in vivo *27*: 843-850 (2013)

Prevalence of HHV-6 and HHV-8 Antibodies in Patients with Autism Spectrum Disorders

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Abstract. *Background/Aim: The etiology of autism spectrum disorders (ASD) still eludes investigators. Several viral infections have been associated with ASD etiopathogenesis but few studies have ever focused on the role of HHV-6 and HHV-8, two members of the herpesviridae family. The aim of the present study was to evaluate seropositivity rate and levels of antibodies to HHV6 and HHV-8 in children with ASD compared to controls. Patients and Methods: We measured and compared seropositivity rate and levels of antibodies to HHV-6 and HHV-8 in 30 children with ASD (14 with autistic disorder and 16 with non-autistic disorder ASD) and in 28 healthy controls of the same age. Results: Seropositivity rate and levels of the two antibodies were similar in cases and controls. Seropositivity rate and levels of antibodies were not correlated with disease severity in children with ASD. Conclusion: Levels and seropositivity rate of antibodies to HHV-6 and HHV-8 do not differ between children with ASD and controls.*

Autism spectrum disorders (ASD) are neuropsychiatric disorders characterized by significantly abnormal or deficient social interaction, communication abilities and language and considerable narrow pattern of activities and interests (1). Symptoms start before three years of age with evidence of abnormal cognitive functioning, learning, attention, and sensory processing (2). Autism spectrum disorders can be associated with numerous comorbidities such as mental retardation, epilepsy, aggression and self-hurting behavior. Behavioral interventions and psychopharmacological treatment are now the mainstay of treatment of ASD (3). One of the most interesting aspects of pervasive disorders is the significant change in terms of epidemiology reported in the past two decades. The incidence of ASD has dramatically increased in recent years and it now affects 1 out of 88 newborns (4). There is a well-defined higher prevalence in boys, namely 1:51 in boys *vs*. 1:252 in girls (4). However, it remains to be established whether these findings represent a real epidemic of ASD (5), a simple effect of improved diagnostic means, or a combination of both. The exact etiology of ASD is unknown, although specific interactions between genetic and environmental factors have been implicated in the condition (6). A family study provides significant evidence in favor of the genetic component of susceptibility to ASD. In fact, an estimated heritability of 37% has been reported for ASD (7).

Approximately 10% of patients with ASD are reported to be affected by *syndromic autism*, due to known genetic disorders such as Mendelian diseases or monogenic disorders [*e.g*. fragile X syndrome, type 1 neurofibromatosis, tuberous sclerosis, Down syndrome, *etc*. (8)], extended chromosomal re-arrangements, specific prenatal teratological agents, or exposure to drugs, such as thalidomide, misoprostol, and valproic acid (9-13). The remaining 90% of patients suffer from *non-syndromic* or *idiopathic autism*. In these cases, it is thought that the etiology lies in the interaction of multiple genes with one or more environmental factors (6, 14).

Altered neurodevelopment during the first and second trimester of prenatal life has been recognized as the underlying neuropathological cause of ASD in most patients (15). On the contrary, Rogers depicts autism as a disorder with gradual onset whose core etiopathogenetic mechanisms may develop during the first two to three years of life (16).

Among the environmental factors thought to be involved in the genesis of ASD, it has been proposed that infections, especially viral infections, may be able to trigger the disorder (17, 18). Vaccinations have also been implicated in ASD

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Key Words: Autism spectrum disorder, HHV-6 , HHV-8.

etiopathogenesis, but this theory has been refuted (19). Several reports have linked viral infections to ASD, mainly herpesviridae [herpes simplex (HSV), cytomegalovirus (CMV) (10), varicella zoster (VZV) (13, 20-30)], mumps (31), influenza (32), lymphocytic choriomeningitis (33) and polyomaviruses (34, 35).

The aim of our study was to evaluate the seropositivity rate and antibody titers against Human Herpes Virus-6 and Human Herpes Virus-8 in a cohort of children with ASD compared to a cohort of healthy same-age controls.

Patients and Methods

Patients. Patients admitted as in- or out-patients to the Child and Adolescent Neuropsychiatry Unit at the Second University of Naples and to the Department of Pediatrics of the Federico II University of Naples, Italy, were recruited between January 2010 and June 2011. The children's parents/legal guardians supplied consent forms and the Ethics Committee of the Federico II University of Naples approved the study (protocol number: 85/09). Inclusion criteria for cases were diagnosis of ASD according to the Diagnostic and Statistical Manual of Mental Disorders, Fourth edition, Text Revision (DSM-IV-TR) (1) and informed consent signed by parents/guardians; the only exclusion criterion was the inability to sign an informed consent form.

