

Transforming Growth Factor Beta 1 869T/C and 915G/C Polymorphisms and Risk of Autism Spectrum Disorders

Mohammad Reza Khakzad^{*1,2}, Farhad Salari³, Maryam Javanbakht⁴,
Maryam Hojati⁵, Abdolreza Varasteh¹, Mojtaba Sankian³, Mojtaba Meshkat⁶

Abstract

Background: Transforming growth factor- β 1 (TGF- β 1) has been found to play a crucial role in early central nervous system development. Several studies have illustrated decreased TGF- β 1 levels in sera and brains of autistic children. Two point mutations in the TGF- β 1 signal peptide at 869T/C and 915G/C have been reported to influence TGF- β 1 expression. The aim of the present study was to investigate the correlation of TGF- β 1 polymorphisms and their haplotypes with autism.

Methods: This study was performed on 39 autistic patients and 35 age- and sex-matched normal controls in an Iranian population, using the sequence specific primed-polymerase chain reaction (PCR-SSP) technique. Patients were divided into mild-to-moderate and severe groups according to the childhood autism rating scale.

Results: No significant differences were observed for allele, genotype, or haplotype frequencies between the autistics and controls. Only a slight difference was observed in GC25 between the controls and all children with autism.

Conclusion: Thus, these results indicate that the polymorphisms in TGF- β 1 gene may not play an important role in the development of autism.

Keywords: Autism spectrum disorders, Development, Polymorphism, Transforming Growth Factor beta 1

Introduction

Autism spectrum disorders (ASDs) are neurodevelopmental disorders characterized by difficulties in social interactions and communication, and repetitive and stereotyped patterns of behaviors and interests with various levels of severity occurring before three years of age (1). Although the exact cause of these disorders remains poorly understood, immunological factors have been proposed to have a major role in their pathophysiology (2). Several studies have shown a correlation between immune

system abnormalities and autism spectrum disorders. These abnormalities include inappropriate immune regulation resulting in abnormalities in the functional immune cell subsets and autoimmunity such as autoantibodies generated against the central nervous system (CNS) (2-4). It is assumed that aberrant immune responses during the critical neurodevelopmental period may result in the development of neurological disorders (5). The plausibility of this hypothesis with regard to immune

1: Allergy Research Center, Mashhad University of Medical Sciences, Mashhad, Iran

2: Department of Immunology, Mashhad Branch, Islamic Azad University, Mashhad, Iran

3: Immunology Research Center, Medical School, Mashhad University of Medical Sciences, Mashhad, Iran

4: Department of Psychiatric, Islamic Azad University, Mashhad Branch, Mashhad, Iran

5: Noor Hedayat Center of Autism Spectrum Disorders, Mashhad, Iran

6: Department of Biostatistic, Shahid Beheshti University of medical sciences, Tehran, Iran

Co-first authors: Mohammad Reza Khakzad and Farhad Salari

*Corresponding author: Mohammad Reza Khakzad; Tel: +98 9151176352; Fax: +98 51- 32250048, E-mail: mr.khakzad@gmail.com

Received: May 12, 2014; Accepted: July 17, 2014

system abnormalities in autistics has been derived from the pivotal role of the immune system in neurodevelopment and the ability of these alterations to influence the CNS (5). Both the immune and nervous systems are highly evolved systems that cross talk via cytokines and neuro-mediators such as neuropeptides (6, 7).

Transforming growth factor- β 1 (TGF- β 1) is an important immune regulator critical for immune homeostasis. Accumulating evidence suggests that TGF- β 1 has a crucial regulatory role in CNS development and potential implications for neurogenesis in a variety of TGF- β 1-related CNS diseases (5, 8). TGF- β 1 knockout mice were shown to have severe cortical developmental impairment with pervasive increased neuronal cell death and microgliosis complication (9). Furthermore, decreased levels of TGF- β 1 in serum have been observed in autistic children (10, 11). These reports suggest that immune system aberrations may lead to abnormal immune responses, autoimmunity, or adverse neuroimmune interactions during brain development.

