Metabolic Brain Disease (2019) 34:1049–1060
https://doi.org/10.1007/s11011-019-00420-5

ORIGINAL ARTICLE

Determination of neuroinflammatory biomarkers in autistic and neurotypical Saudi children

Najat O. Hamed[1,2] · Laila-Al-Ayadhi[3,4,5] · Mohamed A. Osman[6,7] · Abdalla O. Elkhawad[1,7] · Geir Bjørklund[8] · Hanan Qasem[4] · Naima Zayed[9] · Afaf El-Ansary[4,5,9,10]

Received: 14 December 2018 / Accepted: 17 April 2019 / Published online: 30 May 2019

Abstract

To identify neuroinflammatory biomarkers in patients with various severity of autism spectrum disorder (ASD) increases the insight about the pathogenesis and pathophysiology of this neurodevelopmental disorder. The aim of the present study was to analyze the levels in plasma of TGFβ2, Heat shock protein 70 (HSP70), and hematopoietic prostaglandin D2 synthase (H-PGDS) in Saudi ASD children and healthy age-matched neurotypical controls. Also, it was in the present study examined the correlation among these neuroinflammatory biomarkers and the sensory deficit exhibited by the ASD children. Blood samples from 38 Saudi children with ASD and 32 age-matched neurotypical controls were withdrawn after an overnight fast. For the blood taking 3 mL EDTA containing blood collection tubes was used. The samples were centrifuged for 20 min (4 °C; 3000×g) directly after the blood sampling. The harvested plasma was used for in vitro quantification of TGF-β2, HSP70, and H-PGDS by using the sandwich enzyme immunoassay. Receiver operating characteristic (ROC) analysis and predictiveness curves showed that each of TGF-β2, HSP70 or H-PGDS alone could not be used as a predictive neuroinflammatory biomarker for ASD. However, when TGF-β2 and HSP70 were combined in one ROC curve, the AUC was increased to an appreciable value that makes them together robust predictors of variation between the ASD and neurotypical control groups. Overall, it was in the present study found significant differences for TGF-β2 and HSP70 when the ASD and neurotypical control groups were compared, independently of the sensory deficit level. In conclusion, the present study highlights the usefulness of TGF-β2, HSP70, and H-PGDS as diagnostic tools to differentiate between ASD and neurotypical control children, but not among subgroups of ASD children exhibiting different severity levels of sensory dysfunction. The presented data also suggest the effectiveness of ROC as a powerful statistical tool, which precisely can measure a combined effect of neuroinflammatory biomarkers intended for diagnostic purposes.

Keywords Autism · Transforming growth factor-β · Neuroinflammation · Heat shock protein 70 · Hematopoietic prostaglandin D2 synthase · Oxidative stress

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental/−behavioral disorder that affects children before three years of age. It is characterized by deficits in social and communicative functions, repetitive behaviors, and sensory abnormalities (APA 2013). Currently, one in 68 children in the US is affected by ASD (Christensen et al. 2016). Although the ASD

✉ Geir Bjørklund
bjorklund@conem.org

[1] University of Medical Sciences and Technology, Khartoum, Sudan

[2] Almaaref Colleges for Sciences and Technology, Riyadh, Saudi Arabia

[3] Department of Physiology, Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia

[4] Autism Research and Treatment Center, Riyadh, Saudi Arabia

[5] Shiek Al-Amodi Autism Research Chair, King Saud University, Riyadh, Saudi Arabia

[6] Kirkwood College, Cedar Rapids, IA, USA

[7] Sudan Medical and Scientific Research Institute, Khartoum, Sudan

[8] Council for Nutritional and Environmental Medicine, Toften 24, 8610 Mo i Rana, Norway

[9] Therapeutic Chemistry Department, National Research Centre, Dokki, Cairo, Egypt

[10] Central Laboratory, Female Center for Scientific and Medical Studies, King Saud University, Riyadh, Saudi Arabia

etiology is unknown, the interaction between genetic and environmental has been proposed to influence the occurrence of ASD (Krumm et al. 2014). One additional mechanism that has received serious consideration is an abnormality of immune function (Bjørklund et al. 2016).

Immunological abnormalities have been linked to ASD. In particular, the generations of cytokines and immune component dysregulation have been proved in ASD (Ashwood and Van de Water 2004; Ashwood et al. 2011). However, these abnormalities are generated by brain inflammation. Research shows a decreased number of lymphocytes, skewed cytokine profiles, and imbalanced serum levels of immunoglobulins (Ashwood et al. 2006). Vargas et al. (2005) have been found increased neuroinflammation in ASD specimens including blood, and central nervous system (CNS). Also, the gene expression related to the immune system is increased in ASD patients compared to matched neurotypical controls (Garbett et al. 2008). Immune system dysfunction includes changes in the numbers and activities of macrophages, T cells, B cells, and Natural killer cell activity have been concluded in many studies on ASD (Vojdani et al. 2008; Enstrom et al. 2009). Also, the abnormalities observed in the production of interleukin-2 (IL-2) and gamma interferon, and elevated production of IL-4 (Gupta et al. 1998). It has previously been reported that the adaptive and innate immune responses in ASD children were linked to increased concentrations of TNF-α, IL-1, and/or IL-6 above the control mean value (Bjørklund et al. 2016). These findings suggest immune system changes and neuroinflammation in children with ASD.

The transforming growth factor-β (TGF-β) family includes three structurally and functionally related mammalian isoforms of a cytokine (i.e., TGF-β1, TGF-β2, and TGF-β3). The different isoforms of TGF-β are crucial in CNS development and cell fate determination (García-Campmany and Martí 2007), neurogenesis (Vogel et al. 2010), differentiation of glial cells (Stipursky et al. 2014), as well as neuronal migration and survival (Siegenthaler and Miller 2004). The formation of the inhibitory and the excitatory synapses in the cerebral cortex are controlled by TGF-β1 (Diniz et al. 2012, 2014). TGFβ as immunosuppressive cytokines also plays a critical role in immune homeostasis (Den Haan et al. 2007; Sonoda et al. 2001). TGFβ1 is a very important immune regulator that controls different types of immune response. Previously, the published finding has been concluded an alteration in the brain levels of TGFβ1 in samples from ASD subjects. This alteration was associated with behavioral impairments that characterized as core symptoms in ASD (Ashwood et al. 2006). The level of TGFβ2 has been shown to be changed in autistic samples (El-Ansary and Al-Ayadhi 2012a, b).

The HSP70 family is highly conserved of protein chaperones that regulate protein homeostasis and enhance cell survival (Hartl 1996). Some types of HSP are fundamentally expressed under normal physiological conditions, whereas other HSPs are induced by stress (Daugaard et al. 2007). Moreover, the creation of reactive oxygen species (ROS) during cell differentiation and proliferation increases the production of different HSP family members (Simon et al. 2000). The major stress-induced human HSP70 is expressed when the cell is exposed to stress such as heat shock or UV radiation. It is also important in the control of cell signaling for growth, differentiation, as well as apoptosis (Nylandsted et al. 2004). HSP 70 also identifies the natural killer cells by their recognition structures (Multhoff 2002). Milani et al. (2002) have been proposed the role of HSP70 in antigen presentation and immune stimulation.

Exposure of cells to heavy metals, ischemia, microbial infections, nitric oxide, hormones, and antibiotics also induce the expression of different HSPs. Increased expression of HSPs has been shown protective in many cultured cells and animal tissues (Kregel 2002). The HSPs' capacity probably mediates this protection through molecular chaperones, which prevent inappropriate aggregation of proteins. Asea et al. (2000) proposed that HSP70 stimulates cytokine production demonstrating its dual role as a chaperone and cytokine.