Controls were enrolled at the Division of Pediatric Surgery where they were admitted for minor surgical treatments (*e.g.* phimosis, hernia, cryptorchidism, vesicoureteral reflux, hydrocele testis, *etc.*).They underwent an interview to rule out possible ASD and, if such a disorder was identified, the affected children were excluded from the study.

To authenticate the diagnosis of autism, cases were administered the Autism Diagnostic Interview, revised version (36), the Childhood Autism Rating Scales (CARS) (37) and the Autism Diagnostic Observation Schedule (ADOS)-Generic (38).

Virological tests. IgG antibodies against HHV-6 and HHV-8 were detected using an enzyme-linked immunosorbent assay (ELISA) technique (indirect ELISA for HHV-6 IgG and indirect ELISA for KSHV/HHV-8 IgG; Advanced Biotechnologies Inc. Columbia, MD,USA). In detail, to detect specific antibodies, 8-well strips were coated with optimal amounts of solubilized KSHV/HHV-8 whole-virus extracts or HHV-6 antigen. Three incubation steps were needed: a) tested human samples were added to the microtiter wells and any specific antibodies to HHV-6 or HHV-8 present in the sample bind to the antigen-coated plates, thereby forming immunological complexes. After the incubation, the strips were washed to remove unbound sample components; b) anti-human IgGs conjugated to horseradish peroxidase (HRP) were added to the wells and during incubation combined with the antibody-antigen complexes already present in the wells. After incubation, the wells were washed to remove unbound conjugate; c)HRP substrate tetramethylbenzidine was added to the wells. During incubation, enzyme-mediated cleavage of the substrate resulted in color change. A stop reagent was added, after which, the wells with positive samples changed color with an intensity proportional to the levels of antibodies, which was measured spectrophotometrically. To obtain a suitable cut-off value, we averaged the readings of three negative control wells and multiplied the result by 3 (HHV-8) or 2 (HHV-6), according to the instructions provided by the manufacturer . We calculated the optical density (O.D.) ratios by dividing the reading of each sample well by the cutoff value. O.D. ratios ≤0.75 correspond to negative samples, O.D. ratios ≥1.00 correspond to positive samples, while O.D. ratios between 0.75-1 are considered borderline.

Statistical analysis. For quantitative variables, the Kolmogorov-Smirnov test was used to check for Gaussian distribution. In this case, data are given as mean±standard deviation (SD) and the Student's *t*-test for unpaired variables was applied for comparisons. In cases of non-Gaussian distribution, data are reported as the median and interquartile range (IQR) and the Mann-Whitney *U*-test was used for comparisons. To compare quantitative data among more than two groups [controls, autistic disorder (AD), and non-AD ASD], comparisons were performed using the ANOVA or Kruskal-Wallis test, in case of Gaussian or non-Gaussian distribution respectively. As age may significantly impact on antibody titer, a generalized linear model (GLM) was used to correct antibody titer for age and sex. The χ^2 test with Yates correction (or Fisher's exact test where appropriate) was used for categorical variables. Spearman's rho test was used for correlations between continuous variables. A *p*-value of 5% or less in the two-sided test was considered statistically significant. Logistic regression analysis was used to correct seropositivity rate for age and sex. All statistical analyses were carried out using the Statistical Package for the Social Sciences, version 18.0 (SPSS Inc. Chicago, IL, USA).

Results

We enrolled 58 children in the study, 30 with ASD (14 with AD and 16 with non-AD ASD) and 28 controls. None of the controls was found to be affected by a neuropsychiatric disorder. The median age was 5.83 years for cases (IQR=4.16-8.42 years) and 5.88 for controls (IQR=3.16-8.40 years) (p=0.798). Males outnumbered females, both among cases (26/30, 86.7%) and controls (25/28, 89.3%, p=0.999).

We assessed the rate of seropositivity for the two human herpesviruses (HHVs) in cases and controls. As shown in Table I, the rate of seropositivity was similar in cases and controls (p=0.999 both for HHV-6 and HHV-8). Logistic regression analysis showed that neither age nor gender affected the seropositivity status in relation to the two antibodies. We measured and compared O.D. ratios for HHV-6 and HHV-8 in cases and controls. Respective OD ratios are reported in Table II. Although O.D. ratios cannot be considered strictly titers, the data obtained are indicative of antibody concentration. As shown in Table II, the OD ratios of antibodies against the two HHVs did not differ between cases and controls. The GLM revealed that neither age nor gender affected the level of the two antibodies.