The human TGF- β 1 gene, located on chromosome 19q13.1–3, contains five described polymorphisms: two in the promoter region at positions 800 G/A and 509 C/T and three located in the coding sequence at positions 869 T/C, 915 G/C and 1628 C/A (12–15). The point mutations at positions 869 T/C and 915 G/C result in amino acid substitutions in the signal peptide at codons 10 (Leu/Pro) and 25 (Arg/Pro), respectively. It has been found that these polymorphisms genetically control TGF- β 1 serum concentrations. Moreover, the presence of leucine and arginine at codons 10 and 25 determines the high-producer genotype, while proline at both codons is associated with relatively lower levels of TGF- β 1. However, there are some conflicting results concerning codon 10 polymorphisms. Some studies demonstrated that leucine is a high producer (14), while others (15, 16) reported that proline at codon 10 results in the high-producer genotype. However, there is no controversy that the polymorphisms at codons 10 and 25 affect TGF- β 1 production. In the present study, two coding polymorphisms within exon 1 of the TGF- β 1 gene at positions 869 T/C and 915 G/C were analyzed to determine the prevalence of particular TGF- β 1 genotypes in autistic children. Aberrations in immune

system regulation or impairment in immune homeostasis may result in chronic inflammation, autoimmunity, or inappropriate immune responses. These may cause inflammation in the CNS or brain leading to altered neurodevelopment. In addition, TGF- β 1 is a potent immunosuppressive cytokine as well as a crucial regulator in brain development (5). Furthermore, it is believed that TGF- β 1 protects the brain from neuronal degeneration during inflammation in the CNS (17–19). Therefore, TGF- β 1 has been widely recognized as a cytokine that responds to brain injury. Several studies have demonstrated altered TGF- β 1 levels in brains and sera of autistics (3, 11). It has also been shown that the 509C/T and 869T/C point mutations in TGF- β 1 lead to altered TGF- β 1 production and/or activity, which may modulate an individual's susceptibility to autism. Given the key role of these polymorphisms in cytokine production and the low TGF- β 1 serum levels in autistics, we investigated whether TGF- β 1 polymorphisms are risk factors for the development of autism. Our research focused on point mutations involved in TGF- β 1 production; namely alleles T (leucine) and C (proline) at codon 10 and alleles G (arginine) and C (proline) at codon 25.

Materials and Methods

Patients

Thirty-nine autistic children, aged 7–13, were included in this study. The Childhood Autism Rating Scale (CARS) was used to confirm the diagnosis of autism and assess its severity. A 29.5 cut-off point was employed to diagnose autism. Scores of 30–36.5 and 37–60 were classified as mild-to-moderate and severe, respectively. In addition, criteria from the Diagnostic and Statistical and Manual of Mental Disorders 4th Edition (DSM-IV) were also used to differentiate autistic children with CARS > 30 from other developmental disorders e.g. Rett, Asperger's, and childhood disintegrative disorder (20). The control group included 35 age-matched, healthy children with IQs of 90–110. None of the children in the control group had neurological or psychological deficits. Informed consents were obtained from parents in both groups. The study design was in accordance with the tenets of the Helsinki Declaration, and was approved by the Research Ethics Committee of Azad University of Medical Sciences, Mashhad Branch. Demographic

data, previous medical histories, and clinical signs and symptoms of all children were also obtained.