When GSH is used as a cofactor, hematopoietic prostaglandin D2 synthase (H-PGDS) metabolizes PGH2 to PGD2 that can be pro or anti-inflammatory lipid mediators depending on disease etiology (Rajakariar et al. 2007). It has a crucial regulative role of anti-inflammatory and pro-inflammatory cytokines. Some H-PGDS metabolites inhibit a range of proinflammatory signaling pathways, including NF-κB. Trivedi et al. (2006) reported that the suppressive effects of H-PGDS on lymphocyte function might depend on the inhibition of NF-kB activity. Inoue et al. (2003) proposed the PGD2 produced by H-PGDS in Th2 cells and human mast cells increases inflammation and allergy responses through stimulation of CRTH2.

Since the discovery of ASD as a neurological disease, sensory dysfunction is now considered an autistic feature (Ben-Sasson et al. 2009; Lane et al. 2010). It is currently estimated that 45–96% of ASD children have sensory difficulties. The behaviors reported by the parents of ASD children are linked to problems with sensory processing, which create social deficits for these children and limit their daily life activeness (Schaaf et al. 2011).

In the present study the plasma levels of TGFβ2, HSP70, and H-PGDS in ASD children and neurotypical children were investigated. To further study whether sensory dysfunctions is affected in correlation with these

biomarkers and gain a clearer explanation of cytokine activities in ASD individuals. Also, the impact of age on immune dysfunction in ASD has been included in the present study.

Material and methods

Patients and treatment

The Ethical Committee of the Faculty of Medicine, King Saud University, approved the present study. After obtaining informed consent, samples of plasma were taken from 38 male children with ASD (age range 2–12 years) and 32 neurotypical control males (age range 2–14 years). The Autism Diagnostic Observation Schedule, Autism Diagnostic Interview-Revised (ADI-R), 3DI (Developmental, Dimensional Diagnostic Interview), as well as the Childhood Autism Rating Scale (CARS) were used to diagnose autism. (Schopler et al. 1986; Kiang et al. 2010). Autism Research and Treatment Center Clinic (ART) has provided autistic samples, while the pediatric clinic at the King Saud medical city has provided control samples. All ASD patients were interviewed by using these scales. All recruited participants had normal urine analysis and sedimentation rate. The selected sample of participants was based on how convenient and readily available the group of participants was (i.e., convenience sampling). Participants were excluded from the study if they had a diagnosis of fragile X syndrome, epileptic seizures, obsessive-compulsive disorder, affective disorders, or any additional psychiatric or neurological diagnoses.

The 38-item Short Sensory Profile (SSP) (Dunn 1999), which is a questionnaire used to determine the variation in sensory impairments. Each SSP item is measured using a five-point scale (Likert). The measured domain scores are in tactile sensitivity areas, the sensitivity of taste/smell, seeking sensation, movement sensitivity, auditory filtering, levels of low energy, as well as visual/auditory sensitivity. The overall sensory responses and domain scores are in SSP categorized as a typical performance, typical performances from probable differences, or typical performances from definite differences. A score lower than 142 indicates a severe performance (typical performance from definite difference), mild to moderate performance is indicated with scores 142–152 (typical performance from probable difference). A typical performance is indicated with scores 153–190. SSP provides information about the levels of sensory processing in ASD children and may be used to evaluate and plane appropriate interventions (Tomchek and Dunn 2007).

Blood sample collection

Blood samples from 38 ASD and 32 matched neurotypical children were drawn after overnight fasting. Blood was taken into 3 mL EDTA containing tubes for blood collection. The samples were centrifuged directly after the blood sampling for 20 min at 4 °C at 3000×g. Until analysis, the plasma was kept at −80 °C.

Biochemical analysis

Heat shock protein 70

Quantitative measurement of HSP70 was done by using the Sandwich ELISA kit from Uscn Life Science Inc., Wuhan, China.

Human transforming growth factor β-2

Human TGFβ2 was measured by using a solid-phase sandwich ELISA kit from Thermo Fisher Scientific (Rockford, IL, USA).

Human hematopoietic prostaglandin D2 synthase determination

To measure hematopoietic prostaglandin D2 synthase (H-PGDS), a double-antibody Sandwich ELISA kit from Uscn, Life Science Inc., USA was used.

Data analysis

The statistical analysis package was used for all data analysis, and all values were expressed as mean ± SD. All statistics were calculated by using the Mann-Whitney (Nonparametric Test) between the different groups (i.e., ASD and control/ typical to moderate and severe sensory profile/ age). A P value of <0.05 was considered significant. Analysis of Receiver Operating Characteristic (ROC) was used to investigate the tested biomarkers accuracy. In addition, predictiveness curves were drawn. The area under the curve (AUC) gave a useful measure to compare the biomarker's diagnostic value. An AUC value that was close to 1 indicated that the diagnostic biomarker was excellent, while a curve showing AUC of 0.5–0.6 was without diagnostic value. The statistical analysis was performing using an SPSS program, which included the Pearson correlation test. The correlation between all investigated parameters was done using the Pearson correlation test.

