A study of serum interleukin-12 in a sample of autistic children in Egypt

Soha Ibrahim[a], Tarek El-Waleely[a], Nermine Zakaria[a], Rania Ismail[b]

[a]Department of Neuropsychiatry, Faculty of Medicine, [b]Department of Pediatrics, and Clinical Pathology, Police Hospital, Alexandria, Egypt

Correspondence to Soha Ibrahim, PhD Neuropsychiatry Department, Faculty of Medicine, Alexandria University, 23225 Alexandria, Egypt
Tel: +20 100 733 5929;
e-mail: sohaghobashy@hotmail.com

Received 24 October 2014
Accepted 24 December 2014

Egyptian Journal of Psychiatry
2015, 36:81–87

Introduction
Autistic spectrum disorders (ASDs) prevalence varies widely by sex and the racial/ethnic group. The male-to-female ratio ranged from 3 to 4 : 1. Some consider ASD to be an autoimmune disorder, in which the autoimmune response to the developing brain myelin may impair anatomical development of neural pathways in autistic children; this affects the speed of impulse transmission. Interleukin-12 (IL-12) is an interleukin that is naturally produced by dendritic cells and macrophages in response to antigenic stimulation; it plays an important role in the activities of natural killer cells and T lymphocytes involved in the immune system.

Aim of the work
The present study was conducted to compare the level of serum IL-12 between children with autistic disorder (AD) and healthy control children, and also to study the relation of serum IL-12 with the severity of autistic symptoms.

Participants and methods
A cross-sectional study was conducted on two groups; group I included 20 patients with AD and group II included 20 normal children matched for age and sex, recruited from the Child and Adolescent Outpatients Clinic at Al Hadra University Hospital. All children were subjected to a complete psychiatric history, physical and neurological examination, psychometric assessment by Childhood Autistic Rating Scale, and estimation of serum IL-12 using the enzyme-linked immunosorbent assay method.

Results
The mean serum level of IL-12 was significantly higher in AD children than in controls and was related to a younger age, male sex, a positive family history and ante/natal/postnatal history, nondevelopment of spoken language, the presence of comorbidities, and higher Childhood Autistic Rating Scale mean scores.

Conclusion
The study pointed out an immunological impairment in the form of an elevated serum level of IL-12 in autistic children and its positive relation to autistic symptom severity. This supports the immunological etiology of ASD.

Keywords:
autistic disorder, autoimmunity, Childhood Autistic Rating Scale, serum interleukin-12

Egypt J Psychiatr 36:81–87

1110-1105

Introduction

Autistic spectrum disorders (ASDs) are neurodevelopmental disorders characterized by impaired communication and social interaction, and restricted, stereotyped interests that manifest in early childhood [American Psychiatric Association (APA), 1994, 2013]. Autistic disorder (AD) is one of the ASDs that is associated with impairments in social and language functioning and repetitive behaviors (Filipek and Filipek, 1999). ASD is one of the most common childhood developmental disabilities. AD and related conditions affect 11.3 in 1000 (one in 88) children, age less than 8 years, living in 14 communities monitored by the US Centers for Disease Control and Prevention (CDC). Overall ASD prevalence estimates vary widely across the 14 monitored communities (range 4.8–21.2 in 1000 children, age less than 8 years) (Autism and Developmental Disorders Monitoring Network, United States, 2006; MMWR Surveillsumm, 2009). However, in the majority of the cases, the etiology is not known and likely involves complex interactions between genetic, epigenetic, and environmental factors (Trottier *et al.*, 1999; Glasson *et al.*, 2004; Gillberg and Cederlund, 2005; James *et al.*, 2006; Abrahams and Geschwind, 2008, 2010; Cannell, 2008; Grant and Soles, 2009; Hallmayer *et al.*, 2011).

