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Regular Article

Understanding the roles of glutamine synthetase, glutaminase, and glutamate decarboxylase autoantibodies in imbalanced excitatory/inhibitory neurotransmission as etiological mechanisms of autism

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Aim: Autism is a heterogeneous neurological disorder that is characterized by impairments in communication and social interactions, repetitive behaviors, and sensory abnormalities. The etiology of autism remains unclear. Animal, genetic, and post-mortem studies suggest that an imbalance exists in the neuronal excitation and inhibition system in autism. The aim of this study was to determine whether alterations of the measured parameters in children with autism are significantly associated with the risk of a sensory dysfunction.

Methods: The glutamine synthetase (GS), kidney-type glutaminase (GLS1), and glutamic acid decarboxylase autoantibody levels were analyzed in 38 autistic children and 33 age- and sex-matched controls using enzyme-linked immunosorbent assays.

Results: The obtained data demonstrated significant alterations in glutamate and glutamine cycle enzymes, as represented by GS and GLS1, respectively. While the glutamic acid decarboxylase autoantibodies levels were remarkably increased, no significant difference was observed compared to the healthy control participants.

Conclusion: The obtained data indicate that GS and GLS1 are promising indicators of a neuronal excitation and inhibition system imbalance and that combined measured parameters are good predictive biomarkers of autism.

Key words: autism, excitation, glutamic acid decarboxylase autoantibodies, glutaminase kidney isoform, glutamine synthetase.

AUTISM IS A heterogeneous neurological disorder characterized by impairments in communication and social interaction, repetitive behaviors, and sensory abnormalities.[1] The most recently reported prevalence is 1 per 68 children[2] however, the precise etiological mechanism of autism is unknown. One of the accepted hypotheses regarding the pathophysiology of autism posits that an imbalance exists in the magnitude and timing of the excitatory/inhibitory neurotransmitter system. Growing evidence suggests that environmental pollution and maternal infection

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lead to the upregulation of pro-inflammatory cytokines, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and IL-1β, which may be involved in the development of autism.[3,4] This upregulation is likely related to the disruption in the inhibitory/excitatory balance of GABAergic/glutamatergic transmission previously reported in autism.[5,6]

Because approximately 500 mL of cerebrospinal fluid is absorbed into the blood daily, plasma can be considered a complex body fluid that contains a variety of proteins (e.g., enzymes, receptors, and transporters), peptides, lipids, and metabolites that reflect the physiological and pathological activity of the brain.[7]

Although the excitatory/inhibitory imbalance is a highly reproducible phenomenon in autism, this imbalance is not well understood.[8–11] Many studies have shown a remarkable decrease in GABA receptors in different brain areas, such as the cerebellum, fusiform gyrus, and parietal, frontal, and anterior cingulate cortex, in autistic patients. Additionally, a few studies have demonstrated altered enzymatic activities in the glutamate and gamma aminobutyric acid (GABA) signaling pathways.[12–14] The interconnectivity among glutamate, glutamine, and GABA is maintained by transporters, receptors, and enzymes involved in the production, degradation, and transportation of these metabolites. However, these metabolites perform vastly different functions and are implicated in different theories of psychopathology, including the hyper-glutamatergic and hypo-GABAergic receptor dysfunction theory.[15,16] Numerous published studies have indicated that the glutamate–glutamine cycle is affected in a variety of neurological disorders and conditions, including autism. In neurons, glutamate is synthesized from glutamine via glutaminase. Then, glutamate is released, taken up by astrocytes, and is converted into glutamine by glutamine synthetase; subsequently, glutamine is transported and reused in neurons. This glutamate–glutamine cycle is an important component of the glutamatergic neurotransmission system. Based on previous findings, the glutamate–glutamine cycle has been hypothesized to be impaired in the brains of autistic individuals, and the enzymes associated with this cycle are postulated to be dysregulated. These enzymes play important roles in the central nervous system (CNS) by preventing the excessive accumulation of glutamate in synaptic regions, thereby inhibiting the development of glutamate neurotoxicity.[17–20]

