surnames in Albania: a study of the population of Albania through isonymy. *Ann Hum Genet.* **77**, 232-243.

Miousse I.R., Chalbot M.C.G., Lumen A., Ferguson A., Kavouras I.G., Koturbash I. (2015). Response of transposable elements to environmental stressors. *Mutat Res Rev Mutat Res.* **765**, 19-39.

Nellaker C., Yao Y., Jones-Brando L., Mallet F., Yolken R.H., Karlsson H. (2006). Transactivation of elements in the human endogenous retrovirus W family by viral infection. *Retrovirology.* **3**, 44.

Nexø BA., Christensen T., Frederiksen J., Møller-Larsen A., Oturai AB., Villesen P., et al. (2011) The etiology of multiple sclerosis: genetic evidence for the involvement of the human endogenous retrovirus HERV-Fc1. *PLoS One.* **6**, e16652.

Parner E.T., Baron-Cohen S., Lauritsen M.B., Jørgensen M., Schieve L.A., YearginAllsopp M., et al. (2012). Parental age and autism spectrum disorders. *Ann Epidemiol.* **22**, 143-150.

Pedersen C.B., Mors O., Bertelsen A., Waltoft B.L., Agerbo E., McGrath J.J., et al. (2014). A comprehensive nationwide study of the incidence rate and lifetime risk for treated mental disorders. *JAMA Psychiatry.* **71**, 573-581.

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.* **2**, e201.

Perron H., Lang A. (2010). The human endogenous retrovirus link between genes and environment in multiple sclerosis and in multifactorial diseases associating neuroinflammation. *Clin Rev Allergy Immunol.* **39**, 51-61.

Robinson E.B., Samocha K.E., Kosmicki J.A., McGrath L., Neale B.M., Perlis R.H., et al. (2014). Autism spectrum disorder severity reflects the average contribution of de novo and familial influences. *Proc Natl Acad Sci USA.* **111**, 15161-15165.

Ruprecht K., Obojes K., Wengel V., Gronen F., Kim K.S., Perron H., et al. (2006). Regulation of human endogenous retrovirus W protein expression by herpes simplex virus type 1: implications for multiple sclerosis. *J Neurovirol.* **12**, 65-71.

Sandin S., Lichtenstein P., Kuja-Halkola R., Larsson H., Hultman C.M., Reichenberg A. (2014). The familial risk of autism. *JAMA.* **311**, 1770-1777.

Shelton J.F., Tancredi D. J., and Hertz-Picciotto I. (2010). Independent and dependent contributions of advanced maternal and paternal ages to autism risk. *Autism Research.* **3**, 30-39.

Sullivan P.F., Magnusson C., Reichenberg A., Boman M., Dalman C., Davidson M., et al. (2012). Family history of schizophrenia and bipolar disorder as risk factors for autism. *Arch Gen Psychiatry.* **69**, 1099-1103.

Suntsova M., Gogvadze EV., Salozhin S., Gaifullin N., Eroshkin F., Dmitriev SE., et al. (2013) Human-specific endogenous retroviral insert serves as an enhancer for the schizophrenia-linked gene PRODH. *Proc Natl Acad Sci USA.* **110**, 19472-19477.

Sutkowski N., Conrad B., Thorley-Lawson D.A., Huber B.T. (2001). Epstein-Barr virus transactivates the human endogenous retrovirus HERV-K18 that encodes a superantigen. *Immunity.* **15**, 579-589.

Tordjman S., Somogyi E., Coulon N., Kermarrec S., Cohen D., Bronsard G., et al. (2014). Gene×Environment interactions in autism spectrum disorders: role of epigenetic mechanisms. *Front. Psychiatry.* **5**, 1-17.

Turner G., Barbulescu M., Su M., Jensen-Seaman M.I., Kidd K.K., Lenz J. (2001). Insertional polymorphisms of full-length endogenous retroviruses in humans. *Curr Biol.* **11**, 1531-1535.

Uher R. (2009). The role of genetic variation in the causation of mental illness: an evolution-informed framework. *Mol Psychiatry.* **14**, 1072-1082.

van der Kuyl A.C. (2012). HIV infection and HERV expression: a review. *Retrovirology.* **9**, 1-10.

Van Naarden Braun K., Christensen D., Doernberg N., Schieve L., Rice C., Wiggins L., et al. (2015). Trends in the prevalence of autism spectrum disorder, cerebral palsy, hearing loss, intellectual disability, and vision impairment, metropolitan atlanta, 1991-2010. *PLoS One.* 10, e0124120.