Recent studies have described abnormal cytokine findings implicating immune dysfunction in ASD (Singh *et al.*, 1998; Licinio and Alvarado, 2002; Scaefer and Lutz, 2006; Carmody and Lewis, 2010; Costantino *et al.*, 2010; Sacco, 2010). Many potential immune disturbances have been suggested as mediators in the pathogenesis of ASD: one disturbance is autoimmunity. Autoimmunity is manifested by a flare in many cytokines (Singh, 2004, 2005; Singh and Hanson, 2006). Interleukin-12 (IL-12) is a cytokine that is naturally produced by dendritic

 DOI: 10.4103/1110-1105.158115

cells, macrophages, and human B-lymphoblastoid cells in response to antigenic stimulation. It is composed of a bundle of four a-helices. It is a heterodynamic cytokine encoded by two separate genes: IL-12A (p35) and IL-12B (p40). The active heterodimer and a homodimer of p40 are formed after protein synthesis. The normal level of IL-12 (p70) is 0–7.9 pg/ml and that of IL-12 (p40) is 39–170 pg/ml (Wang *et al.*, 2000). IL-12 has multiple biological functions and, importantly, it bridges the early nonspecific innate resistance and the subsequent antigen-specific adaptive immunity. Its expression during infection regulates innate responses and determines the type of adaptive immune responses. IL-12 induces interferon-g (IFN-g) production and triggers $CD4^+$ T cells to differentiate into type 1 T-helper (Th-1) cells. Autoimmune diseases and chronic inflammatory reactions can all be accompanied by an increase in the quantity of IL-12 (Warren *et al.*, 1986; Gately *et al.*, 1998; Wang *et al.*, 2000). In this work, we hypothesized that an increase in IL-12 among autistic children would suggest an autoimmune etiology for ASD and will be related to the autistic severity.

Aim of the work

The present study was conducted to compare the level of serum IL-12 between children with AD and healthy control children. Also, this work aimed to study the relation of serum IL-12 with the severity of autistic symptoms.

Participants and methods

Participants

This study had a cross-sectional design with nonrandom sampling, and was conducted over 1½ years (January 2012 to July 2013) at the Child and Adolescent Outpatients Clinic at Al Hadra University Hospital. Two groups were recruited: group I included 20 children diagnosed with AD and group II included 20 healthy control children matched for age and sex. The sample was taken in the period of 1 year from the onset of the study from the Child and Adolescent Outpatients Clinic at Al Hadra University Hospital. The first 20 children who fulfilled the following inclusion and exclusion criteria were recruited. Inclusion criteria were an AD diagnosed according to DSM-IV-TR diagnostic criteria (American Psychiatric Association, 2000), age 3–12 years, both sex, and written informed consent from the parents. Exclusion criteria were concurrent physical disease and infections altering the immune system and physical or neurological debilitating diseases.

Methods

The recruited sample was subjected to (a) complete history taking and clinical examination (physical and neurological), (b) a child psychiatric interview (the diagnosis was made according to DSM-IV-TR diagnostic criteria) ([], 2000), (c) psychometric assessment by the Childhood Autistic Rating Scale (CARS) (Schopler *et al.*, 1980), and (d) assessment of serum IL-12 (p40) (performed by enzyme-linked immunosorbent assay (ELISA)], which is a test that uses antibodies and color change to identify a substance (Lequin, 2005).

Childhood Autistic Rating Scale (Schopler *et al.*, 1980).

All autistic children were subjected to assessment using the CARS, which helps in the identification of children (2 years and older) with autism, specifically distinguishing them from developmentally handicapped children who are not autistic. In addition, it distinguishes between mild-to-moderate and severe autism. Its brevity makes it a very useful tool to help the recognition and the classification of autistic children.[122] It is a four-Likert scale for various criteria, ranging from normal to severe, and yields a composite score ranging from nonautistic to mild, moderate or autistic, or severely autistic. This scale was used to observe and subjectively rate 15 items, mainly relationship to people, imitation, emotional response, body use, object use, adaptation to change, visual response, listening response, taste–smell–touch response and use, fear and nervousness, verbal communication, nonverbal communication, activity level, level and consistency of intellectual response, and general impression. Each of the 15 criteria listed above is rated with the following scores: 1, normal for the child's age; 2, mildly abnormal; 3, moderately abnormal; and 4, severely abnormal. Midpoint scores of 1.5, 2.5, and 3.5 are also used. Total CARS scores range from 15 to 60, with a minimum score of 30 serving as the cutoff for the diagnosis of autism on the mild end of the autism spectrum. CARS has also been shown to have 100% predictive accuracy when distinguishing between groups of autistic and intellectually disabled children, which was superior to the diagnostic checklist (Schopler *et al.*, 1980).