We also evaluated the levels and seropositivity rate of the two antibodies in three specific categories, children with AD, children with non-AD ASD and controls. As shown in Table II, neither the seropositivity rate for the two viruses nor antibody titers for HHV-8 differed significantly among the

Table I. *Rate of seropositivity to HHV-6 and HHV-8 antigens in cases and controls and in autistic disorder (AD) and non-AD autism spectrum disorders (ASD).*

Seropositivity against	Cases		Controls	*p*-Value
HHV-8	1/30 (3.3%)	AD 0/14 (0%)	0/28 (0%)	0.999 (F)†
		Non-AD ASD 1/16 (6.3%)		0.999 (F)‡
HHV-6	25/30 (83.3%)	AD 11/14 (78.6%)	23/28 (81.2%)	0.999 (F)†
		Non-AD ASD 14/16 (87.5%)		0.642 (F)‡

F: Fisher's exact test; †cases *vs*. controls; ‡AD *vs*. non-AD ASD *vs*. controls.

Table II. *OD ratios of antibodies to HHV-6 and HHV-8 in cases and controls, and in autistic disorder (AD) and non-AD autism spectrum disorders (ASD).*

	Cases		Controls	*p*-Value
HHV-8	0.046 (0.044-0.052) n=30	AD 0.048 (0.045-0.054) n=14	0.046 (0.044-0.048) n=28	0.487 (U)†
(OD ratios)		Non-AD ASD 0.045 (0.043-0.051) n=16		0.133 (U)‡
HHV-6	2.433 (0.737-2.738) n=30	AD 1.523 (0.528-2.584) n=14	2.366 (0.856-2.728) n=28	0.938 (U)†
(OD ratios)		Non-AD ASD 2.678 (1.421-2.747) n=16		0.046 (U)‡

Data are given as median (interquartile range). U: Mann-Whitney *U*-test; †cases *vs*. controls; ‡AD *vs*. non-AD ASD *vs*. controls. OD: optical density.

three groups, whereas antibody titers for HHV-6 differed significantly between children with AD and those with non-AD ASD. At multivariate analysis (GLM), no variable was independently associated with anti-HHV-6 OD. In detail, *p*-values for age, sex and AD condition *vs*. non-AD ASD condition were 0.704, 0.095, 0.358, respectively.

Therefore, neither antibody level nor seropositivity rate was associated with ASD status.

Discussion

Viruses of the herpesviridae family have several characteristics that make them an intriguing topic of study and research. In fact, they employ a plethora of mechanisms to circumvent clearance by the host immune system. They are able to cause persistent infection, and are characterized by frequent reactivations during the host's life. According to Stack *et al*. (39), HHVs are able to activate immune inhibitory pathways that control autoimmunity and limit excessive responses to harmless antigen in mucosal surfaces. In this way, they can antagonize T-cell responses both during acute infection and during persistence in mucosal tissues by dampening anti-viral immunity and promoting virus survival and dissemination.

HHVs have also been implicated in several disorders, especially in autoimmune diseases, because they are known to act as triggers of immune deregulation. For instance, similar events are thought to be involved in the genesis of multiple sclerosis (40, 41), rheumatoid arthritis (42), autoimmune thrombocytopenia (43) and systemic lupus erythematosus (44, 45). Many theories, ranging from direct cytolysis of target tissue cells to such immunological processes as antigen mimicry and polyclonal lymphocyte activation have been invoked to explain the correlation between HHV infections and these autoimmune disorders. However, the exact mechanisms involved continue to elude investigators.

ASD is widely described as being a disorder of immune deregulation (18, 46-50) that, in an early or late stage of brain development, causes neurological damage in one or more areas of the brain. Numerous studies focused on virus infection, as a specific co-factor in the triggering of this deranged immune response in ASD pathogenesis (17, 18, 31, 33, 34, 51-54). Several members of herpesviridae family, *i.e*., HSV (20, 55-58), CMV (13, 21-30, 59, 60) and VZV (61), have been reported to be associated with ASD, based on clinical studies or case series. Indeed, HHVs can trigger the production of a variety of pro-inflammatory cytokines during the acute phase of infection (62-64), as demonstrated by high levels of interferon in brain tissue during HSV-induced encephalitis (65). Despite its high prevalence, studies of HHV-6 in ASD are scarce (66-68).Yet, this virus is closely associated with a complex immune deregulation condition called drug-induced hypersensitivity syndrome (69-71). This disorder is an infectious disease due to HHV-6, an allergic systemic reaction to medicines and a complex event of deregulation of the immune system (69).