PCR Method

Peripheral blood was obtained and genomic DNA extracted by the salting-out method as described previously (21). To determine TGF- β 1 genotyping at codons 10 (T/C) and 25 (G/C), sequence-specific primed-polymerase chain reaction (PCR-SSP) was performed. The following primers were used to amplify codons 10 (T/C) and 25 (G/C):

5'-CGGGCTGCGGCTGCTGCC-3' (T10),

5'-CGGGCTGCGGCTGCTGCT-3' (C10),

5'-TTTCGTTGTGGGTTTCCACCATTA-3'

(common codon 10) and

5'-GTGCTGACGCCTGGCCG-3' (G25),

5'-GTGCTGACGCCTGGCCC-3' (C25),

5'-GGCTCCGGTTCTGCACTC-3' (common codon

25). The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene was amplified as an internal control for the genomic DNA preparation. The optimized PCRs were performed in 20 μ l reactions containing 50 ng of genomic DNA, 10 nmol of specific and control primer mixes, 200 μ M of each deoxynucleotide triphosphate (dNTP), 1x ammonium-sulfate-based PCR buffer, 1.5 mM MgCl₂, 5% DMSO, and 0.5 U of HS-Taq DNA polymerase (Parstous, Iran). Amplifications were performed on a Corbett Research Thermocycler (Corbett, Australia), using the following conditions: initial denaturation at 94 °C for 10 min followed by 35 cycles of 30 s at 94 °C, 20 s at 65 °C for 869T/C and 30 s at 63 °C for 915G/C, 30 s at 72 °C and a final extension at 72 °C for 5 min. The PCR products were analyzed in 2% agarose gel stained with green viewer and visualized under UV.

Statistical analysis

All statistical analyses were performed using SPSS version 11.5 (SPSS Inc. Chicago, IL, USA). Genotype and allele frequencies were compared between the study groups by χ^2 test, Fisher's exact test and odds ratios (OR) with 95% confidence intervals (CIs). Demographic and clinical data between groups were compared by χ^2 test and Student's t-test. A p-value less than 0.05 was considered statistically significant.

Results

The demographic and clinical characteristics of all groups are summarized in Table 1. The genotype and

allele frequencies for the TGF- β 1 polymorphisms in the autistics, which were divided into mild-to-moderate and severe, and controls are shown in Table 2. The genotype distribution among the autistics and controls was in Hardy-Weinberg equilibrium. In this study, two polymorphic positions within the TGF- β 1 codon 10 T/C and 25 G/C were analyzed. No significant differences were observed in the distribution of TGF- β 1 genotypes and allele frequencies between patients and controls. Only a slight difference was observed in GC25 between the controls (n = 35) and all children with autism (n = 39, p = 0.082). In addition, to investigate whether any specific haplotypes would relate to the development of autism, all possible haplotypes composed of these polymorphisms were examined (Table 3). The result showed that CC/CC (low producer) haplotype was not detected in any group. Moreover, the TC/CC low producer was detected in only one subject in the control group. The major haplotypes in both groups were those polymorphisms considered as high producers (TT/GG and TC/GG). Furthermore, no significant differences in TGF- β haplotypes were identified between autistic patients and controls (Table 3).

Discussion

In this study we focused on point mutations of alleles that were reported to be involved in TGF- β 1 expression; namely alleles T (leucine) and C (proline) at codon 10 and alleles G (arginine) and C (proline) at codon 25. No significant differences were found in the distributions of TGF- β 1 alleles, genotypes, or haplotype frequencies between autistics and controls.

Over the past decade, numerous reports have noted abnormalities or alterations of immune system activity in autistics; these include increased serum levels of inflammatory cytokines and factors such as tumor necrosis factor- α (TNF- α), interferon- γ (IFN- γ) and high sensitivity C-reactive protein (hsCRP) (22-25). It has been reported that autoantibodies against brain and CNS proteins exists in 30-70% of autistic patients (4, 26-28). In addition, decreases in lymphocytes and T cell mitogen responses, and an imbalance of serum immunoglobulin levels have been reported in a significant number of autistic children (2, 29). Overall, the data suggests that immune dysfunction and excessive inflammation play important pathophysiologic roles in autism disorders.

Table 1. Comparison of demographic and clinical data as well as abnormalities in CT-scans between autism (n = 39) and control (n = 35) groups (*Not significant).