Statistical analysis of the data

Data were fed to the computer and analyzed using the IBM SPSS software package version 20.0 (Belmont, California, Wadsworth Cengage Learning 2013). Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum), mean, SD, and median. Comparison between different groups regarding categorical variables

was performed using the χ^2-test. When more than 20% of the cells had an expected count less than 5, correction for χ^2 was conducted using Fisher's exact test or the Monte Carlo correction. The distribution of quantitative variables was tested for normality using the Kolmogorov–Smirnov test, the Shapiro–Wilk test and the D'Agostino test; a histogram and the QQ plot were also used for the vision test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, nonparametric tests were used. For normally distributed data, comparison between the three studied groups was performed using the *F*-test (analysis of variance) and the post-hoc test (Scheffe). For abnormally distributed data, comparison between two independent populations was performed using the Mann–Whitney test, whereas the Kruskal–Wallis test was used to compare different groups and the pairwise comparison was assessed using the Mann–Whitney test. The significance of the obtained results was judged at the 5% level (Kotz *et al.*, 2006; Kirkpatrick and Feeney 2013).

Results

Descriptive data

In the present study, there were 20 autistic patients with a mean age of 5.73 ± 2.42 years in group I [16 (80%) of them were boys and four (20%) were girls] and 20 normal children with a mean age 6.75 ± 2.57 years in group II [12 (60%) were boys and eight (40%) girls]. Both groups were matched for age (t = 1.298, P = 0.202) and sex (χ^2 = 1.905, P = 0.168). On comparing IL-12 (p40) among the two groups, it was estimated to be 285.22 ± 159.43 pg/l in the autistic sample and 52.34 ± 43.76 pg/l among controls. The higher IL-12 serum level among autistic children reached a statistically significant difference ($P \leq 0.001$). Likewise, the CARS mean score was higher among autistic children and the difference was statistically significant ($P \leq 0.001$) (Table 1). Other clinical data were collected from the history taking: autistic children had more reported positive ante/natal/postnatal complications (eclampsia, pathological jaundice) and a positive family history for pervasive developmental disorders (PDD) compared with controls, but only the difference in the family history reached statistical significance ($P \leq 0.008$) (Table 1). Only four (20%) autistic children were developing language compared with all [20 (100%)] control children, showing a statistical significance ($P \leq 0.001$) (Table 1).

Correlative study

In the present study, we tried to study the factors related to IL-12 increase. First, we found that the serum IL-12 level increased among autistic children below the age of 7 years more than in children above 7 years. Also, the serum IL-12 level was higher in boys than in girls. However, both positive relations were not statistically significant, but none reached statistical difference (age: P = 0.156, sex: P = 0.450) (Table 2). Again, positive relations were seen as an increased serum IL-12 was related to a positive ante/natal/postnatal history, a positive family history, nondevelopment of spoken language, and the presence of comorbidities

