in vivo *28*: 667-672 (2014)

Prevalence of Herpes Simplex Virus 1 and 2 Antibodies in Patients with Autism Spectrum Disorders

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Abstract. *Background/Aim: The etiology of autism spectrum disorder (ASD) is unknown, even though it is hypothesized that a viral infection could trigger this disorder. The aim of this study was to evaluate the seropositivity rate and antibody level of Herpes Simplex Virus 1 (HSV1) and Herpes Simplex Virus 2 (HSV2) in children with ASD compared to same-aged healthy controls. Patients and Methods: We compared seropositivity rate and levels of antibodies to HSV1/2 in 54 children with ASD (19 with autistic disorder and 35 with non-autistic ASD) and in 46 controls. Results: Seropositivity rate and levels of anti-HSV1/2 were not dissimilar between cases and controls. Exposure to HSV2 was minimal. Conclusion: Rate of contact with HSV1 and HSV2 assessed by the mean of detection of specific antibodies was similar between children with ASD and healthy controls.*

The autism spectrum disorders (ASD) are developmental disorders defined by significantly abnormal social interaction, impaired communication, language abilities, and narrow pattern of interests. According to the Diagnostic and Statistical Manual of Mental Disorders, IV edition-text revision (1), Autistic Disorder (AD) is the most severe form of ASD. Asperger syndrome, Rett syndrome, Childhood Disintegrative Disorder and Pervasive Developmental Disorder Not Otherwise Specified are considered milder forms of ASD. The American Psychiatric Association has just published the fifth edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM-5) (2). The diagnostic criteria for ASD have been modified based on the literature and clinical experience in the 19 years since the DSM-IV was published in 1994. In particular, the diagnosis will be called Autism Spectrum Disorder and there no longer will be sub-diagnoses. Nevertheless, at the time this study was performed, the new manual had not been published, therefore we decided to refer to the previous version.

The prevalence of ASD is increasing and the current prevalence rate is alarming. In fact one in 88 newborns is currently affected by ASD in the USA, with a specific male dominance (5:1 ratio between males and females) (3). However, a recent study performed in the UK suggests lower figures (annual incidence rate of about 1.2/1,000 boys and 0.2/1,000 girls) (4).

Only about 10% of patients with a diagnosis of ASD have a defined aetiology (so-called syndromic autism, secondary to fragile X syndrome, neurofibromatosis, exposure to thalidomide) (5, 6), while 90% of ASD cases are considered idiopathic (*i.e.* without a definite aetiological agent) (3). In these cases, genetic factors are known to be relevant. However, genetics cannot explain the rapid increase of the prevalence observed in recent years. For this reason, most authors consider that the aetiology of ASD lies in an interaction between genetic and environmental factors (7, 8). The absence of a definite causative agent makes the set-up of preventative measures for ASD impossible.

Several studies have assessed the role of infections, measles vaccine, vitamin D deficiency, or oxidative stress (6, 9-19) as aetiological agents of ASD but none provided definite conclusions. We recently proposed that ASD is due to deranged immune system responses that in individuals with a genetic predisposition to autoimmune disorders and environmental susceptibility (likely associated with vitamin D deficiency) are triggered by a viral infection and lead to the impairment of specific areas in the central nervous system.

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Key Words: Autism spectrum disorder, etiopathogenesis, HSV1, HSV2.

Some case reports show an association of herpes simplex virus (HSV) infection and ASD onset (20, 21).

Two prospective studies assessed the prevalence of antibodies to HSV in a cohort of children with psychiatric disorders and in healthy controls and found discordant results (17, 22).

The aim of this study was to evaluate and compare the prevalence of HSV exposure (assessed *via* the presence of specific antibodies) in a cohort of patients with ASD and in healthy controls. Moreover, we compared the levels of antibodies to HSV in the two groups.

Patients and Methods

Patients. We recruited patients among those admitted 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, between January 2010 and January 2013. Informed consent was obtained from all children's parents or legally authorized representatives and identifying information was removed from each sample. 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 was 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 of the Federico II University of Naples, Italy, where they were admitted for minor surgical treatments (*e.g.* phimosis, hernia, cryptorchidism, vesicoureteral reflux, and hydrocele testis). 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 (23), the Childhood Autism Rating Scales (CARS) (24) and the Autism Diagnostic Observation Schedule (ADOS)-Generic (25).

Adaptive functioning was assessed by the mean of Vineland Adaptive Behavior Scales (26). Developmental quotient was determined using the Griffiths Mental Developmental Scales (27) .

Virological tests. For the determination of specific IgG antibodies to HSV1 and -2 in human serum, an indirect chemiluminescence immunoassay (CLIA) was performed (LIAISON® HSV-1/2 IgG assay; DiaSorinS.p.A., Saluggia,VC, Italy). This test is unable to discriminate between HSV1 and HSV2 antibodies. For this reason, we refer to these antibodies as total anti-HSV. To discriminate among the two viruses, sample which tested positive at this first test were analysed with a specific HSV2 antibody test (LIAISON® HSV-2 IgG assay; DiaSorinS.p.A., Saluggia,VC, Italy).