Variables	Autism group	Control group	p-value
Mean age (Years) \pm SD	8.54 \pm 1.68	7.9 \pm 3.1	NS
Gender (boys/girls)	25/14	20/15	NS
BMI (kg/m ²) \pm SD	22.85 \pm 2.19	21.14 \pm 2.45	NS
Age of diagnosis	2.8	–	–
Severity (%)			
Severe	29 (74.4%)	–	–
Mild/moderate	10 (25.6%)	–	–
Obstetric complications (%)	4 (25%)	6 (37.5%)	NS
Another disease (%)	8 (20.5%)	2 (5.7%)	NS
Family history (%)	8 (20.5%)	0 (0.0%)	0.008
Epilepsy (%)	12 (30.8%)	0 (0.0%)	0.001
Hypoxia (%)	3 (7.7%)	0 (0.0%)	NS
Abnormal CT-scan (%)	1 (2.6%)	0 (0.0%)	NS

Table 2. Genotype and allele frequencies for the TGF- β 1 Codon 10 and 25 polymorphisms in autistic patients and controls (*Fisher's exact test).

Genotype	Autism cases(n=39)		Controls (n=35) (%)	(p)*	OR p	OR (CI 95%)
	Mild/ Moderate 10 (%)	Severe 29 (%)				
Codon 10 T>C						
TT (%)	6 (60%)	14 (48.3%)	11 (31.4%)	(0.51)		1.0 (reference)
TC (%)	3 (30%)	10 (34.5%)	17 (48.6%)		0.162	0.324 (0.06- 1.57)
CC (%)	1 (10%)	5 (17.2%)	7 (20%)		0.258	0.262 (0.02- 2.66)
χ ² HW (p)	0.4 (0.52)	1.62 (0.2)	0.008 (0.92)			
Allele						
T (%)	15 (75 %)	38 (65.5%)	39 (55.7%)	(0.26)		1.0 (reference)
C (%)	5 (25 %)	20 (34.5%)	31 (44.3%)		0.086	0.435 (0.16- 1.12)
Codon 25 G>C						
GG (%)	7 (70%)	20 (69%)	30 (85.7%)	(0.12)		1.0 (reference)
GC (%)	2 (20%)	9 (31%)	4 (11.4%)		0.082	3.05 (0.86- 10.73)
CC (%)	1 (10%)	0 (0%)	1 (2.9%)		0.942	1.11 (0.06-18.64)
χ ² HW (p)	1.40(0.23)	0.97 (0.32)	2.56 (0.10)			
Allele						
G (%)	16 (80%)	49 (84.48%)	64 (91.42%)	(0.27)		1.0 (reference)
C (%)	4 (20%)	9 (15.51%)	6 (8.57%)		.099	2.667 (0.83- 8.55)

Table 3. Distribution of TGF- β 1 (codon 10 T/C and codon 25 G/C) haplotypes among autistic patients and healthy controls.

Production level	Haplotype	Control	Mild/ Moderate	Severe	χ^2 (p)
High	TT/GG	11 (31.4%)	5 (50%)	8 (27.6%)	7.14 (0.128)
	TC/GG	14 (40%)	2 (20%)	8 (27.6%)	
Intermediate	TT/GC	0 (0.0%)	0 (0.0%)	6 (20.7%)	
	TC/GC	2 (5.7%)	1 (10%)	2 (6.9%)	
	CC/GG	5 (14.3%)	0 (0.0%)	4 (13.8%)	
	TT/CC	0 (0.0%)	1 (10%)	0 (0.0%)	
Low	TC/CC	1 (2.9%)	0 (0.0%)	0 (0.0%)	
	CC/GC	2 (5.70%)	1 (10%)	1 (3.4%)	
	CC/CC	0 (0.0%)	0 (0.0%)	0 (0.0%)	

TGF- β 1 is considered to be one of the critical immunosuppressive cytokines in immune homeostasis and T cell activated unresponsiveness (30-32). Furthermore, evidence suggests that during the brain development, glial and neuronal cells produce TGF- β 1, which plays a crucial role in the regulation of early CNS development such as astrocyte differentiation (33, 34), synaptogenesis (35), neuronal migration in the cerebral cortex (36), neuronal survival (37, 38), neuronal death, microgliosis control (9), wound healing, and immunosuppression (39).