Table 1 Comparison of clinical data among the studied groups

Clinical data	Group I (n = 20)	Group II (n = 20)	Test of significance	P value
IL-12			MW test: U = 14.0, Z = 5.035	<0.001*
Minimum–maximum	128.40–562.50	18.20–171.0		
Mean ± SD	285.22 ± 159.43	52.34 ± 43.76		
Median	247.10	38.50		
CARS			t = 10.659	<0.001*
Minimum–maximum	30.0–57.0	15.0–21.0		
Mean ± SD	9.88 ± 41.10	2.24 ± 16.95		
Median	37.50	16.50		
Antenatal, natal and postnatal history [n (%)]			χ^2 = 2.506	0.113
Negative	8 (40)	13 (65)		
Positive	12 (60)	7 (35)		
Family history [n (%)]			χ^2 = 8.485	0.008*
Negative	13 (65)	20 (100)		
Positive	7 (35)	0 (0)		
Spoken language [n (%)]				
Developed	4 (20)	20 (100)	χ^2 = 26.667	<0.001*
Not developed	16 (80)	0 (0)		

CARS, Childhood Autistic Rating Scale; IL-12, interleukin-12; MW, Mann–Whitney test (U and Z are the coefficients); t, Student t-test, Statistically significant at $P \leq 0.05$, *Statistically significant.

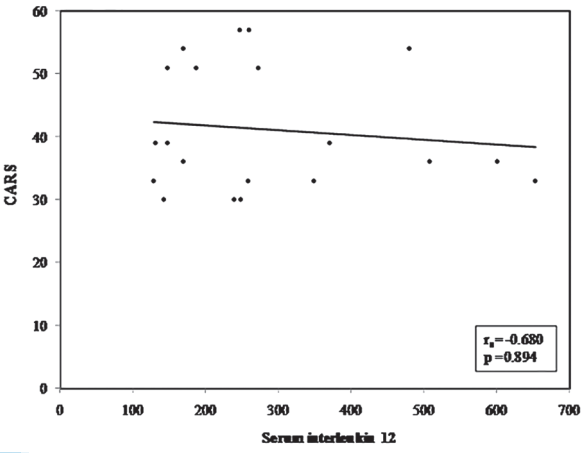
[intellectual disabilities (three children), epilepsy (nine children)]. The latter relations, however, showed no statistical significance (Table 3).

Lastly, our results showed a positive correlation between an increased serum IL-12 level and the severity of autistic symptoms as shown by CARS mean scores. The Spearman coefficient indicated a positive relation; however, it did not reach statistical significance ($r = 0.032$, $P = 0.894$) (Fig. 1).

Discussion

Current theories have focused on environmental factors, the genetic etiology, immune dysfunction, neurodevelopmental abnormalities, and yet other undiscovered factors (Fabry *et al.*, 1994). Immunologic evaluation on autistic children showed results indicating that it is one of the possible etiological theories in this disability (Todd *et al.*, 1988). The current study found significantly higher levels of serum IL-12 among autistic children compared with controls ($P \leq 0.001$). Our results seem to be in agreement with many studies examining IL-12 and other cytokines in autism (Singh, 1996; Croonenberghs *et al.*, 2002; Ashwood, 2011; Suzuki *et al.*, 2011). IL-12 was investigated in (Singh, 1996) a study that included 20 randomly selected autistic children (mean age 10.7; 16 male and four female) and 20 normal controls (mean age 10.4; 13 male and seven female). At the time of blood draw, none of them was taking any medications that alter the immune function. The quantitation of IL-12, IFN-g, IFN-a, and IL-6 was performed by ELISA. That study stated that the plasma concentration of IL-12 was ~20-fold higher in autistic patients when compared with normal controls. Approximately one-half of the

Figure 1



The correlation between serum interleukin-12 and Childhood Autistic Rating Scale (CARS).

Table 2 The relation between serum interleukin-12 with age and sex in group I

Age and Sex	Serum interleukin-12			Mann–Whitney test	*P* value
	Minimum–maximum	Mean ± SD	Median		
Age					
≤7	128.40–652.50	307.89 ± 168.98	253.40	*U* = 17.0	0.156
>7	130.50–258.10	194.55 ± 67.10	194.80	*Z* = 27.0	
Sex					
Male	128.40–652.50	299.25 ± 170.02	252.85	*U* = 24.0	0.450
Female	130.50–370.30	299.10 ± 105.79	207.80	*Z* = 34.0	

MW, Mann–Whitney test (*U* and *Z* are the coefficients), *P* value for the Mann–Whitney test statistically significant at $P \leq 0.05$.