For the determination of total HSV antibodies (or HSV2 antibodies), HSV recombinant proteins (or HSV-2 specific gG2 recombinant protein) were used for coating magnetic particles (solid phase), while a mouse monoclonal antibody was linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, total HSV antibodies (or HSV2 antibodies), present in calibrators, samples or controls, bound to the solid phase. During the second incubation, the antibody conjugate reacted with total HSV IgG (or HSV2 IgG), already bound to the solid phase. After each incubation, the unbound material was removed with a wash cycle.

Subsequently, the starter reagents were added and a flash chemiluminescence reaction was thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, was measured by a photomultiplier as relative light units (RLU) and was indicative of HSV IgG (or HSV2 IgG), concentration present in calibrators, samples or controls.

Test of assay-specific calibrators allowed to adjust the assigned master curve. The analyzer automatically calculated total HSV IgG levels (or HSV2 IgG levels), expressed as index value and graded the results.

The cut-off value discriminating between the presence and the absence of total HSV IgG (or HSV2 IgG), has an index value of 1. Sample results were interpreted as follows: Samples with total HSV IgG levels (or HSV2 IgG levels) below an index value of 0.9 were graded as negative; those with levels ranging between 0.9 and 1.1 considered equivocal by the manufacturer were also graded as negative in the present study; those with an index value of 1.1 or more were graded as positive. Index values <0.5 were considered as 0.5 for statistical analysis.

Statistical analysis. Quantitative variables were checked for Gaussian distribution using Kolmogorov–Smirnov test. In cases of Gaussian distribution, data are presented as mean±standard deviation (SD), while non-Gaussian distributed data are given as median and interquartile range (IQR). Student's *t*-test for unpaired variables (or ANOVA for comparisons of more than two groups) or Mann–Whitney *U*-test (or Kruskal–Wallis test for comparisons of more than two groups) were used for comparison of quantitative variables in cases of Gaussian or non-Gaussian distribution, respectively. For comparison of qualitative variables, the χ^2 test (or Fisher's exact test in case of expected counts fewer than 5) was used. Spearman's rho test was used for correlations between VABS and GMDS. A multivariate analysis for ASD status was carried out using logistic regression analysis models including age, gender and presence or levels of HSV antibodies. A *p*-value less than 0.05 on two-sided testing was considered statistically significant. Statistical analysis was performed using the Statistical Package for the Social Sciences, version 18.0 (SPSS Inc. Chicago, IL, USA).

Table I. *Demographic and clinical features of children with Autism Spectrum Disorders*.

	Median (interquartile range)
GMDS (Developmental quotient)	50 (43-59)
VABS (Adaptive quotient)	50 (38-58)
ADOS (Language)	5 (4-6)
ADOS (Interaction)	11 (8-12)
ADOS (Total score)	16 (13-18)
CARS (Total score)	36 (33-40)

GMDS: Griffith mental developmental scales.VABS: Vineland adaptive behavior scales. ADOS: Autism diagnostic observation schedule. CARS: Childhood autism rating scales.

Table II. *Rate of seropositivity to total HSV IgG and HSV2 IgG in cases and controls and in patients with autistic disorder (AD) or non-autistic disorder autism spectrum disorders (non-AD ASD).*

Seropositivity against		Cases	Controls	*p*-Value
Total HSV-IgG	16/54 (29.6%)	AD 8/19 (42.1%)	10/46 (21.7%)	0.370(χ^2)†
		Non-AD ASD 8/35 (22.9%)		0.236 (F)‡
HSV2-IgG	1/52 (1.9%)	AD 0/19 (0%)	0/40 (0%)	1.000 (F)†
		Non-AD ASD 1/33 (3%)		0.565 (F)‡

χ^2: χ^2 test; F: Fisher exact test; †cases *vs*. controls; ‡AD *vs*. non-AD ASD *vs*. controls.

Table III. *Total HSV IgG and HSV2 IgG levels [median (interquartile range)] in cases and controls and in patients with autistic disorder (AD) or non-autistic disorder autism spectrum disorders (non-AD ASD).*

		Cases	Controls	*p*-Value
Total HSV-IgG (Index)	0.5 (0.5-6.28)	AD 0.6 (0.5-15.6)	0.5 (0.5-0.67)	0.346 (U)†
		Non-AD ASD 0.5 (0-0.52)		0.094 (KW)‡
HSV2-IgG (Index)	0.5 (0.5-0.5)	AD 0.5 (0.5-0.5)	0.5 (0.5-0.5)	0.701 (U)†
		Non-AD ASD 0.5 (0.5-0.5)		0.850 (KW)‡

U: Mann–Whitney *U*-test; KW: Kruskal–Wallis test; †cases *vs*. controls; ‡AD *vs*. non-AD ASD *vs*. controls.