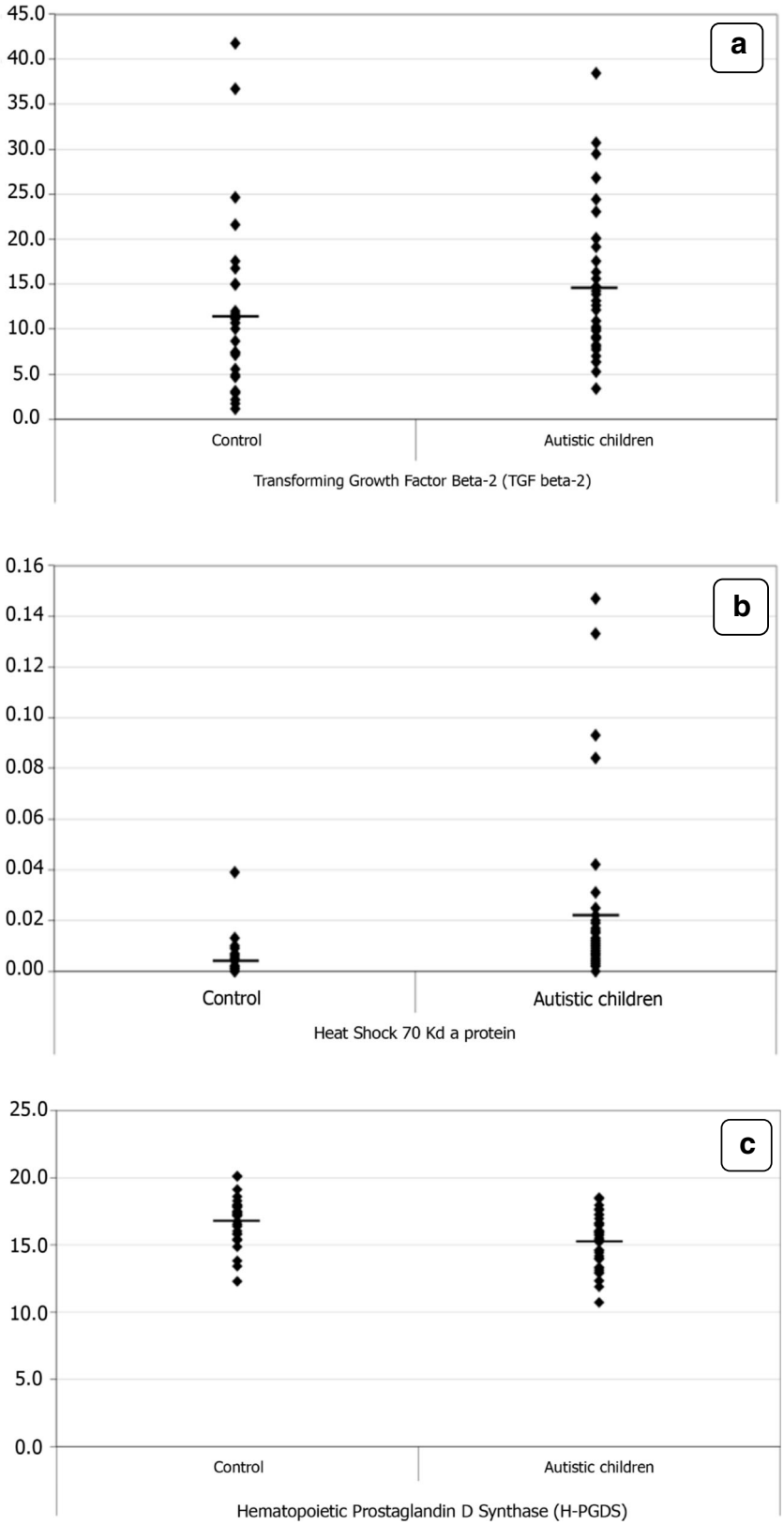


Fig. 1 Plasma cytokine concentrations of ASD and neurotypical children. **Panel A**. compare between the autistic group and the control group. TGF-β concentration TGF-β was significantly higher (*P = 0.001)* in the autistic group relative to the control, TGF-β concentration in pg/mL. **Panel B**. compares between the autistic group and the control group in heat shock protein 70 (HSP70) concentration, HSP70 was higher in the autistic group relative to control, HSP70 concentration in ng/mL. **Panel C**. compare between the autistic group and the control group in H-PGDS concentration, H-PGDS was significantly higher (*P = 0.001)* in the control group relative to autistic, H-PGDS concentrations in ng/mL

Results

Data for all the subjects are presented as mean ± SD. Significant differences found in the groups of ASD patients in the present study are shown in Fig. 1 and Table 1. The HSP70 concentrations in ASD subjects were significantly different in comparison to neurotypical controls ($P < 0.001$). A significant decrease of H-PGDS by 10% ($P < 0.001$) was found in children with ASD from control values of 16.83 ± 1.71 ng/mL, and the range was 12.28–20.1 ng/mL (Table 1). In ASD children, plasma TGFβ2 concentration increased by 27.3% from a mean of 11.49 ± 9.68 to 14.63 ± 8.11 pg/mL in the neurotypical group (Fig. 1). Figure 1 demonstrates the mean value for the three studied parameters presented by line (left side) and the percent change for ASD groups compared to the neurotypical group. Also, Fig. 1 presents the lowest and highest level of these biomarkers for ASD patients and neurotypical controls.

Specificity and sensitivity are basic measures of a diagnostic test's accuracy. These two parameters give information about a test's ability to diagnose the disease correctly when the disease is present and to rule out the disease when it is totally absent. ROC shows that AUC for HSP was 0.832 and 0.738 for H-PGDS while TGFβ2 was 0.645 (Table 2 and Fig. 2). Combined ROC Curve between HSP 70 and H-PGDS in the ASD group showed higher AUC compared with each one (Table 3 and Fig. 3).

The predictiveness curves are in Fig. 4 demonstrated as a performance measure of transforming growth factor-beta 2 (TGF-β2), HSP70, and H-PGDS in the prediction of autism risk in the Saudi population. The three investigated parameters were shown not to have enough predictive power.

The results of the present study showed a significantly different correlation between TGFβ2 and HSP70 (Table 4). Statistical analysis revealed no association between sensory dysfunction severity, age, and elevations in TGFβ-2 and HSP70 or decreasing in H-PGDS ($P > 0.05$).

Discussion

The current study explored some biomarkers as the early diagnosis of autism which is present in plasma and was measured via ELISA. Previous studies were showing that TGF-β2, HSP70, and H-PGDS are associated with neuroinflammation in individuals with ASD.

During early postnatal cerebellar development, many biological responses such as proliferation, apoptosis, neuronal migration, and differentiation are regulated by different cytokines (Lindholm et al. 1992; Buisson et al. 2003). Among them; TGF-β which plays critical functions in the development of the CNS (King et al. 2004), including many biological processes (Wyss-Coray et al. 1995). The expression of TGF-β1 and TGF-β2 differed in the postnatal stage and remain constant until adulthood. The increased level of TGF-β2 in patients (Table 1 and Fig. 1) can be due to brain damage with

Table 1 Plasma concentration of TGF-β2 (pg/mL), HSP70 (ng/mL) and H-PGDS (ng/mL) in the investigated groups of neurotypical and ASD children

Parameters		Groups	N	Min	Max	Mean ± S.D.	Percent change	*P* value
HSP70 (ng/mL)	Groups	Control	30	0.00	0.04	0.004 ± 0.007	100.00%	0.001
		Autistic	38	0.00	0.15	0.022 ± 0.034	506.98%	
	Sensory	Typical	8	0.00	0.03	0.013 ± 0.010	300.00%	0.608
		Severe	14	0.00	0.15	0.035 ± 0.050	809.30%	
	Age Groups	<= 7	16	0.00	0.15	0.025 ± 0.046	576.74%	0.977
		> 7	9	0.00	0.09	0.018 ± 0.029	413.95%	
H-PGDS (ng/mL)	Groups	Control	32	12.28	20.10	16.83 ± 1.71	100.00%	0.001
		Autistic	40	10.73	18.50	15.26 ± 1.89	90.68%	
	Sensory	Typical	8	13.34	18.41	15.71 ± 1.84	93.37%	0.651
		Severe	15	10.73	18.50	15.12 ± 1.90	89.87%	
	Age Groups	<= 7	17	11.86	18.50	15.45 ± 1.77	91.81%	0.435
		> 7	9	10.73	17.64	14.58 ± 2.55	86.62%	
TGFβ-2 (pg/mL)	Groups	Control	29	1.15	41.76	11.49 ± 9.68	100.00%	0.049
		Autistic	34	3.41	38.40	14.63 ± 8.11	127.30%	
	Sensory	Typical	7	6.97	26.81	14.92 ± 6.34	129.82%	0.684
		Severe	11	6.35	38.40	17.28 ± 9.60	150.37%	
	Age Groups	<= 7	14	5.26	38.40	13.74 ± 9.75	119.53%	0.571
		> 7	9	3.41	19.14	12.52 ± 4.52	108.93%	

Mann-Whitney (nonparametric test) analysis for plasma TGFβ-2, HSP70 and H-PGDS concentrations in control and autistic children

Table 2 Receiver operating characteristic curve of the investigated parameters in the group of ASD children

Parameters	The area under the curve	Cutoff value	Sensitivity %	Specificity %	OR (CI)	PPV	NPV
HSP70	0.832	0.0025	89.50%	63.30%	14.68 (4.10–52.52)	75.60%	82.60%
H-PGDS	0.738	16.672	80.00%	62.50%	0.15 (0.05–0.43)	72.70%	71.40%
TGFβ-2	0.645	7.574	85.30%	44.80%	4.71 (1.42–15.62)	64.40%	72.20%

an expression of this cytokine in injured brain tissues and increases in the central nervous system after brain damage induced by ischemia (Stipursky et al. 2014; Lesné et al. 2003). Some studies reported an altered level of TGF-β1 in the brain (Ashwood et al. 2008) while some showed a decreased level of TGF-β1 in plasma of autistic patients (Okada et al. 2007).