Table 3 The relation between serum interleukin-12 and clinical data

Clinical data	Serum IL-12 (pg/l)			Mann–Whitney test	*P* value
	Minimum–maximum	Mean ± SD	Median		
Ante/natal/postnatal history					
Negative	130.50–348.80	226.08 ± 73.84	243.35	*U* = 36.0 *Z* = 0.926	0.355
Positive	128.40–652.50	324.65 ± 190.24	252.90		
Family history					
Negative	128.40–600.10	260.32 ± 149.19	239.10	*U* = 32.0 *Z* = 1.070	0.285
Positive	147.70–652.50	331.46 ± 179.26	258.10		
Spoken language					
Not Developed	143.0–258.10	221.95 ± 53.20	243.35	*U* = 25.0 *Z* = 0.661	0.508
Developed	128.40–652.50	301.04 ± 174.05	252.90		
Psychiatric comorbidities					
Absent	130.50–348.80	226.08 ± 73.84	243.35	*U* = 36.0 *Z* = 0.926	0.355
Present	128.40–652.50	324.65 ± 190.24	252.90		

IL-12, interleukin-12; MW, Mann–Whitney test (*U* and *Z* are the coefficients), *P* value for the Mann–Whitney test statistically significant at $P \leq 0.05$.

autistic patients showed an increase in this cytokine. It also showed the increase in IFN-g to be about three-fold greater in autistic patients as compared with controls (Singh, 1996).

Similarly, another study investigated evidence of differential cytokine release in plasma samples obtained from 2- to 5-year-old children with ASD compared with controls, and they included 97 participants with a confirmed diagnosis of ASD using standard assessments (DSM-IV criteria and ADOS, ADI-R) and 87 controls. Plasma was isolated and cytokine production was assessed by multiplex Luminex analysis. It stated that levels of IL-6 and IL-12 were significantly higher in children with ASD compared with controls (Ashwood, 2011). Also, another study was conducted and a multiplex assay for cytokines and chemokines was applied to plasma samples from 28 male patients with a high functioning ASD and matched 28 controls; among the total of 48 analytes examined, plasma concentrations of IL-1b, IL-5, IL-8, IL-12, IL-13, and IL-17 were significantly higher in individuals with ASD compared with the corresponding values of matched controls after correction for multiple comparisons. Plasma levels of IL-4, IL-7, IFN-c, and tumor necrosis factor-a (TNF-a) tended to be greater in the ASD group than in the control group, but after correction for multiple comparisons, the differences did not reach the level of statistical significance. Mean levels of fold changes of the cytokines differed significantly between the two groups (Suzuki *et al.*, 2011).

Croonenberghs *et al.* (2002) conducted a study on cytokines such as IFN-g, IL-6, TNF-a, and IL-1 in the whole blood of autistic children and compared them with normal controls. The study agreed with our results as they found that cytokines significantly increased in autism, especially IFN-g and IL-1, and there was a trend towards a significantly increased production of IL-6 and TNF-a in the whole blood of autistic children. There were no significant differences in the serum concentrations of IL-2 and IL-1 between autistic and normal children (Croonenberghs *et al.*, 2002).