Recently, in accordance with several other publications, we found (unpublished data) that serum levels of TGF- β 1 are significantly lower in autistics than in age and gender-matched controls (10, 11). These findings are consistent with the hypothesis that reduced levels of this cytokine may lead to an inappropriate regulation of immune responses as well as the development of neuroinflammation disorders such as autism spectrum disorders. However, it is not yet demonstrated that reduction of TGF- β 1 is a primary cause of autism or simply a secondary reflection of the disorder.

Given the key role of TGF- β 1 in brain development and inflammation, we investigated the association between TGF- β 1 gene polymorphisms and autism. Consequently, estimated alleles, genotypes, and haplotypes frequencies were compared between autistic patients and normal controls in an Iranian population. We found no association between the TGF- β 1 gene polymorphisms and autism. It is well established that polymorphisms at 509C/T and 869T/C influence TGF- β 1 production and resultant serum levels. It has also been demonstrated that the TT/GG and TC/GG haplotypes are associated with high TGF- β 1 expression. The TC/GC, CC/GG, and TT/GC

haplotypes are associated with intermediate expression, and the CC/GC, CC/CC, TT/CC and TC/CC haplotypes are known as low expressors. Based on the relatively low TGF- β 1 expression in our study (unpublished data), we predicted that the subjects in our study with the CC/GC, CC/CC, TT/CC, TC/CC polymorphisms would be low expressors. Surprisingly the CC/CC and TC/CC polymorphisms were not detected in autistics, and only the TC/CC polymorphism was detected in one control subject. Our results also demonstrated that the dominant haplotypes in autistics were TT/GG and TC/GG, which contribute to high and intermediate expressors, respectively. Therefore, it seems difficult to assess only the influence of different genotype variants for the TGF- β 1 serum levels in autistics. On the other hand, considering the relatively high rates of autoimmunity and inflammatory diseases in autistics and their families (40), decreased TGF- β 1 levels in sera and brains may be explained by other factors such as defects in regulatory T cell development. Regulatory T cells are responsible for TGF- β 1 production, self-tolerance, and immune homeostasis.

In conclusion, we found no association between autism and TGF- β 1 gene polymorphisms in codons 10 and 25. Considering the influence of polymorphisms in TGF- β 1 expression, further studies may be required to determine the effects of decreased levels of TGF- β 1. More importantly, due to the small sample size, we believe the findings of the present study should be tested with a larger number of patients. Undoubtedly, more findings should be taken into account in the assessment of autism and these will help us to understand the underlying clinical and molecular mechanisms of the disorder.

Acknowledgements

The authors thank the Noor Hedayat Autism Center, Zakaria Research Center, and Islamic Azad University (Mashhad branch) for their kind support in

conducting this study. We also appreciate the patients and their families and healthy volunteers for their participation in this study.