A plasma sample from ASD patients showed high expression of TGF-β2, which may be due to inflammation as TGF-β2 can regulate inflammation. Likewise, Wyss-Coray et al. (2001) reported that injection of antiserum against TGF-β1 decreases the neuroinflammation after traumatic damage. In addition to this Vargas et al. (2005) showed that high level of TGF-β1 in brain of ASD patients can lead to inflammation which can also be linked to the generation of amyloid β (Aβ), which previously have been recorded in ASD patients (Frackowiak et al. 2011; Al-Ayadhi et al. 2012). Lesné et al. 2003) have been concluded that TGF-β promotes perivascular inflammation in human astrocyte cultures; it interacts with and increases the production of the amyloid β precursor protein (AβPP) that generates Aβ. This mechanism of action can affect brain functions such as memory, thinking and sensory process which consider as autistic features.

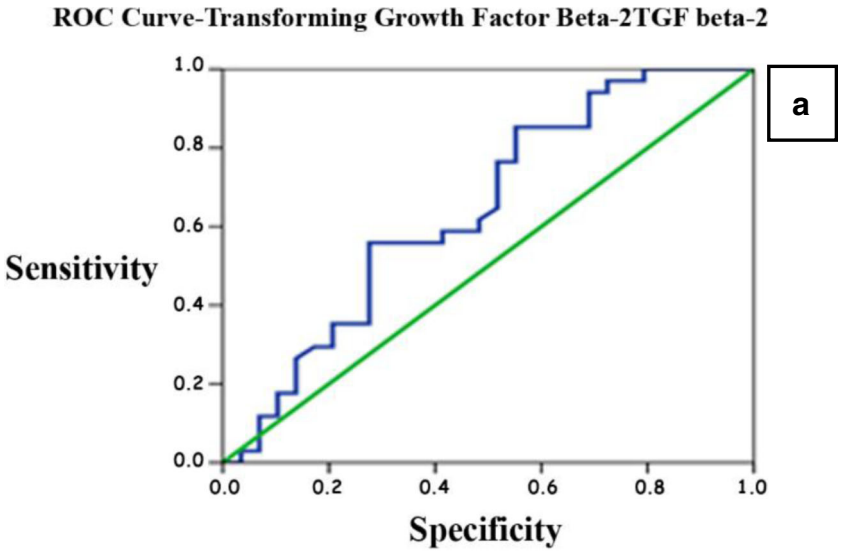
The significant increase in HSP70 concentration in ASD children (Table 1) can be referred to its dual roles as an anti-apoptotic and anti-inflammatory cytokine. It elevates anti-apoptotic protein expression, B cell lymphoma 2 (Bcl-2), by blocking the apoptosis-inducing factor, and interferes with apoptosis protease-activating factor-1(Lesné et al. 2006). Some studies on the brains of individuals with schizophrenia or ASD have been shown changes in HSP70 mRNA levels (Vargas et al. 2005; Yenari et al. 2005). HSP70 overexpression has in different nervous system injury models been shown to have a protective role. However, HSP70 has in some diseases been considered harmful (Arion et al. 2007).

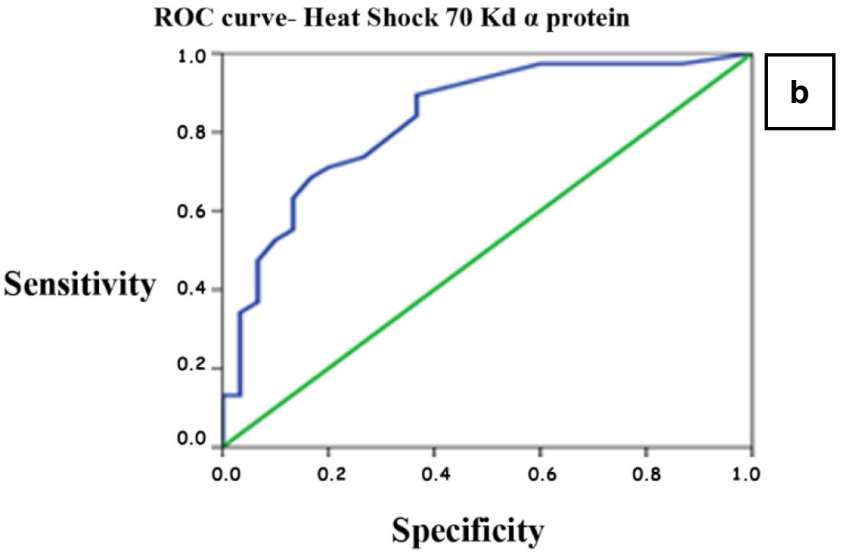
Oxidative stress is considered an important mechanism in the ASD etiology. Although HSPs have a protective role, they only have a beneficial role when expressed at the right time at the right place, and the co-chaperones are present in appropriate levels (Moore et al. 2012). TNF-mediated apoptosis can be initiated by HSP70 by impairing nuclear factor-kappa B (NF-κB) and binding IκB kinase (IKK) and survival signaling because of inactivation after phosphorylation (Kalmar and Greensmith 2009). Elevation of heavy metal toxicity has been proved in ASD patients and among them mercury. Lymphocytes that have been cultured from ASD children when treated with ethyl mercury responded with an up-regulation of numerous Hsp transcripts (Ran et al. 2004).

Interestingly, brain excitotoxicity as an important process in autism etiology can be attenuated through many steps; one of them is up-regulation of HSP70 (Walker et al. 2006). This hypothesis may be supported by the consideration of the recent study by El-Ansary et al. (2011). Additionally, many studies have shown that the elevated HSP70 suppresses NF-κB activity which proves its role in reducing inflammation (do Amaral e Silva Müller et al. 2013; Feinstein et al. 1997; Guzhova et al. 1997; Andrés et al. 2002; Malhotra and Wong 2002).

Increased H-PGDS expression by selenium, which is dose-dependent, was reported in RAW264.7 macrophages, and studies of organic non-bioavailable selenium forms, as well as manipulation of genes in cellular machinery with selenium incorporation, have suggested that selenoproteins are necessary for the expression of H-PGDS (Gandhi et al. 2011). It is also possible that expressions of NF-κB-dependent microsomal PGE2 synthase and thromboxane synthase were regulated down by selenium. This may suggest that selenium is of importance in the AA metabolism toward prostaglandin D 2 (PGD2) production metabolites that can be of clinical importance. Based on this information, the significant lower concentration in the present study of H-PGDS (Table 3) may be due to deficiency of selenium, which repeatedly have been reported in ASD patients compared to neurotypical control subjects (Sajdel-Sulkowska et al. 2008; Lakshmi Priya and Geetha 2011; Blaurock-Busch et al. 2012; El-Ansary et al. 2017).

Recent results indicate that PGD2 protects neonatal mouse brain from hypoxic-ischemic injury (Taniguchi et al. 2007) and inhibits the expression of inducible nitric oxide synthase (iNOS) in rat vascular smooth muscle cells (Trivedi et al. 2006; Nagoshi et al. 1998).