Glutamine synthetase (GS) is an adenosine triphosphate-dependent enzyme located in brain astrocytes that helps eliminate ammonia by interacting with glutamate to yield glutamine. GS plays a critical role in the maintenance of the appropriate levels of glutamate and glutamine.[21–23] Neurological researchers have shown that glutamate and glutamine levels are altered in individuals with an autism spectrum disorder (ASD). In a recent literature review,[24] all studied levels of glutamate and glutamine in autistic children were compared to those in matched controls. The authors showed that the glutamate and glutamine levels in autistic children differed from those in normally developing children. GS is associated with the glutamate–glutamine cycle and has been found to be decreased in post-mortem brain tissues from an autistic individual. This finding provides strong evidence that a dysfunction exists in the glutamate–glutamine cycle in autism.[25,26]

Glutaminase is an inner mitochondrial membrane enzyme predominant in the brain.[27] In neurons, glutaminase is the predominant glutamine-utilizing and glutamate-producing enzyme; hence, glutaminase has the potential to induce neurotoxicity by elevating glutamate.[25] There are two glutaminase isozymes as follows: kidney-type glutaminase (GLS1) and liver-type glutaminase (GLS2). While GLS1 has been identified as kidney-type, it is highly expressed in the brain.[28] Glutaminase, which is an enzyme involved in the glutamate–glutamine cycle, has been found to be significantly lower in the anterior cingulate cortex in post-mortem brains of autistic individuals.[25] Biochemical, electroencephalography, and post-mortem studies have suggested that the dysfunction in the excitatory/inhibitory system is a mechanism causatively linked to cognitive disability in autism.[29–32]

Glutamic acid decarboxylase (GAD) is an important enzyme related to glutamate excitotoxicity, which may be involved in the etiological mechanism of autism. GABA is one of the most important neurotransmitters in the inhibitory system when the CNS is excited. GABA is formed via the decarboxylation of glutamate as a mechanism to prevent the accumulation of glutamate in brain cells. GABA is the rate-limiting enzyme in the glutamate/GABA cycle through which GABA, as an inhibitory neurotransmitter, is synthesized from the excitatory neurotransmitter glutamate; thus, GABA is likely involved in the pathophysiology of autism. There are two isoforms of GAD – GAD65 and GAD67 – that are

derived from two unlinked genes in the adult brain.[33] Because GAD67 is located on chromosome 2q31.1, which is one of the susceptibility loci in autism, the GAD67 gene might play a potential role in the GABAergic abnormalities repeatedly observed in autism.[6,34] Fatemi *et al.*[35] reported that the GAD65 and GAD67 protein levels were reduced in the cerebellum and parietal cortex in the brains of autistic individuals. This finding was further supported by a study performed by Yip *et al.*[36] in which the authors observed a remarkable reduction in the mRNA expression of GAD67 in Purkinje cells in autistic brains.[37] Antibodies against GAD65 are detected in serum from patients with several neurological disorders,[38–40] but information regarding GAD65 autoantibodies in autism is lacking.[41] Previous studies have identified antibodies directed against several CNS proteins in individuals with autism. These antibody targets include glial myelin basic proteins,[42] serotonin receptors,[43] nerve growth factor,[44] cerebellar peptides, brain-derived neurotrophic factor[45] and brain endothelial cells.[46] Scholars have concluded that compared to controls, autoantibodies against brain proteins are increased in autism.[47] Autoantibodies against brain proteins are associated with neurobehavioral problems. These autoantibodies may cross the blood–brain barrier (BBB) during inflammation and exposure to environmental agents. Antibodies against molecules regulating brain function may disturb the excitatory and inhibitory balance.[48]

Several studies suggest that autoimmunity in the CNS is related to the pathogenesis of neurodiseases, and several attempts have been made to investigate the presence of antineuronal antibodies, including anti-GAD autoantibodies (anti-GAD-ab),[49] anti-glutamate receptor (anti-GluR) antibodies, and seven types of antiganglioside antibodies, in children with neurodevelopmental disorders.[50] Anti-GAD-ab are assumed to be involved in the pathogenesis of neurodevelopmental disorders. *In vitro*, GAD-ab from patients with neurological syndromes induce the suppression of GABA release. Therefore, GAD-ab likely play a pathogenic role in these diseases.[51,52]

Fein *et al.*[53] have specifically challenged the assumption that autism is a static and lifelong condition and provide strong evidence that recovering from autism is indeed possible, introducing the possibility of improvement, even without optimal normalization.[54] While several studies have attempted to elucidate the exact mechanisms by which certain typical children develop autism and certain children recover from autism following intervention, it is currently well established that specific medical problems are associated with the severity of the condition and that successfully addressing these comorbidities often leads to significant improvements in overall functioning. Many studies have attempted to identify the best biomedical methods to diagnose autism and distinguish between autism and other mental disorders.[55] The biomedical abnormalities found in autism include neuroinflammation, abnormal gut flora, autonomic dysfunction, immune dysregulation, oxidative stress, and mitochondrial dysfunction. These abnormalities are considered pathological consequences and have a clear negative impact on behavior, intellectual ability, and neurological functioning.