References

1. Bertoglio K, Hendren RL. New developments in autism. *The Psychiatric clinics of North America*. 2009;32(1):1-14.
2. Ashwood P, Wills S, Van de Water J. The immune response in autism: a new frontier for autism research. *Journal of leukocyte biology*. 2006;80(1):1-15.
3. Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA. Neuroglial activation and neuroinflammation in the brain of patients with autism. *Annals of neurology*. 2005;57(1):67-81.
4. Cohly HH, Panja A. Immunological findings in autism. *International review of neurobiology*. 2005;71:317-41.
5. Gomes FC, Sousa Vde O, Romao L. Emerging roles for TGF-beta1 in nervous system development. *International journal of developmental neuroscience : the official journal of the International Society for Developmental Neuroscience*. 2005;23(5):413-24.
6. Zablocka A. [Cooperation between the immune and nervous systems]. *Postepy higieny i medycyny doswiadczalnej*. 2001;55(1):3-15.
7. Ziemssen T, Kern S. Psychoneuroimmunology—cross-talk between the immune and nervous systems. *Journal of neurology*. 2007;254 Suppl 2:i8-11.
8. Wachs FP, Winner B, Couillard-Despres S, Schiller T, Aigner R, Winkler J, et al. Transforming growth factor-beta1 is a negative modulator of adult neurogenesis. *Journal of neuropathology and experimental neurology*. 2006;65(4):358-70.
9. Brionne TC, Tesseur I, Masliah E, Wyss-Coray T. Loss of TGF-beta 1 leads to increased neuronal cell death and microgliosis in mouse brain. *Neuron*. 2003;40(6):1133-45.
10. Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, et al. Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. *Journal of neuroimmunology*. 2008;204(1-2):149-53.
11. Okada K, Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, et al. Decreased serum levels of transforming growth factor-beta1 in patients with autism. *Progress in neuro-psychopharmacology & biological psychiatry*. 2007;31(1):187-90.
12. Fujii D, Brissenden JE, Derynck R, Francke U. Transforming growth factor beta gene maps to human chromosome 19 long arm and to mouse chromosome 7. *Somatic cell and molecular genetics*. 1986;12(3):281-8.
13. Awad MR, El-Gamel A, Hasleton P, Turner DM, Sinnott PJ, Hutchinson IV. Genotypic variation in the transforming growth factor-beta1 gene: association with transforming growth factor-beta1 production, fibrotic lung disease, and graft fibrosis after lung transplantation. *Transplantation*. 1998;66(8):1014-20.
14. Hutchinson IV, Turner D, Sankaran D, Awad M, Pravica V, Sinnott P. Cytokine genotypes in allograft rejection: guidelines for immunosuppression. *Transplantation proceedings*. 1998;30(8):3991-2.
15. Suthanthiran M, Li B, Song JO, Ding R, Sharma VK, Schwartz JE, et al. Transforming growth factor-beta 1 hyperexpression in African-American hypertensives: A novel mediator of hypertension and/or target organ damage. *Proceedings of the National Academy of Sciences of the United States of America*. 2000;97(7):3479-84.
16. Yamada Y, Miyauchi A, Goto J, Takagi Y, Okuizumi H, Kanematsu M, et al. Association of a polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to osteoporosis in postmenopausal Japanese women. *Journal of bone and mineral research : the official journal of the American Society for Bone and Mineral Research*. 1998;13(10):1569-76.
17. Lindholm D, Castren E, Kiefer R, Zafra F, Thoenen H. Transforming growth factor-beta 1 in the rat brain: increase after injury and inhibition of astrocyte proliferation. *The Journal of cell biology*. 1992;117(2):395-400.
18. Logan A, Frautschy SA, Gonzalez AM, Sporn MB, Baird A. Enhanced expression of transforming growth factor beta 1 in the rat brain after a localized cerebral injury. *Brain research*. 1992;587(2):216-25.
19. Prehn JH, Backhauss C, Kriegstein J. Transforming growth factor-beta 1 prevents glutamate

neurotoxicity in rat neocortical cultures and protects mouse neocortex from ischemic injury in vivo. *Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism*. 1993;13(3):521-5.

20. Schopler E, Reichler RJ, DeVellis RF, Daly K. Toward objective classification of childhood autism: Childhood Autism Rating Scale (CARS). *Journal of autism and developmental disorders*. 1980;10(1):91-103.

21. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic acids research*. 1988;16(3):1215.

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

23. Jyonouchi H, Sun S, Le H. Proinflammatory and regulatory cytokine production associated with innate and adaptive immune responses in children with autism spectrum disorders and developmental regression. *Journal of neuroimmunology*. 2001;120(1-2):170-9.

24. Zimmerman AW, Jyonouchi H, Comi AM, Connors SL, Milstien S, Varsou A, et al. Cerebrospinal fluid and serum markers of inflammation in autism. *Pediatric neurology*. 2005;33(3):195-201.