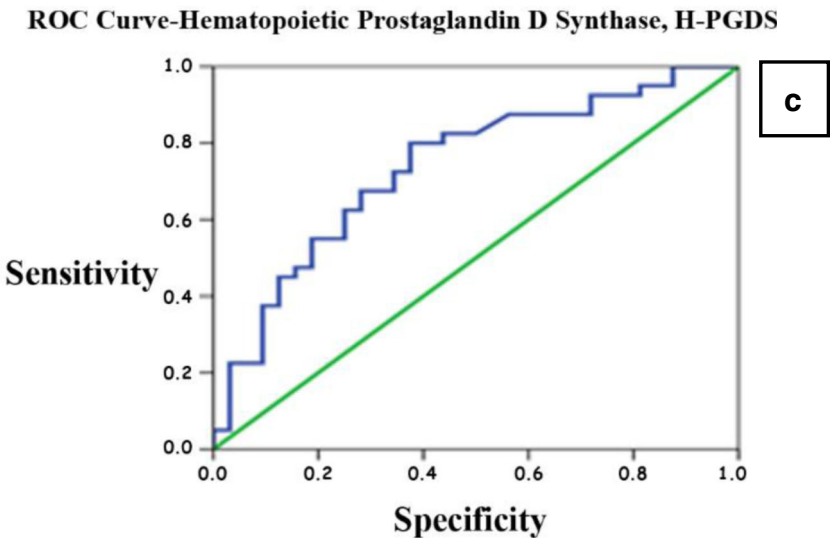


Fig. 2 Receiver operating characteristic (ROC) curve of all parameters for the autistic group. Panel A: ROC curve of TGF-β. Panel B: ROC curve of HSP70. Panel C: RO curve of H-PGDS

Table 3 * Combined receiver operating characteristic curve between HSP70 and H-PGDS in ASD children

	HSP70	H-PGDS	Combining
Area under the curve	0.832	0.738	0.861
Cutoff value	0.0025	16.672	
Sensitivity %	89.50%	80.00%	94.70%
Specificity %	63.30%	62.50%	63.30%
OR (CI)	14.68 (4.10–52.52)	0.15 (0.05–0.43)	31.09 (6.24–154.90)
PPV	75.60%	72.70%	76.60%
NPV	82.60%	71.40%	90.50%

* to indicate increase in area under the curve and sensitivity in combined ROC curve between HSP-70 and H-PGDS

H-PGDS mice are bearing a delayed-type hypersensitivity reaction that displays an exaggerated inflammatory response, which fails to resolve. In the present study, the observed significant decrease of H-PGDS in ASD patients compared to neurotypical control participants may be a result of neuroinflammation as an etiological mechanism in ASD. Lower H-PGDS accompanied with a lower level of the protective PGD2 can be easily related to the elevated nitric oxide and the exaggerated inflammatory response previously recorded in patients with ASD (El-Ansary and Al-Ayadhi 2012a, 2012b). Moreover, ASD patients demonstrate increased blood level of nitric oxide (NO), nitrites, and nitrates which might increase the permeability of BBB and intestinal permeability, as common features in ASD (Sweeten et al. 2004).

Table 2 and Fig. 2 show the ROC analysis for all the measured parameters. Among these parameters, HSP70 recorded the much higher AUC, specificity and sensitivity which help to suggest them as a good marker to discriminate between ASD and neurotypical subjects. In the same way, predictiveness curves give information about the distribution of scores for ROC curves as well as providing a scale to evaluate the validity of biomarkers. Also, to evaluate biomarker validity, predictiveness curves may be utilized to define the optimal threshold scores to select biomarkers for testing the effectiveness of any treatment approach. Fig. 4 shows moderate effectiveness of the measured parameters as predictive biomarkers in ASD. This can be ascertained with the presented ROC curves in which the highest AUC recorded was 0.832 showing an acceptable but not excellent usefulness as biomarkers.

Table 3 demonstrates the combined ROC in the present study. The analysis of receiver operating characteristic (ROC) indicated that the combination HSP70 and H-PGDS produced the best specificity and sensitivity for ASD diagnosis. Therefore, these three markers can be very useful in the diagnosing of ASD.

Limitations

The findings of the present study are hindered by the somewhat small sample size, and will, therefore, need to be replicated by higher numbers of participants. Also, all the recruited participants in this study were males, so the findings cannot be anticipated to be present in females with autism. The inclusion of both sexes in our future studies

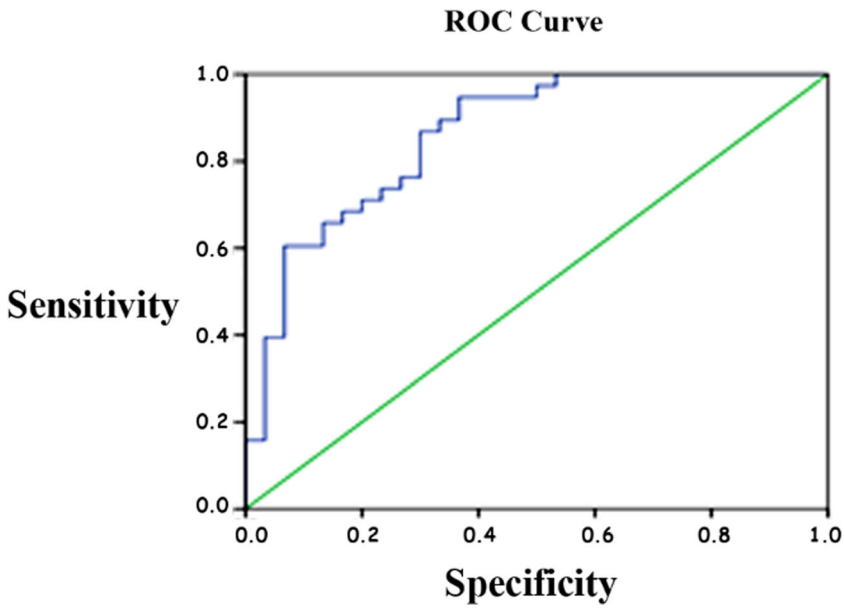


Fig. 3 Combined receiver operating characteristic curve between HSP70 and H-PGDS in the autistic group