The current immune theory in ASD explains that cytokine abnormalities may be linked to genes that encode immune-related proteins that are at fault in ASD, in response to environmental toxins. Cytokines influence aspects of brain development and synaptic functions, and therefore, any disturbance in cytokines may negatively impact neuronal function, differentiation, migration, proliferation, behavioral impairments, and clinical outcomes relevant to ASD (Goines and Ashwood, 2013). Further, cytokine abnormalities may be markers for genetic and/or environmental contributor(s) to ASD (Goines and Ashwood, 2013). Many potential immune disturbances have been suggested as mediators in the pathogenesis of ASD; one disturbance is autoimmunity. The significant increase in the serum IL-12 level in our study and in the literature indicates that immunological disorders occur more in autistic children compared with normal children, possibly due to autoimmunity. It was suggested that autism involves the T-helper cells 1 (Th-1) immune response, and that it could be related to an autoimmune pathology because IL-12 is a well-known inducer of autoimmune diseases. Immune activation leads to spontaneous proliferation of peripheral blood mononuclear cells, increased expression of activation markers on peripheral blood mononuclear cells, and increased accumulation of blood mononuclear cell-derived soluble antigens, mainly cytokines, cytokine receptors, and adhesion molecules (Singh, 2003). On the basis of these considerations, immune activation occurs naturally in autistic children because they have elevated levels of immune activation antigens such as IL-2, IL-12, and IFN-g, and their blood contains activated T cells (Costantino *et al.*, 2010). Thus, it is reasonable to conclude that the increase in IL-12 in autistic children points toward antigenic stimulation of Th-1 cells, which through IFN-g may induce autoimmunity. The IL-12 cytokine selectively promotes the development of T-helper (Th-1) cells, and Th-1 cells initiate the pathogenesis of organ-specific autoimmune diseases (Neumann *et al.*, 1995; Warren *et al.*, 1996).

Other studies investigating different cytokines have been found debating the immune etiology (El-Ansary *et al.*, 2011; Napolioni, 2013). In opposition to this study rationale, El-Ansary *et al.* (2011) reported different levels of cytokines in ASD patients compared with the control group. The study was conducted to examine K^+, Ca^{2+}/Mg^{2+} together with IL-6, and TNF-a as proinflammatory cytokines in the plasma of 25 Saudi autistic male patients compared with 16 age-matched and sex-matched control samples. Their study stated that concentrations of IL-6 and TNF-a were significantly lower in children with autism compared with controls. In contrast, K^+ was significantly increased in plasma samples of children with autism compared with age-matched and sex-matched controls, recording 2.3-fold higher values. Further, Napolioni (2013), studied cytokines in autistic children using an array-based multiplex sandwich ELISA for simultaneous quantitative measurement of 40 unique targets and found negative data. They also analyzed the correlations between cytokine levels and clinically relevant quantitative traits (the Vineland Adaptive Behavior Scale in Autism composite score, the Social Responsiveness

Scale total *T* score, the head circumference, and the full intelligence quotient). In addition, because of the high phenotypic heterogeneity of ASD, they defined four subgroups of patients (those who were nonverbal, those with gastrointestinal issues, those with regressive autism, and those with a history of allergies), which encompass common and/or recurrent endophenotypes in ASD, and tested the cytokine levels in each group. The study stated that none of the measured parameters [(IL-2+IFN-g+TNF-a), Th-2 (IL-4+IL-5+IL-6+IL-10+IL-13), and (IL-6+IL-17) cytokine levels] showed significant differences between the ASD group and the control group, and it was not related to the autistic severity. The apparent difference between our results and the previously mentioned two opposing results might be contributed to the different cytokines investigated, to the nonhomogeneity of the diagnoses or the different sample size and methodology in our study (Napolioni, 2013).

In the present study, a higher IL-12 was related positively, but not statistically significantly, to the severity of AD according to its relation to the CARS mean score (r = 0.032, P = 0.894). Further, it was related positively, but nonstatistically significantly, to several parameters indicating the severity of AD such as the development of spoken language, the presence of comorbidities, and a positive family history of PDD. Similarly, Al-Ayadhi and Moustafa (2012) investigated autoimmunity as indicated by IL-17 and its relation to the autistic severity among a sample of 45 autistic children and compared it with 40 matched control children. They found that a high serum level of IL-17 related significantly to the severity of autistic symptoms on CARS (Al-Ayadhi and Moustafa, 2012). Furthermore, Enstrom *et al.* (2009a) reported, in accordance with our results, elevated plasma levels of several cytokines, one of which was IL-12. They found these elevated levels to be related to the deviant communication and the impaired behavioral outcome in their autistic sample (Enstrom *et al.*, 2009a). One might think that the increased IL-12 reflects the severity of the pathological process underlying AD in these children or that the increased IL-12 has a direct or indirect relation to the affection of neuronal functioning in AD.