25. Khakzad MR, Javanbakht M, Shayegan MR, Kianoush S, Omid F, Hojati M, et al. The complementary role of high sensitivity C-reactive protein in the diagnosis and severity assessment of autism. *Research in Autism Spectrum Disorders*. 2012;6(3):1032-7.

26. van Gent T, Heijnen CJ, Treffers PD. Autism and the immune system. *Journal of child psychology and psychiatry, and allied disciplines*. 1997;38(3):337-49.

27. Singer HS, Morris CM, Williams PN, Yoon DY, Hong JJ, Zimmerman AW. Antibrain antibodies in children with autism and their unaffected siblings. *Journal of neuroimmunology*. 2006;178(1-2):149-55.

28. Singh VK, Warren RP, Odell JD, Warren WL, Cole P. Antibodies to myelin basic protein in children with autistic behavior. *Brain, behavior, and immunity*. 1993;7(1):97-103.

29. Heuer L, Ashwood P, Schauer J, Goines P, Krakowiak P, Hertz-Picciotto I, et al. Reduced levels of immunoglobulin in children with autism correlates with behavioral symptoms. *Autism research: official*

Journal of the International Society for Autism Research. 2008;1(5):275-83.

30. den Haan JM, Kraal G, Bevan MJ. Cutting edge: Lipopolysaccharide induces IL-10-producing regulatory CD4+ T cells that suppress the CD8+ T cell response. *J Immunol*. 2007;178(9):5429-33.

31. Sonoda KH, Faunce DE, Taniguchi M, Exley M, Balk S, Stein-Streilein J. NK T cell-derived IL-10 is essential for the differentiation of antigen-specific T regulatory cells in systemic tolerance. *J Immunol*. 2001;166(1):42-50.

32. Marra LE, Zhang ZX, Joe B, Campbell J, Levy GA, Penninger J, et al. IL-10 induces regulatory T cell apoptosis by up-regulation of the membrane form of TNF-alpha. *J Immunol*. 2004;172(2):1028-35.

33. de Sampaio e Spohr TC, Martinez R, da Silva EF, Neto VM, Gomes FC. Neuro-glia interaction effects on GFAP gene: a novel role for transforming growth factor-beta1. *The European journal of neuroscience*. 2002;16(11):2059-69.

34. Sousa Vde O, Romao L, Neto VM, Gomes FC. Glial fibrillary acidic protein gene promoter is differently modulated by transforming growth factor-beta 1 in astrocytes from distinct brain regions. *The European journal of neuroscience*. 2004;19(7):1721-30.

35. Feng Z, Ko CP. Schwann cells promote synaptogenesis at the neuromuscular junction via transforming growth factor-beta1. *The Journal of neuroscience : the official journal of the Society for Neuroscience*. 2008;28(39):9599-609.

36. Siegenthaler JA, Miller MW. Transforming growth factor beta1 modulates cell migration in rat cortex: effects of ethanol. *Cereb Cortex*. 2004;14(7):791-802.

37. Unsicker K, Kriegstein K. TGF-betas and their roles in the regulation of neuron survival. *Advances in experimental medicine and biology*. 2002;513:353-74.

38. Roussa E, Farkas LM, Kriegstein K. TGF-beta promotes survival on mesencephalic dopaminergic neurons in cooperation with Shh and FGF-8. *Neurobiology of disease*. 2004;16(2):300-10.

39. Pratt BM, McPherson JM. TGF-beta in the central nervous system: potential roles in ischemic injury and neurodegenerative diseases. *Cytokine & growth factor reviews*. 1997;8(4):267-92.

40. Atladóttir HO, Pedersen MG, Thorsen P, Mortensen PB, Deleuran B, Eaton WW, et al. Association of family history of autoimmune diseases and autism spectrum disorders. *Pediatrics*. 2009;124(2):687-94.