Although exanthema subitum is the most common manifestation of HHV-6 infection, this virus is known as a neurotropic virus (72-74) and can result in prolonged, recurrent seizures and meningitis in a minority of children. It can also cause encephalitis in otherwise healthy children. These children exhibit an altered level of consciousness, seizures, psychosis, or cranial nerve deficits (75-77), and a case of developmental regression ensuing HHV-6 encephalitis has been described (78). Bozzola *et al*. reported three cases of HHV-6 meningoencephalitis in previously healthy children followed-up for 10 years. Two of the patients presented invalidating sequelae (79). In detail, one patient developed speech disturbance and the other persistent hemiplegia and bilateral visual deficit. The authors concluded that HHV-6 meningoencephalitis could be associated with a wide range of clinical outcomes, from long-term neurological sequelae to a benign post-infectious clinical course (79). Binstock hypothesized that several ASD subgroups could derive from intra-monocyte chronic infections with pathogens such as measles virus, CMV or HHV-6 (80).

Two studies that evaluated the prevalence or titer of antibodies to HHV-6 in serum of patients with ASD *vs*. controls (66, 68), did not show significant differences between the two groups. Results for serological association between HHV-6 and brain autoantibodies were conflicting. In the first study, Singh *et al*. found higher rates of antibodies to basic myelin protein and neuron-axon filament protein in ASD (84% of ASD cases with serum positive for HHV-6-IgG were also positive for auto-antibodies to myelin basic protein and 72% of HHV-6-IgG-positive were also positive for anti-neuron–axon filament protein) (66). On the contrary, the second article showed no serological association for HHV-6 and several brain auto-antibodies (beta-amyloid protein, brain stem; cerebral cortex; cerebellum; caudate nucleus; 2',3'-cyclic nucleotide phosphohydrolase; galactocerebroside; glial filament acidic protein; hippocampus; myelin basic protein; neuron-axon filament protein; S100 protein) (68).

Similarly to these studies, we found a comparable prevalence and levels of antibodies to HHV-6 between cases and controls. Moreover, we first looked for an association between rate of positivity or antibody levels and severity of the diseases assessed with severity scales. We found no difference between more severe and milder forms of ASD. In fact, neither the seropositivity rate nor antibody titers for HHV-6 differed significantly between children with AD and those with non-AD ASD. In fact, the finding of higher levels of antibodies to HHV-6 observed at univariate analysis in non-AD ASD *versus* AD was not confirmed at multivariate analysis. Taken together, these results do not definitively rule out a role of HHV-6 in ASD pathogenesis because we cannot exclude that a genetic and environmental predisposition may make these children more vulnerable to the virus (18). However, our data fail to provide any evidence of such an association.

No study has yet assessed whether HHV-8 is associated with ASD. No study evaluated its possible role in the onset of ASD, although it belongs to the herpesviridae family and shares with the other members of this family specific immunopathogenetic mechanisms regarding persistence and triggering of immune disorders. In particular, a study on the contribution of innate immunity to immune surveillance of this virus revealed substantial alterations of the Natural Killer cell receptor repertoire in healthy HHV-8 carriers and these observations are consistent with distinct immune evasion mechanisms that allow HHV-8 to elude Natural Killer cell responses in a stepwise fashion, first at the early stages of infection to facilitate the maintenance of viral latency, and later to promote tumor cell growth (81). Our study demonstrates that HHV-8 exposure is minimal among children regardless of their health status. This prevalence (1 out of 58 children) reflects data on HHV-8 prevalence (82, 83) which is reported to increase with age (84, 85).

In conclusion, our study shows that the rate of previous infections with HHV-8 is minimal in both healthy children and those with ASD and that the rate of HHV-6 exposure is not dissimilar between these children, nor it is related to the severity of ASD. New strategies, including the simultaneous study of co-factors implicated in ASD (18) and the use of animal models of ASD are needed to elucidate the role (if any) of HHV-6 infection in the pathogenesis of ASD.

Acknowledgements

The Authors thank Jean Ann Gilder (Scientific Communication SRL) for text editing.

References

1 American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Washington, DC: American Psychiatric Association, 2000.

2 Yeargin-Allsopp M, Rice C, Karapurkar T, Doernberg N, Boyle C and Murphy C: Prevalence of autism in a US metropolitan area. JAMA *289*: 49-55, 2003.

3 Nazeer A: Psychopharmacology of autistic spectrum disorders in children and adolescents. Pediatr Clin North Am *58*: 85-97, 2011.