Results

We enrolled 100 children in the study, 54 with ASD (19 with AD) and 35 with non-AD ASD) and 46 controls. None of the controls was found to be affected by a neuropsychiatric disorder. The mean age was 6.1 (±2.5) years for cases and 5.9 (±2.8) years for controls (p=0.775). Males out numbered females, both among cases (41/54, 75.9%) and controls (39/46, 84.8%, p=0.270). The neuropsychiatric and clinical features of the children with ASD are shown in Table I.

We assessed the rate of seropositivity for the two types of HSV in cases and controls. As shown in Table II, this rate was similar in the two groups (for total HSV, 29.6% *vs*. 21.7%; for HSV2, 1.9% *vs*. 0%, respectively).

We measured and compared antibody levels of total HSV and HSV2 in cases and controls and, as shown in Table III, they did not differ between the two groups.

In a logistic regression analysis model, which included age and gender, it was confirmed that the presence or the titre of total HSV antibody was not an independent predictor for health condition (ASD status or health).

We also evaluated the levels and seropositivity rate for the two antibodies in three specific categories (children with AD, children with non-AD ASD and controls). As shown in Table II and III, neither the seropositivity rate nor antibody levels differed significantly among the three groups.

Among children with ASD, we correlated severity scores, namely GMDS and VABS, with HSV antibody level using Spearman's Rho test. None of them showed a significant correlation with the antibody levels. For the total HSV, p-values were –0.175 (p=0.210) and –0.161 (p=0.249) and for HSV2 were – 0.025 (p=0.861) and –0.031 (p=0.831) for GMDS and VABS, respectively.

Finally, we evaluated the median values of severity scales in HSV seropositive and seronegative children with ASD. GMDS was 47 (IQR=37-54) *vs*. 53.5 (IQR=44.5-60.8, p=0.282) and VABS was 45 (IQR=35-55) *vs*. 52 (IQR=40.3-63.3, p=0.221) in seropositive and seronegative patients, respectively.

Discussion

Some researchers have hypothesized a viral trigger in the aetiopathogenesis of ASD (8). The research for such a trigger must include studies on the Herpesviridae family. In fact, these viruses have both a tropism for the central nervous system and, more importantly, they set up complex interactions with host immune system and are suspected to trigger other immune disorders (28-30)

Several authors reported clinical cases about the onset of ASD following herpes encephalitis (20-22, 31-33) In one case, ASD recovered after recovery from HSV-related encephalitis (31).

Our study shows that patients with ASD and same-aged controls have similar rates of total HSV exposure. These findings were confirmed using a multivariate model that took into account age and gender. However, a trend for high HSV antibody levels in those with AD *vs*. non-AD ASD and controls (p=0.094 for difference in the three groups) was noticed.

Our findings are similar to a study by Sylvester Jorgensen *et al*. who found a similar prevalence of antibodies to HSV in psychiatric patients and healthy individuals. However, the population of patients enrolled in their study was quite heterogeneous as it was made-up of patients with conduct disorder, emotional disorder, hyperkinetic syndrome, anorexia nervosa, infantile autism and borderline schizophrenia in childhood. Our study enrolled only patients with autism.

In contrast, a study by Mora *et al*. found a higher prevalence of anti-HSV IgM antibodies in children with ASD than in healthy controls (17). Interestingly, in this study the autistic patients with antibodies to HSV had also a higher rate of anti-encephalon antibodies than autistic children without antibodies to HSV, thus suggesting a role of viral infection in triggering an immune-mediated damage (17).

Concerning HSV2, it is noteworthy that only one out of 100 patients enrolled in our study was found to have a previous HSV2 infection (a child with non-AD ASD). Therefore nearly all those with total antibodies to HSV had actual exposure to HSV1.

The negative findings of the present study do not rule-out a role of HSV for two reasons: i) the non-significant trend of higher levels of antibodies to HSV in those with AD than non-AD ASD or controls deserves further investigation due to the relatively small size of this study; ii) even with a similar prevalence between children with ASD and healthy controls, it cannot be excluded that the virus acts as a trigger or as a causative agent in some children who have genetic or environmental predisposition to these disorders (8). Under this light, a recent study found that HSV1 interacts and modifies the expression of several human genes creating a host/pathogen 'interactome' of 1347 genes (34). These genes can be involved in modulating the risk of several diseases such as schizophrenia or autism (34).

In conclusion, the rate of seroprevalence and level of total antibodies to HSV are similar in those with ASD and in same-aged healthy controls. HSV2 exposure was minimal in the overall population enrolled in the study.

Conflicts of Interest

Each Author certifies that they have no commercial associations that might pose a conflict of interest related to this study.

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Received February 4, 2014
Revised March 3, 2014
Accepted March 4, 2014