Our study confirmed the increase in IL-12 in autistic children mentioned in the literature, indicating an immune dysfunction, possibly autoimmunity. The cytokine level was related positively with the severity of AD as indicated by the CARS mean score and the nondevelopment of spoken language. Further studies replicating our results are recommended involving a larger number of ASD children and more types of cytokines. Future research on this topic in Egypt must focus on the implications of the study results over the management of ASD and whether the use of new immune therapies should be added.

Acknowledgements

Conflicts of interest

There are no conflicts of interest.

References

Abrahams BS, Geschwind DH (2008). Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 9:341–355.

Abrahams BS, Geschwind DH (2010). Connecting genes to brain in the autism spectrum disorders. Arch Neurol 67:395–399.

Al-Ayadhi LY, Mostafa GA (2012). Elevated serum levels of interleukin-17A in children with autism. J Neuroinflammation 9:158.

American Psychiatric Association (APA) (1994). *Diagnostic and statistical manual of mental disorders: DSM-IV-TR.* 4th ed. Washington, DC: American Psychiatric Association.

American Psychiatric Association (2013). DSM-5 development, autism spectrum disorder. Proposed revision.

American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders.* 4th ed. Washington, DC: American Psychiatric Association.

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van deWater J (2011). Elevated plasma cytokines in autism spectrum disorders provide evidence of immune dysfunction and are associated with impaired behavioral outcome. Brain Behav Immun 25:40–45.

Autism and Developmental Disorders Network, United States (2009). Prevalence of Autistic Spectrum Disorders. Surveill Summary 194:500–509.

Autism and Developmental Disorders Monitoring Network (2006). Prevalence of autism spectrum disorders United States Autism and Developmental Disorders Monitoring Network.

Cannell JJ (2008). Autism and vitamin D. Med Hypotheses 70:750–759.

Carmody DP, Lewis M (2010). Regional white matter development in children with autism spectrum disorders. Dev Psychobiol 52:755–763.

Costantino JN, Zhang Y, Frazier T, Abacchi AM, Law P (2010). Sibling recurrence and the genetic epidemiology of autism. Am J Psychiatry 167:1349–1356.

Croonenberghs J, Bosmans E, Deboutte D, Kenis G, Maes M (2002). Activation of the inflammatory response system in autism. Neuropsychobiology 45:1–6.

El-Ansary AK, Ben Bacha AG, Al-Ayadhi LY (2011). Proinflammatory and proapoptotic markers in relation to mono and di-cations in plasma of autistic patients from Saudi Arabia. J Neuroinflammation 8:142.

Enstrom A, Onore C, Traver A, Hertz-Picciotto I, Hansen R, *et al.* (2009a). Autoimmunity in autism. Curr Opinion 10:463–473.

Fabry Z, Raine CS, Hart MN (1994). Nervous tissue as an immune compartment: the dialect of the immune response in the CNS. Immunol Today 15:218–224.

Filipek PA, Filipek PJ (1999). The screening and diagnosis of autistic spectrum disorders. J Autism Deve Disord 29:439–484.

Gately MK, Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, Presky DH (1998). The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. Annu Rev Immunol 16:495–521.

Gillberg C, Cederlund M (2005). Asperger syndrome: familial and pre- and perinatal factors. J Autism Dev Disord 35:159–166.

Glasson EJ, Bower C, Petterson B, de Klerk N, Chaney G, Hallmayer JF (2004). Perinatal factors and the development of autism: a population study. Arch Gen Psychiatry 61:618–627.

Goines PE, Ashwood P (2013). Cytokines dysregulation in ASD. Neurotoxicol Teratol 36:67–81.

Grant WB, Soles CM (2009). Epidemiologic evidence supporting the role of maternal vitamin D deficiency as a risk factor for the development of infantile autism. Dermatoendocrinol 1:223–228.