4 Autism and Developmental Disabilities Monitoring Network Surveillance Year 2008 Principal Investigators; Centers for Disease Control and Prevention: Prevalence of autism spectrum disorders-Autism and Developmental Disabilities Monitoring Network, 14 sites, United States, 2008. MMWR Surveill Summ *61*: 1-19, 2012.

5 Fombonne E: Epidemiology of autism. *In*: Tremblay RE, Boivin M, Peters RDeV (eds.). Encyclopedia on Early Childhood Development [online]. Montreal, Quebec: Centre of Excellence

for Early Childhood Development and Strategic Knowledge Cluster on Early Child Development, 2012.
6 Chaste P and Leboyer M: Autism risk factors: Genes, environment, and gene -environment interactions. Dialogues Clin Neurosci *14*: 281-292, 2012.
7 Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, Miller J, Fedele A, Collins J, Smith K, Lotspeich L, Croen LA, Ozonoff S, Lajonchere C, Grether JK and Risch N: Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry *68*: 1095-1102, 2011.
8 Zafeiriou DI, Ververi A, Dafoulis V, Kalyva E and Vargiami E: Autism spectrum disorders: The quest for genetic syndromes. Am J Med Genet B Neuropsychiatr Genet *162*: 327-366, 2013.
9 Chess S, Fernandez P and Korn S: Behavioral consequences of congenital rubella. J Pediatr *93*: 699-703, 1978.
10 Christianson AL, Chesler N and Kromberg JG: Fetal valproate syndrome: Clinical and neuro-developmental features in two sibling pairs. Dev Med Child Neurol *36*: 361-369, 1994.
11 Miller MT, Stromland K, Ventura L, Johansson M, Bandim JM and Gillberg C: Autism associated with conditions characterized by developmental errors in early embryogenesis: A mini review. Int J Dev Neurosci *23*: 201-219, 2005.
12 Persico AM and Bourgeron T: Searching for ways out of the autism maze: Genetic, epigenetic and environmental clues. Trends Neurosci *29*: 349-358, 2006.
13 Yamashita Y, Fujimoto C, Nakajima E, Isagai T and Matsuishi T: Possible association between congenital cytomegalovirus infection and autistic disorder. J Autism Dev Disord *33*: 455-459, 2003.
14 Herbert MR, Russo JP, Yang S, Roohi J, Blaxill M, Kahler SG, Cremer L and Hatchwell E: Autism and environmental genomics. Neurotoxicology *27*: 671-684, 2006.
15 DiCicco-Bloom E, Lord C, Zwaigenbaum L, Courchesne E, Dager SR, Schmitz C, Schultz RT, Crawley J and Young LJ: The developmental neurobiology of autism spectrum disorder. J Neurosci *26*: 6897-6906, 2006.
16 Rogers SJ: What are infant siblings teaching us about autism in infancy? Autism Res 2: 125-137, 2009.
17 Libbey JE, Sweeten TL, McMahon WM and Fujinami RS: Autistic disorder and viral infections. J Neurovirol *11*: 1-10, 2005.
18 Gentile I, Zappulo E, Militerni R, Pascotto A, Borgia G and Bravaccio C: Etiopathogenesis of autism spectrum disorders: Fitting the pieces of the puzzle together. Med Hypotheses *81*: 26-35, 2013.
19 Gentile I, Bravaccio C, Bonavolta R, Zappulo E, Scarica S, Riccio MP, Settimi A, Portella G, Pascotto A and Borgia G: Response to measles-mumps-rubella vaccine in children with autism spectrum disorders. In Vivo *27*: 377-382, 2013.
20 Mora M, Quintero L, Cardenas R, Suarez-Roca H, Zavala M and Montiel N: Association between HSV-2 infection and serum anti-rat brain antibodies in patients with autism. Invest Clin *50*: 315-326, 2009. Article in Spanish.
21 Stubbs EG: Autistic symptoms in a child with congenital cytomegalovirus infection. J Autism Child Schizophr *8*: 37-43, 1978.
22 Markowitz PI: Autism in a child with congenital cytomegalovirus infection. J Autism Dev Disord *13*: 249-253, 1983.