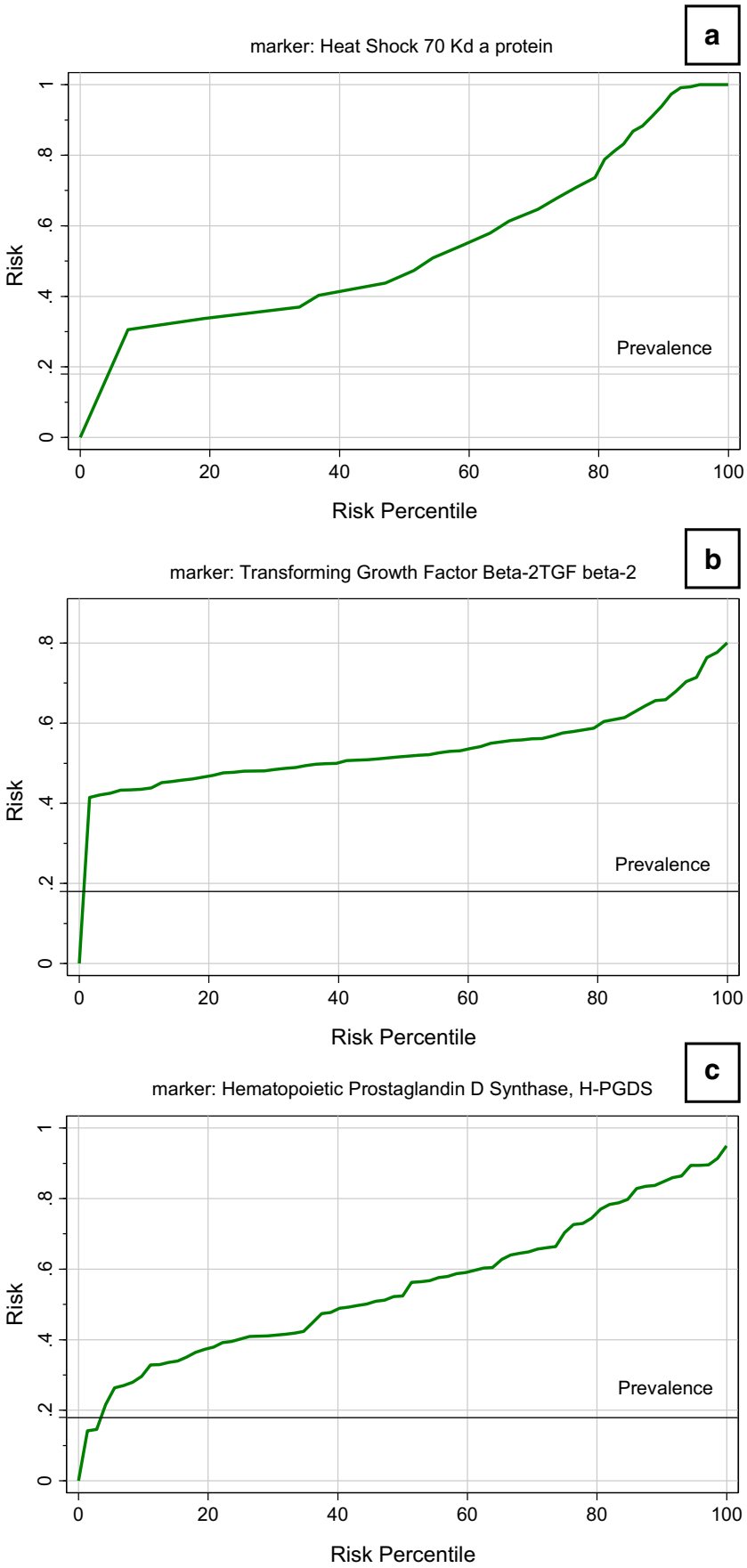


Fig. 4 Predictiveness curve of all parameters for the autistic group. Panel A: Predictiveness curve of TGF-β. Panel B: Predictiveness curve of HSP70. Panel C: Predictiveness curve of H-PGDS

Table 4 Pearson correlation parameters

Parameters	R (Person Correlation)	Sig.	
HSP 70 ~ TGFβ-2	0.377**	0.003	P[a]
HSP 70 ~ H-PGDS-	−.203	.096	p[b]
TGFβ-2~PGDS-H	−.190	0.136	p[b]

[a] Positive Correlation

[b] Negative Correlation

** Correlation is significant at $P = 0.01$

* Correlation is significant at $P = 0.05$

may help to understand the sex differences in autism with males being more vulnerable to develop neuroinflammation compared to females.

Conclusions

The present study highlights the usefulness of the measured parameters to discriminate between ASD patients and neurotypical controls but not between subgroups of patients with different severity of sensory dysfunction or age. Also, it proves the suitability of combining ROC as a statistical tool which might help in grouping a set of markers together with more accurate and potential diagnostic value.

Acknowledgements This project was funded by the National Plan for Science Technology and Innovation (MAARIFAH), King Abdulaziz City for Science and Technology, Kingdom of Saudi Arabia, Award number: 08-MED 510-02. We would like to acknowledge Mrs. Ramesa Shafi Bhat (Biochemistry Department, King Saud University) for revising the manuscript.

Authors' contributions All authors contributed equally to the conception, design, analysis, drafting, and revising of the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare no potential conflicts of interest with respect to the authorship, and/or publication of this article.

Ethical approval All procedures performed were in accordance with the ethical standards of the institutional and/or national research committee, and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Abbreviations ADI-R, Autism Diagnostic Interview-Revised; ASD, autism spectrum disorder; AUC, Area under the curve; CARS, Childhood Autism Rating Scale; H-PGDS, Hematopoietic prostaglandin D2 synthase; HSP70, Heat shock protein 70; HRP, Horseradish peroxidase; IFN-γ, Interferon- γ; IL-2, Interleukin-2; ROC, Receiver operating characteristics; ROS, Reactive oxygen species; TGF-β, Transforming growth factor-β; TNF-α, Tumor necrosis factor-a.

References

Al-Ayadhi LY, Ben Bacha AG, Kotb M, El-Ansary A (2012) A novel study on amyloid b peptide 40, 42 and 40/42 ratio in Saudi autistics. Behav Brain Funct 8(1):4. https://doi.org/10.1186/1744-9081-8-4

Andrés D, Díez-Fernández C, Castrillo A, Cascales M (2002) Relationship between the activation of heat shock factor and the suppression of nuclear factor-kappaB activity in rat hepatocyte cultures treated with cyclosporine a. Biochem Pharmacol 64(2):247–256. https://doi.org/10.1016/S0006-2952(02)01115-2

APA - American Psychiatric Association (2013) Diagnostic and Statistical Manual of Mental Disorders, 5th ed.; American Psychiatric Publishing: Arlington

Arion D, Unger T, Lewis DA, Levitt P, Mirnics K (2007) Molecular evidence for increased expression of genes related to immune and chaperone function in the prefrontal cortex in schizophrenia. Biol Psychiatry 62(7):711–721. https://doi.org/10.1016/j.biopsych.2006.12.021

Asea A, Kraeft SK, Kurt-Jones EA, Stevenson MA, Chen LB, Finberg RW, Koo GC, Calderwood SK (2000) HSP70 stimulates cytokine production through a CD14-dependent pathway, demonstrating its dual role as a chaperone and cytokine. Nat Med 6(4):435–442. https://doi.org/10.1038/74697

Ashwood P, Van de Water J (2004) A review of autism and the immune response. Clin Dev Immunol 11(2):165–174. https://doi.org/10.1080/10446670410001722096

Ashwood P, Wills S, Van de Water J (2006) The immune response in autism: a new frontier for autism research. J Leukoc Biol 80(1):1–15. https://doi.org/10.1189/jlb.1205707

Ashwood P, Enstrom A, Krakowiak P, Hertz-Picciotto I, Hansen RL, Croen LA, Ozonoff S, Pessah IN, Van de Water J (2008) Decreased transforming growth factor beta1 in autism: a potential link between immune dysregulation and impairment in clinical behavioral outcomes. J Neuroimmunol 204(1–2):149–153. https://doi.org/10.1016/j.jneuroim.2008.07.006

Ashwood P, Krakowiak P, Hertz-Picciotto I, Hansen R, Pessah I, Van de Water 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(1):40–45. https://doi.org/10.1016/j.bbi.2010.08.003. Epub 2010 Aug 10

Ben-Sasson A, Hen L, Fluss R, Cermak SA, Engel-Yeger B, Gal E (2009) A meta-analysis of sensory modulation symptoms in individuals with autism spectrum disorders. J Autism Dev Disord 39(1):1–11. https://doi.org/10.1007/s10803-008-0593-3

Bjørklund G, Saad K, Chirumbolo S, Kern JK, Geier DA, Geier MR, Urbina MA (2016) Immune dysfunction and neuroinflammation in autism spectrum disorder. Acta Neurobiol Exp 76(4):257–268

Blaurock-Busch E, Amin OR, Dessoki HH, Rabah T (2012) Toxic metals and essential elements in hair and severity of symptoms among children with autism. Maedica (Buchar) 7(1):38–48

Buisson A, Lesne S, Docagne F, Ali C, Nicole O, MacKenzie ET, Vivien D (2003) Transforming growth factor-beta and ischemic brain injury. Cell Mol Neurobiol 23(4–5):539–550

Christensen DL, Bilder DA, Zahorodny W, Pettygrove S, Durkin MS, Fitzgerald RT, Rice C, Kurzius-Spencer M, Baio J, Yeargin-Allsopp M (2016) Prevalence and characteristics of autism Spectrum disorder among 4-year-old children in the autism and developmental disabilities monitoring network. J Dev Behav Pediatr 37(1):1–8. https://doi.org/10.1097/DBP.0000000000000235