Hallmayer J, Cleveland S, Torres A, Phillips J, Cohen B, Torigoe T, *et al.* (2011). Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry 68:1095–1102.

James SJ, Melnyk S, Jernigan S, Cleves MA, Halsted CH, Wong DH, *et al.* (2006). Metabolic endophenotype and related genotypes are associated with oxidative stress in children with autism. Am J Med Genet B Neuropsychiatr Genet 141B:947–956.

Kirkpatrick LA, Feeney BC (2013). *A simple guide to IBM SPSS statistics for version 20.0*. Student ed. Belmont, California: Wadsworth, Cengage Learning.

Kotz S, Balakrishnan N, Read CB, Vidakovic B (2006). *Encyclopedia of statistical sciences*. 2nd ed. Hoboken, NJ: Wiley-Interscience.

Lequin RM (2005). Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). Clin Chem 51:2415–2418.

Licinio J, Alvarado I (2002). Autoimmunity in autism. Mol Psychiatry 7:229–236.

Napolioni V, Ober-Reynolds B, Szelinger S, Corneveaux JJ, Pawlowski T, Ober-Reynolds S, *et al.* (2013). Plasma cytokine profiling in sibling pairs discordant for autism spectrum disorder. J Neuroinflammation 10:38.

Neumann H, Cavalie A, Jenne DE (1995). Induction of MHC class I in neurons. Science 269:549–552.

Sacco R, Curatolo P, Manzi B, Militerni R, Bravaccio C, Frolli A, *et al.* (2010). Principal pathogenetic components and biological endophenotypes in autism spectrum disorders. Autism Res 3:237–252.

Schaefer GB, Lutz RE (2006). Diagnostic yield in the clinical genetic evaluation of autism spectrum disorders. Genet Med 8:549–556.

Schopler E, Reichler RJ, DeVellis RF, Daly K (1980). Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). J Autism Dev Disord 10:91–103.

Singh VK (1996). Plasma increase of interleukin-12 and interferon-gamma. Pathological significance in autism. J Neuroimmunol 66:143–145.

Singh VK (2003). Cytokine regulation in autism. In: Kronfol Z, editor *Cytokines and mental health*. Boston, MA: Kluwer Academic Publishers; 369–383.

Singh VK (2004). Autism, vaccines, and immune reactions. Presented at Institute of Medicine (IOM) Conference; Washington, DC, USA: National Academy of Sciences.

Singh VK (2005). Elevation of serum C-reactive protein and S100 proteins for systemic inflammation in autistic children. J Spec Educ Rehabil 3–4: 117–125.

Singh VK, Hanson J (2006). Assessment of metallothionein and antibodies to metallothionein in normal and autistic children having exposure to vaccine-derived thimerosal. Pediatr Allergy Immunol 17:291–296.

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

Suzuki K, Matsuzaki H, Iwata K, Kameno Y, Shimmura C, Kawai S, *et al.* (2011). Plasma cytokine profiles in subjects with high-functioning autism spectrum disorders. PLoS One 6:e20470.

Todd RD, Hickok JM, Anderson GM, Cohen DJ (1988). Antibrain antibodies in infantile autism. Biol Psychiatry 23:644–647.

Trottier G, Srivastava L, Walker CD (1999). Etiology of infantile autism: a review of recent advances in genetic and neurobiological research. J Psychiatry Neurosci 24:103–115.

Wang KS, Frank DA, Ritz J (2000). Interleukin-2 enhances the response of natural killer cells to interleukin-12 through up-regulation of the interleukin-12 receptor and STAT4. Blood 95:3183–3190.

Warren RP, Margaretten NC, Pace NC, Foster A (1986). Immune abnormalities in patients with autism. J Autism Dev Disord 16:189–197.

Warren RP, Singh VK, Averett RE, Odell JD, Maciulis A, Burger RA, *et al.* (1996). Immunogenetic studies in autism and related disorders. Mol Chem Neuropathol 28:77–81.