23 Ivarsson SA, Bjerre I, Vegfors P and Ahlfors K: Autism as one of several disabilities in two children with congenital cytomegalovirus infection. Neuropediatrics *21*: 102-103, 1990.
24 Sweeten TL, Posey DJ and McDougle CJ: Brief report: Autistic disorder in three children with cytomegalovirus infection. J Autism Dev Disord *34*: 583-586, 2004.
25 Cannon MJ and Davis KF: Washing our hands of the congenital cytomegalovirus disease epidemic. BMC Public Health *5*: 70, 2005.
26 Kawatani M, Nakai A, Okuno T, Kobata R, Moriuchi M, Moriuchi H, Tsukahara H and Mayumi M: Detection of cytomegalovirus in preserved umbilical cord from a boy with autistic disorder. Pediatr Int *52*: 304-307, 2010.
27 Fowler KB, Stagno S, Pass RF, Britt WJ, Boll TJ and Alford CA: The outcome of congenital cytomegalovirus infection in relation to maternal antibody status. N Engl J Med *326*: 663-667, 1992.
28 Alford CA, Stagno S, Pass RF and Britt WJ: Congenital and perinatal cytomegalovirus infections. Rev Infect Dis *12(Suppl 7)*: S745-753, 1990.
29 Stagno S and Whitley RJ: Herpesvirus infections of pregnancy. Part I: Cytomegalovirus and Epstein-Barr virus infections. N Engl J Med *313*: 1270-1274, 1985.
30 Adler SP, Nigro G and Pereira L: Recent advances in the prevention and treatment of congenital cytomegalovirus infections. Semin Perinatol *31*: 10-18, 2007.
31 Ciaranello AL and Ciaranello RD: The neurobiology of infantile autism. Annu Rev Neurosci *18*: 101-128, 1995.
32 Fatemi SH, Folsom TD, Reutiman TJ, Abu-Odeh D, Mori S, Huang H and Oishi K: Abnormal expression of myelination genes and alterations in white matter fractional anisotropy following prenatal viral influenza infection at E16 in mice. Schizophr Res *112*: 46-53, 2009.
33 Bonthius DJ and Perlman S: Congenital viral infections of the brain: Lessons learned from lymphocytic choriomeningitis virus in the neonatal rat. PLoS Pathog *3*: e149, 2007.
34 Lintas C, Altieri L, Lombardi F, Sacco R and Persico AM: Association of autism with polyomavirus infection in postmortem brains. J Neurovirol *16*: 141-149, 2010.
35 Gentile I, Altieri L, Lintas C, Sacco R, Curatolo P, Benvenuto A, Muratori F, Santocchi E, Bravaccio C, Lenti C, Faggioli R, Rigardetto R, Gandione M, Portella G, Zappulo E, Borgia G and Persico A: Urinary polyomavirus infections in neuro-developmental disorders. Open J Psychiatr *3*: 18-25, 2013.
36 Lord C, Rutter M and Le Couteur A: Autism Diagnostic Interview-Revised: a revised version of a diagnostic interview for caregivers of individuals with possible pervasive developmental disorders. J Autism Dev Disord *24*: 659-685, 1994.
37 Schopler E, Reichler RJ and Renner BR: The Childhood Autism Rating Scale (CARS). Los Angeles, CA. Western Psychological Service Inc, 1988.
38 Lord C, Risi S, Lambrecht L, Cook EH Jr., Leventhal BL, DiLavore PC, Pickles A and Rutter M: The autism diagnostic observation schedule-generic: A standard measure of social and communication deficits associated with the spectrum of autism. J Autism Dev Disord *30*: 205-223, 2000.
39 Stack G, Stacey MA and Humphreys IR: Herpesvirus exploitation of host immune inhibitory pathways. Viruses *4*: 1182-1201, 2012.
40 Jankosky C, Deussing E, Gibson RL and Haverkos HW: Viruses and vitamin D in the etiology of type 1 diabetes mellitus and multiple sclerosis. Virus Res *163*: 424-430, 2012.
41 Virtanen JO and Jacobson S: Viruses and multiple sclerosis. CNS Neurol Disord Drug Targets *11*: 528-544, 2012.