Daugaard M, Rohde M, Jäättelä M (2007) The heat shock protein 70 family: highly homologous proteins with overlapping and distinct functions. FEBS Lett 581(19):3702–3710. https://doi.org/10.1016/j.febslet.2007.05.039

Den Haan R, Rose SH, Lynd LR, van Zyl WH (2007) Hydrolysis and fermentation of amorphous cellulose by recombinant

Saccharomyces cerevisiae. Metab Eng 9(1):87–94. https://doi.org/10.1016/j.ymben.2006.08.005

Diniz LP, Almeida JC, Tortelli V, Vargas Lopes C, Setti-Perdigão P, Stipursky J, Kahn SA, Romão LF, de Miranda J, Alves-Leon SV, de Souza JM, Castro NG, Panizzutti R, Gomes FC (2012) Astrocyte-induced synaptogenesis is mediated by transforming growth factor β signaling through modulation of D-serine levels in cerebral cortex neurons. J Biol Chem 287(49):41432–41445. https://doi.org/10.1074/jbc.M112.380824

Diniz LP, Matias IC, Garcia MN, Gomes FC (2014) Astrocytic control of neural circuit formation: highlights on TGF-beta signaling. Neurochem Int 78:18–27. https://doi.org/10.1016/j.neuint.2014.07.008

do Amaral e Silva Müller G, Vandresen-Filho S, Tavares CP, Menegatti AC, Terenzi H, Tasca CI, Severino PC (2013) Proteomic analysis of the mice hippocampus after preconditioning induced by N-methyl-D-aspartate (NMDA). J Mol Neurosci 50(1):154–164. https://doi.org/10.1007/s12031-012-9888-0

Dunn W (1999) Sensory profile. Psychological Corporation, San Antonio

El-Ansary A, Al-Ayadhi L (2012a) Lipid mediators in plasma of autism spectrum disorders. Lipids Health Dis 11:160. https://doi.org/10.1186/1476-511X-11-160

El-Ansary A, Al-Ayadhi L (2012b) Neuroinflammation in autism spectrum disorders. J Neuroinflammation 9(1):265. https://doi.org/10.1186/1742-2094-9-265

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(1):142. https://doi.org/10.1186/1742-2094-8-142

El-Ansary A, Bjørklund G, Tinkov AA, Skalny AV, Al Dera H (2017) Relationship between selenium, lead, and mercury in red blood cells of Saudi autistic children. Metab Brain Dis 32(4):1073–1080

Enstrom AM, Lit L, Onore CE, Gregg JP, Hansen RL, Pessah IN, Hertz-Picciotto I, Van de Water JA, Sharp FR, Ashwood P (2009) Altered gene expression and function of peripheral blood natural killer cells in children with autism. Brain Behav Immun 23(1):124–133. https://doi.org/10.1016/j.bbi.2008.08.001

Feinstein DL, Galea E, Reis DJ (1997) Suppression of glial nitric oxide synthase induction by heat shock: effects on proteolytic degradation of IkappaB-alpha. Nitric Oxide 1(2):167–176. https://doi.org/10.1006/niox.1997.0117

Frackowiak J, Mazur-Kolecka B, Kuchna I, Brown WT (2011) Accumulation of Amyloid-Beta Peptide Species in Four Brain Structures in Children with Autism. Conference: International Meeting for Autism Research

Gandhi UH, Kaushal N, Ravindra KC, Hegde S, Nelson SM, Narayan V, Vunta H, Paulson RF, Prabhu KS (2011) Selenoprotein-dependent up-regulation of hematopoietic prostaglandin D2 synthase in macrophages is mediated through the activation of peroxisome proliferator-activated receptor (PPAR) gamma. J Biol Chem 286(31):27471–27482. https://doi.org/10.1074/jbc.M111.260547

Garbett K, Ebert PJ, Mitchell A, Lintas C, Manzi B, Mirnics K, Persico AM (2008) Immune transcriptome alterations in the temporal cortex of subjects with autism. Neurobiol Dis 30(3):303–311. https://doi.org/10.1016/j.nbd.2008.01.012

García-Campmany L, Martí E (2007) The TGF beta intracellular effector Smad3 regulates neuronal differentiation and cell fate specification in the developing spinal cord. Development 134(1):65–75. https://doi.org/10.1242/dev.02702

Gupta S, Aggarwal S, Rashanravan B, Lee T (1998) Th1- and Th2-like cytokines in CD4+ and CD8+ T cells in autism. J Neuroimmunol 85(1):106–109

Guzhova IV, Darieva ZA, Melo AR, Margulis BA (1997) Major stress protein Hsp70 interacts with NF-kB regulatory complex in human T-lymphoma cells. Cell Stress Chaperones 2(2):132–139

Hartl FU (1996) Molecular chaperones in cellular protein folding. Nature 381(6583):571–579. https://doi.org/10.1038/381571a0

Inoue T, Irikura D, Okazaki N, Kinugasa S, Matsumura H, Uodome N, Yamamoto M, Kumasaka T, Miyano M, Kai Y, Urade Y (2003) Mechanism of metal activation of human hematopoietic prostaglandin D synthase. Nat Struct Biol 10(4):291–296. https://doi.org/10.1038/nsb907

Kalmar B, Greensmith L (2009) Induction of heat shock proteins for protection against oxidative stress. Adv Drug Deliv Rev 61(4):310–318. https://doi.org/10.1016/j.addr.2009.02.003

Kiang TK, Teng XW, Karagiozov S, Surendradoss J, Chang TK, Abbott FS (2010) Role of oxidative metabolism in the effect of valproic acid on markers of cell viability, necrosis, and oxidative stress in sandwich-cultured rat hepatocytes. Toxicol Sci 118(2):501–509. https://doi.org/10.1093/toxsci/kfq294

King VR, Phillips JB, Brown RA, Priestley JV (2004) The effects of treatment with antibodies to transforming growth factor beta1 and beta2 following spinal cord damage in the adult rat. Neuroscience 126(1):173–183

Kregel KC (2002) Heat shock proteins: modifying factors in physiological stress responses and acquired thermotolerance. J Appl Physiol 92(5):2177–2186. https://doi.org/10.1152/japplphysiol.01267.2001

Krumm N, O'Roak BJ, Shendure J, Eichler EE (2014) A de novo convergence of autism genetics and molecular neuroscience. Trends Neurosci 37(2):95–105. https://doi.org/10.1016/j.tins.2013.11.005

Lakshmi Priya MD, Geetha A (2011) Level of trace elements (copper, zinc, magnesium and selenium) and toxic elements (lead and mercury) in the hair and nail of children with autism. Biol Trace Elem Res 142(2):148–158. https://doi.org/10.1007/s12011-010-8766-2

Lane AE, Young RL, Baker AE, Angley MT (2010) Sensory processing subtypes in autism: association with adaptive behavior. J Autism Dev Disord 40(1):112–122. https://doi.org/10.1007/s10803-009-0840-2

Lesné S, Docagne F, Gabriel C, Liot G, Lahiri DK, Buée L, Plawinski L, Delacourte A, MacKenzie ET, Buisson A, Vivien D (2003) Transforming growth factor-beta 1 potentiates amyloid-beta generation in astrocytes and in transgenic mice. J Biol Chem 278(20):18408–18418

Lesné S, Koh MT, Kotilinek L, Kayed R, Glabe CG, Yang A, Gallagher M, Ashe KH (2006) A specific amyloid-beta protein assembly in the brain impairs memory. Nature 440(7082):352–357. https://doi.org/10.1038/nature04533

Lindholm D, Castrén E, Kiefer R, Zafra F, Thoenen H (1992) Transforming growth factor-beta 1 in the rat brain: increase after injury and inhibition of astrocyte proliferation. J Cell Biol 117(2):395–400