Sensory abnormalities are common features in children with an ASD. These abnormalities affect the tactile, auditory, taste, smell, and visual senses in autistic individuals, and the prevalence of autistic individuals suffering from sensory dysfunction is 30–100% according to psychiatrist observations.[56–58] Autistic children exhibit visual and auditory responses that differ from those exhibited by normally developing children. Sensory abnormalities have been used to differentiate behaviors and distinguish between children with autism and those with other developmental disorders.[59] Sensory abnormalities have been tested using a short sensory profile.[60] Dunn outlined the Short Sensory Profile (SSP) of sensory function, which focuses on the effect of sensory processing on autistic individuals in their daily lives. The principle of this scale is that people respond to sensory events based on a combination of the sensory threshold and response strategies.[61]

In this context, we sought to measure the levels of GS, GLS1, and GAD-abs in plasma from 38 autistic patients and 33 age- and sex-matched healthy children to understand the role of these enzymes in glutamate excitotoxicity and the inhibitory imbalance in the neuronal excitation and inhibition (E/I) signaling repeatedly reported as the pathological mechanisms of autism. In addition, this study attempts to identify the correlation between the E/I system and sensory abnormalities in autism measured by the SSP.

METHODS

Participants

This study followed the Helsinki Declaration ethical guidelines (5th revision, Edinburgh, 2000). All subjects enrolled in this study (38 male autistic children and 33 male control children) assented to participate if developmentally able, and their parents provided written informed consent. The subjects were investigated and diagnosed at the Autism Research and Treatment (ART) Center clinic. The sample population at the ART Center clinic consisted of children diagnosed with an ASD. The diagnosis of ASD was confirmed in all subjects using the Autism Diagnostic Interview – Revised as a generalized assessment tool commonly used in clinical settings,[62] the Autism Diagnostic Observation Schedule[63] and the Developmental, Dimensional, and Diagnostic Interview. The mean age of the autistic patients was 7 years. The pediatric clinic at King Saud Medical City provided the control samples used in our study from children with a mean age of 12 years. Any autistic child with dysmorphic features, a diagnosis of fragile X, or other serious neurological or known physical illnesses was excluded from this study.

Ethical approval

This study was approved by the local Ethical Committee of the Faculty of Medicine, King Saud University, Riyadh, Saudi Arabia.

Chemicals

All chemicals and kits used in this study were of analytical grade and obtained from BioVision (San Francisco, CA, USA), Northwest, DRG International (Springfield, NJ, USA), CUSABIO (College Park, MD, USA), and MyBioSource Company (San Diego, CA, USA).

Blood sample collection

Blood samples were drawn from 38 autistic children and 33 matched controls after an overnight fast. The blood samples were collected in 3-mL blood-collection tubes containing ethylenediaminetetraacetic acid, and the samples were immediately centrifuged at 3000 *g* at 4°C for 20 min. The plasma was stored at –80°C until use.

Biochemical analyses

Determination of GS

GS was measured using an enzyme-linked immunosorbent assay (ELISA) kit obtained from MyBioSource. This assay employs the quantitative sandwich enzyme immunoassay technique, which was performed according to the manufacturer's instructions. The microtiter plate provided in this kit was precoated with an antibody specific to GS. The enzyme-substrate reaction was terminated by the addition of a sulfuric acid solution, and the color change was spectrophotometrically measured at a wavelength of 450 ± 2 nm. The range of the detection limit was 15.6–1000 pg/mL.