42 Kurbanov SA and Mamedov MK: Serological markers of viral infections in patients with rheumatoid arthritis. Georgian Med News: 65-67, 2009. Article in Russian.

43 Gentile I, Bonadies G, Buonomo AR, Minei G, Borrelli F, Foggia M, Chiurazzi F and Borgia G: Resolution of autoimmune thrombocytopenia associated with raltegravir use in an HIV-positive patient. Platelets *6*: 6, 2012.

44 Lossius A, Johansen JN, Torkildsen O, Vartdal F and Holmoy T: Epstein-Barr virus in systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis-association and causation. Viruses *4*: 3701-3730, 2012.

45 Draborg AH, Duus K and Houen G: Epstein-Barr virus and systemic lupus erythematosus. Clin Dev Immunol *370516*: 3, 2012.

46 Bilbo SD, Jones JP and Parker W: Is autism a member of a family of diseases resulting from genetic/cultural mismatches? Implications for treatment and prevention. Autism Res Treat *910946*: 26, 2012.

47 Persico AM, Van de Water J and Pardo CA: Autism: Where Genetics Meets the Immune System. Autism Res Treat *2012*: 1-2, 2012.

48 El-Ansary A and Al-Ayadhi L: Neuroinflammation in autism spectrum disorders. J Neuroinflammation *9*: 265, 2012.

49 Jyonouchi H, Sun S and Le H: Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. J Neuroimmunol *120*: 170-179, 2001.

50 Comi AM, Zimmerman AW, Frye VH, Law PA and Peeden JN: Familial clustering of autoimmune disorders and evaluation of medical risk factors in autism. J Child Neurol *14*: 388-394, 1999.

51 Gregg NM: Congenital cataract following German measles in the mother. 1941. Aust N Z J Ophthalmol *19*: 267-276, 1991.

52 Desmond MM, Wilson GS, Verniaud WM, Melnick JL and Rawls WE: The early growth and development of infants with congenital rubella. Adv Teratol *4*: 39-63, 1970.

53 Stubbs EG: Autistic children exhibit undetectable hemagglutination-inhibition antibody titers despite previous rubella vaccination. J Autism Child Schizophr *6*: 269-274, 1976.

54 Martin WJ: Stealth virus isolated from an autistic child. J Autism Dev Disord *25*: 223-224, 1995.

55 DeLong GR, Bean SC and Brown FR, 3rd: Acquired reversible autistic syndrome in acute encephalopathic illness in children. Arch Neurol *38*: 191-194, 1981.

56 Gillberg C: Onset at age 14 of a typical autistic syndrome. A case report of a girl with herpes simplex encephalitis. J Autism Dev Disord *16*: 369-375, 1986.

57 Greer MK, Lyons-Crews M, Mauldin LB and Brown FR, 3rd: A case study of the cognitive and behavioral deficits of temporal lobe damage in herpes simplex encephalitis. J Autism Dev Disord *19*: 317-326, 1989.

58 Ghaziuddin M, Tsai LY, Eilers L and Ghaziuddin N: Brief report: Autism and herpes simplex encephalitis. J Autism Dev Disord *22*: 107-113, 1992.

59 Kitajima J, Inoue H, Ohga S, Kinjo T, Ochiai M, Yoshida T, Kusuhara K and Hara T: Differential transmission and postnatal outcomes in triplets with intrauterine cytomegalovirus infection. Pediatr Dev Pathol *15*: 151-155, 2012.

60 Dogan Y, Yuksel A, Kalelioglu IH, Has R, Tatli B and Yildirim A: Intracranial ultrasound abnormalities and fetal cytomegalovirus infection: Report of 8 cases and review of the literature. Fetal Diagn Ther *30*: 141-149, 2011.

61 Knobloch H and Pasamanick B: Some etiologic and prognostic factors in early infantile autism and psychosis. Pediatrics *55*: 182-191, 1975.

62 Dalod M, Salazar-Mather TP, Malmgaard L, Lewis C, Asselin-Paturel C, Briere F, Trinchieri G and Biron CA: Interferon alpha/beta and interleukin 12 responses to viral infections: Pathways regulating dendritic cell cytokine expression *in vivo*. J Exp Med *195*: 517-528, 2002.

63 Salazar-Mather TP, Hamilton TA and Biron CA: A chemokine-to-cytokine-to-chemokine cascade critical in antiviral defense. J Clin Invest *105*: 985-993, 2000.

64 Orange JS and Biron CA: An absolute and restricted requirement for IL-12 in natural killer cell IFN-gamma production and antiviral defense. Studies of natural killer and T-cell responses in contrasting viral infections. J Immunol *156*: 1138-1142, 1996.

65 Legaspi RC, Gatmaitan B, Bailey EJ and Lerner AM: Interferon in biopsy and autopsy specimens of brain. Its presence in herpes simplex virus encephalitis. Arch Neurol *37*: 76-79, 1980.

66 Singh VK, Lin SX and Yang VC: Serological association of measles virus and human herpesvirus-6 with brain autoantibodies in autism. Clin Immunol Immunopathol *89*: 105-108, 1998.

67 Nicolson GL, Gan R, Nicolson NL and Haier J: Evidence for *Mycoplasma* ssp., *Chlamydia pneumoniae*, and human herpes virus-6 coinfections in the blood of patients with autistic spectrum disorders. J Neurosci Res *85*: 1143-1148, 2007.