Malhotra V, Wong HR (2002) Interactions between the heat shock response and the nuclear factor-kappaB signaling pathway. Crit Care Med 30(1Supp):S89–S95

Milani V, Noessner E, Ghose S, Kuppner M, Ahrens B, Scharner A, Gastpar R, Issels RD (2002) Heat shock protein 70: role in antigen presentation and immune stimulation. Int J Hyperth 18(6):563–575. https://doi.org/10.1080/02656730210166140

Moore SA, Kim MY, Maiolini A, Tipold A, Oglesbee MJ (2012) Extracellular hsp70 release in canine steroid responsive meningitis-arteritis. Vet Immunol Immunopathol 145(1–2):129–133. https://doi.org/10.1016/j.vetimm.2011.10.021

Multhoff G (2002) Activation of natural killer cells by heat shock protein 70. Int J Hyperth 18(6):576–585. https://doi.org/10.1080/02656730210000017109

Nagoshi H, Uehara Y, Kanai F, Maeda S, Ogura T, Goto A, Toyo-oka T, Esumi H, Shimizu T, Omata M (1998) Prostaglandin D2 inhibits inducible nitric oxide synthase expression in rat vascular smooth muscle cells. Circ Res 82(2):204–209

Nylandsted J, Gyrd-Hansen M, Danielewicz A, Fehrenbacher N, Lademann U, Høyer-Hansen M, Weber E, Multhoff G, Rohde M, Jäättelä M (2004) Heat shock protein 70 promotes cell survival by

inhibiting lysosomal membrane permeabilization. J Exp Med 200(4):425–435. https://doi.org/10.1084/jem.20040531

Okada K, Hashimoto K, Iwata Y, Nakamura K, Tsujii M, Tsuchiya KJ, Sekine Y, Suda S, Suzuki K, Sugihara G, Matsuzaki H, Sugiyama T, Kawai M, Minabe Y, Takei N, Mori N (2007) Decreased serum levels of transforming growth factor-beta1 in patients with autism. Prog Neuro-Psychopharmacol Biol Psychiatry 31(1):187–190

Rajakariar R, Hilliard M, Lawrence T, Trivedi S, Colville-Nash P, Bellingan G, Fitzgerald D, Yaqoob MM, Gilroy DW (2007) Hematopoietic prostaglandin D2 synthase controls the onset and resolution of acute inflammation through PGD2 and 15-deoxyDelta12 14 PGJ2. Proc Natl Acad Sci 104(52):20979–20984. https://doi.org/10.1073/pnas.0707394104

Ran R, Lu A, Zhang L, Tang Y, Zhu H, Xu H, Feng Y, Han C, Zhou G, Rigby AC, Sharp FR (2004) Hsp70 promotes TNF-mediated apoptosis by binding IKK gamma and impairing NF-kappa B survival signaling. Genes Dev 18(12):1466–1481. https://doi.org/10.1101/gad.1188204

Sajdel-Sulkowska EM, Lipinski B, Windom H, Audhya T, McGinnis W (2008) Oxidative stress in autism: elevated cerebellar 3-nitrotyrosine levels. Am J Biochem & Biotech 4(2):73–84

Schaaf RC, Toth-Cohen S, Johnson SL, Outten G, Benevides TW (2011) The everyday routines of families of children with autism: examining the impact of sensory processing difficulties on the family. Autism 15(3):373–389. https://doi.org/10.1177/1362361310386505

Schopler E, Reichler RJ, Renner BR (1986) The childhood autism rating scale (CARS). Western Psychological Services, Los Angeles

Siegenthaler JA, Miller MW (2004) Transforming growth factor beta1 modulates cell migration in rat cortex: effects of ethanol. Cereb Cortex 14(7):791–802. https://doi.org/10.1093/cercor/bhh039

Simon HU, Haj-Yehia A, Levi-Schaffer F (2000) Role of reactive oxygen species (ROS) in apoptosis induction. Apoptosis 5(5):415–418

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

Stipursky J, Francis D, Dezonne RS, de Araújo APB, Souza L, Moraes CA, Gomes FCA (2014) TGF- β1 promotes cerebral cortex radial glia-astrocyte differentiation in vivo. Front Cell Neurosci 8:1–13. https://doi.org/10.3389/fncel.2014.00393

Sweeten TL, Posey DJ, Shankar S, McDougle CJ (2004) High nitric oxide production in autistic disorder: a possible role for interferon-gamma. Biol Psychiatry 55(4):434–437. https://doi.org/10.1016/j.biopsych.2003.09.001

Taniguchi H, Mohri I, Okabe-Arahori H, Aritake K, Wada K, Kanekiyo T, Narumiya S, Nakayama M, Ozono K, Urade Y, Taniike M (2007) Prostaglandin D2 protects neonatal mouse brain from hypoxic- ischemic injury. J Neurosci 27(16):4303–4312. https://doi.org/10.1523/JNEUROSCI.0321-07.2007

Tomchek SD, Dunn W (2007) Sensory processing in children with and without autism: a comparative study using the short sensory profile. Am J Occup Ther 61(2):190–200. https://doi.org/10.5014/ajot.61.2.190

Trivedi S, Newson J, Rajakariar R, Jacques TS, Hannon R, Kanaoka Y, Eguchi N, Colville-Nash P, Gilroy DW (2006) Essential role for hematopoietic prostaglandin D2 synthase in the control of delayed type hypersensitivity. Proc Natl Acad Sci U S A 103(13):5179–5184. https://doi.org/10.1073/pnas.0507175103

Vargas DL, Nascimbene C, Krishnan C, Zimmerman AW, Pardo CA (2005) Neuroglial activation and neuroinflammation in the brain of patients with autism. Ann Neurol 57(1):67–81

Vogel T, Ahrens S, Büttner N, Krieglstein K (2010) Transforming growth factor- beta promotes neuronal cell fate of mouse cortical and hippocampal progenitors in vitro and in vivo: identification of Nedd9 as an essential signaling component. Cereb Cortex 20(3):661–671. https://doi.org/10.1093/cercor/bhp134

Vojdani A, Mumper E, Granpeesheh D, Mielke L, Traver D, Bock K, Hirani K, Neubrander J, Woeller KN, O'Hara N, Usman A, Schneider C, Hebroni F, Berookhim J, McCandless J (2008) Low natural killer cell cytotoxic activity in autism: the role of glutathione, IL-2 and IL-15. J Neuroimmunol 205(1–2):148–154. https://doi.org/10.1016/j.jneuroim.2008.09.005

Walker SJ, Segal J, Aschner M (2006) Cultured lymphocytes from autistic children and non-autistic siblings up-regulate heat shock protein RNA in response to thimerosal challenge. Neurotoxicology 27(5):685–692. https://doi.org/10.1016/j.neuro.2006.06.003

Wyss-Coray T, Feng L, Masliah E, Ruppe MD, Lee HS, Toggas SM, Rockenstein EM, Mucke L (1995) Increased central nervous system production of extracellular matrix components and development of hydrocephalus in transgenic mice overexpressing transforming growth factor-beta 1. Am J Pathol 147(1):53–67

Wyss-Coray T, Lin C, Yan F, Yu GQ, Rohde M, McConlogue L, Masliah E, Mucke L (2001) TGF-beta1 promotes microglial amyloid-beta clearance and reduces plaque burden in transgenic mice. Nat Med 7(5):612–618

Yenari MA, Liu J, Zheng Z, Vexler ZS, Lee JE, Giffard RG (2005) Antiapoptotic and anti-inflammatory mechanisms of heat-shock protein protection. Ann N Y Acad Sci 1053:74–83. https://doi.org/10.1196/annals.1344.007