Determination of human GLS1

GLS1 was measured using an ELISA kit obtained from MyBioSource. This assay employs the quantitative sandwich enzyme immunoassay technique, which was performed according to the manufacturer's instructions. The microtiter plate provided in this kit was precoated with an antibody specific to GLS. The enzyme-substrate reaction was terminated by the addition of a sulfuric acid solution, and the color change was spectrophotometrically measured at a wavelength of 450 ± 2 nm. The range of the detection limit was 0.312–20 ng/mL.

Determination of human GAD-ab

The levels of glutamate decarboxylase autoantibody were measured using an ELISA kit obtained from MyBioSource. This assay employs the quantitative sandwich enzyme immunoassay technique, which was performed according to the manufacturer's instructions. The microtiter plate provided in this kit was precoated with an antibody specific to GAD-Ab. The enzyme-substrate reaction was terminated by the addition of a sulfuric acid solution, and the color change was spectrophotometrically measured at a wavelength of 450 ± 2 nm. The range of the detection limit was 1.56–100 U/mL.

Data analysis

Statistical analyses were performed using SPSS (Chicago, IL, USA) and all values are expressed as the mean ± SD. The parameters were analyzed using the Mann–Whitney *U*-test (nonparametric test) to compare the different groups (i.e., autistic and control/typical to moderate and severe sensory

profile/age). The differences were considered statistically significant at $P < 0.05$. A receiver–operator curve (ROC) analysis was performed to measure the specificity and sensitivity of the three measured markers.[64,65] Briefly, logistic regression models were estimated for each marker individually and for a combination of the three markers to differentiate between individuals with autism and normal healthy controls. ROC were constructed for each logistic regression model, and the area under the curve (AUC) was compared among the markers or marker combinations using a non-parametric method.[66] The AUC provided a useful measure to compare the diagnostic values of the biomarkers. An AUC value close to 1 indicated an excellent diagnostic marker, while a curve with an AUC of 0.5–0.6 had no diagnostic utility. Moreover, curves representing the predictive value of the measured parameters were drawn in which the *x*-axis represents the percentile rank of the biomarker, the *y*-axis represents the probability of identifying the disease, and the horizontal line represents the prevalence of the disease as determined using the BioStat 16 computer program (AnalystSoft, Walnut, CA, USA). In this study, a combined ROC for the measured parameters was calculated to determine whether the collected parameters have a strong diagnostic utility in autism pathology.

RESULTS

The levels of GS, GLS1, and GAD-abs were measured in plasma from autistic patients aged approximately 7 years with different sensory profile severities (mild–moderate or severe) and compared to those in control subjects. The patients were classified according to their recorded SSP (Table 1 and Fig. 1). The data are presented as the mean ± SD of a maximum number of 38 patients with ASD compared to 30 controls, and the significant differences between the groups and subgroups of patients with autism are presented in the table. Two of the three measured parameters significantly differed between the autistic subjects and the controls but not among the subgroups of patients showing differing levels of sensory dysfunction. Moreover, no significant difference related to age was observed. Table 2 shows the AUC, specificity, and sensitivity of the three measured parameters; notably, GS had the highest AUC, followed by GLS1 (0.804 and 0.762, respectively), while GAD-abs had an AUC of only 0.576. As shown in the same table, the AUC of the three combined parameters was remarkably

Table 1. Mann–Whitney *U*-test comparing GS, GLS1, and GAD among the groups

		Groups	*N*	Mean ± SD	*P*-value
GS (pg/mL)	Total autistic	Control	30	10.09 ± 9.25	0.001
		Autistic	38	19.94 ± 9.20	
	SSP score	Typical/mild	8	20.05 ± 9.19	0.891
		Severe	14	21.23 ± 10.39	
	Age (years)	≤7	16	20.03 ± 10.35	0.500
		>7	8	17.27 ± 9.89	
GLS1 (ng/mL)		Control	33	0.07 ± 0.16	0.001
	Total autistic	Autistic	39	0.66 ± 1.35	
	SSP score	Typical/mild	7	0.43 ± 0.51	0.972
		Severe	15	1.08 ± 1.87	
	Age (years)	≤7	17	0.63 ± 1.60	0.907
		>7	8	0.82 ± 1.36	
GAD-ab (U/mL)	Total autistic	Control	30	0.31 ± 0.34	0.333
		Autistic	38	0.34 ± 0.32	
	SSP score	Typical/mild	6	0.27 ± 0.13	0.969
		Severe	15	0.30 ± 0.24	
	Age (years)	≤7	17	0.33 ± 0.28	0.829
		>7	9	0.31 ± 0.19	