68 Singh VK: Phenotypic expression of autoimmune autistic disorder (AAD): A major subset of autism. Ann Clin Psychiatry *21*: 148-161, 2009.

69 Gentile I, Talamo M and Borgia G: Is the drug-induced hypersensitivity syndrome (DIHS) due to human herpesvirus 6 infection or to allergy-mediated viral reactivation? Report of a case and literature review. BMC Infect Dis *10*: 49, 2010.

70 Descamps V: Drug reaction with eosinophilia and systemic symptoms (DRESS). Rev Prat *62*: 1347-1352, 2012.

71 Dreyfus DH: Herpes viruses and the microbiome. J Allergy Clin Immunol. In press

72 Campadelli-Fiume G, Mirandola P and Menotti L: Human herpesvirus 6: An emerging pathogen. Emerg Infect Dis *5*: 353-366, 1999.

73 Braun DK, Dominguez G and Pellett PE: Human herpesvirus 6. Clin Microbiol Rev *10*: 521-567, 1997.

74 Caserta MT, Hall CB, Schnabel K, McIntyre K, Long C, Costanzo M, Dewhurst S, Insel R and Epstein LG: Neuroinvasion and persistence of human herpesvirus 6 in children. J Infect Dis *170*: 1586-1589, 1994.

75 Asano Y, Yoshikawa T, Kajita Y, Ogura R, Suga S, Yazaki T, Nakashima T, Yamada A and Kurata T: Fatal encephalitis/encephalopathy in primary human herpesvirus-6 infection. Arch Dis Child *67*: 1484-1485, 1992.

76 Ishiguro N, Yamada S, Takahashi T, Takahashi Y, Togashi T, Okuno T and Yamanishi K: Meningo-encephalitis associated with HHV-6 related exanthem subitum. Acta Paediatr Scand *79:* 987-989, 1990.

77 Suga S, Yoshikawa T, Asano Y, Kozawa T, Nakashima T, Kobayashi I, Yazaki T, Yamamoto H, Kajita Y, Ozaki T, Nishimura Y, Yamanaka T, Yamada A and Imanishi J: Clinical and virological analyses of 21 infants with *exanthem subitum* (*roseola infantum*) and central nervous system complications. Ann Neurol *33*: 597-603, 1993.

78 Pulickal AS, Ramachandran S, Rizek P, Narula P and Schubert R: Chorea and developmental regression associated with human herpes virus-6 encephalitis. Pediatr Neurol *48*: 249-251, 2013.
79 Bozzola E, Krzysztofiak A, Bozzola M, Calcaterra V, Quondamcarlo A, Lancella L and Villani A: HHV6 meningo-encephalitis sequelae in previously healthy children. Infection *40*: 563-566, 2012.
80 Binstock T: Intra-monocyte pathogens delineate autism subgroups. Med Hypotheses *56*: 523-531, 2001.
81 Dupuy S, Lambert M, Zucman D, Choukem SP, Tognarelli S, Pages C, Lebbe C and Caillat-Zucman S: Human Herpesvirus 8 (HHV8) sequentially shapes the NK cell repertoire during the course of asymptomatic infection and Kaposi sarcoma. PLoS Pathog *8*: e1002486, 2012.
82 Gao SJ, Kingsley L, Li M, Zheng W, Parravicini C, Ziegler J, Newton R, Rinaldo CR, Saah A, Phair J, Detels R, Chang Y and Moore PS: KSHV antibodies among Americans, Italians and Ugandans with and without Kaposi's sarcoma. Nat Med *2*: 925-928, 1996.
83.Whitby D, Luppi M, Barozzi P, Boshoff C, Weiss RA and Torelli G: Human herpesvirus 8 seroprevalence in blood donors and lymphoma patients from different regions of Italy. J Natl Cancer Inst *90*: 395-397, 1998.
84 Cattani P, Cerimele F, Porta D, Graffeo R, Ranno S, Marchetti S, Ricci R, Capodicasa N, Fuga L, Amico R, Cherchi G, Gazzilli M, Zanetti S and Fadda G: Age-specific seroprevalence of human herpesvirus 8 in Mediterranean regions. Clin Microbiol Infect *9*: 274-279, 2003.
85 Serraino D, Toma L, Andreoni M, Butto S, Tchangmena O, Sarmati L, Monini P, Franceschi S, Ensoli B and Rezza G: A seroprevalence study of human herpesvirus type 8 (HHV8) in eastern and Central Africa and in the Mediterranean area. Eur J Epidemiol *17*: 871-876, 2001.

Received July 6, 2013
Revised August 8 2013
Accepted August 9, 2013