GADab glutamic acid decarboxylase autoantibody GLS1 kidneytype glutaminase GS glutamine synthetase SSP Short Sensory Profile

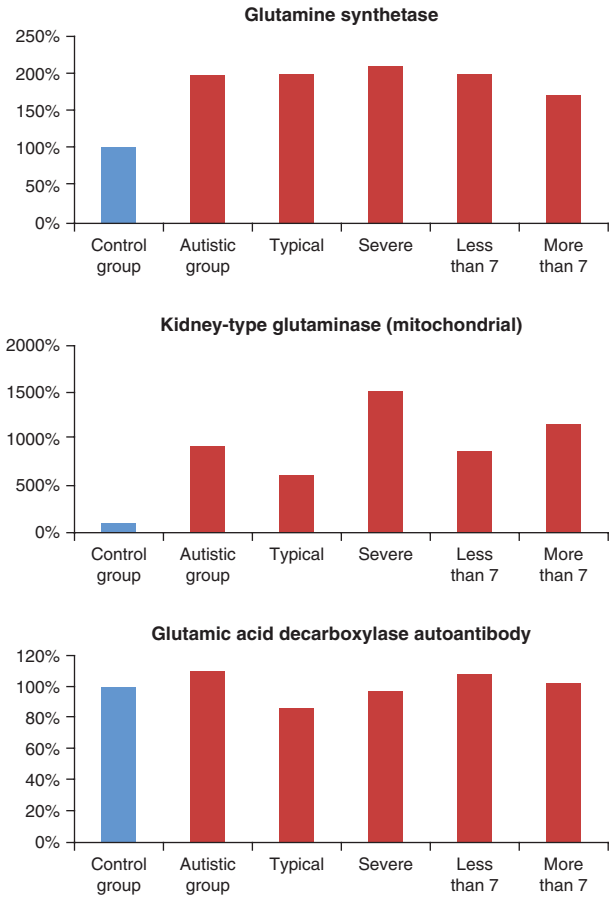


Figure 1. Percentage change of glutamine synthetase, kidney-type glutaminase (mitochondrial), and glutamic acid decarboxylase autoantibody in all groups compared to control.

high (0.833). Figure 2 demonstrates the predictiveness curves of the three measured parameters independently and combined. Positive correlations were observed between GS and GLS1 and between GS and GAD-abs (Table 3 and Fig. 3).

DISCUSSION

Our hypothesis is supported by Ajram *et al.*, who proposed that the glutamate and glutamine–GABA cycle dysregulation plays a critical role in autism and, in turn, leads to an E/I imbalance in this disorder.[67] Horder *et al.* have provided primary evidence suggesting that patients with autism have impairment in brain glutamate and/or glutamine metabolism. These authors have also suggested that this cycle can be a common pathway in this disorder.[18]

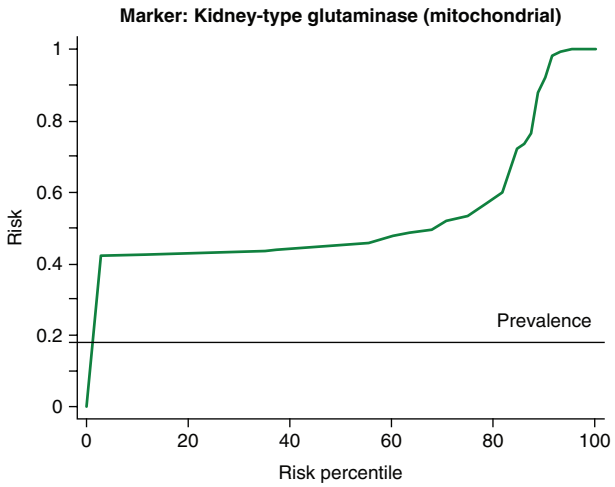
As shown in Table 1, GS and GLS exhibited a nearly 2- and 10-fold increase, respectively, in the plasma of autistic patients, but no significant differences were observed in the autoantibody positivity against GAD. The significant increase in GS, which is an astroglial-specific enzyme, in the plasma from autistic patients compared to the control suggests that astroglial abnormalities may be involved in the etiology of the pathology of autism.

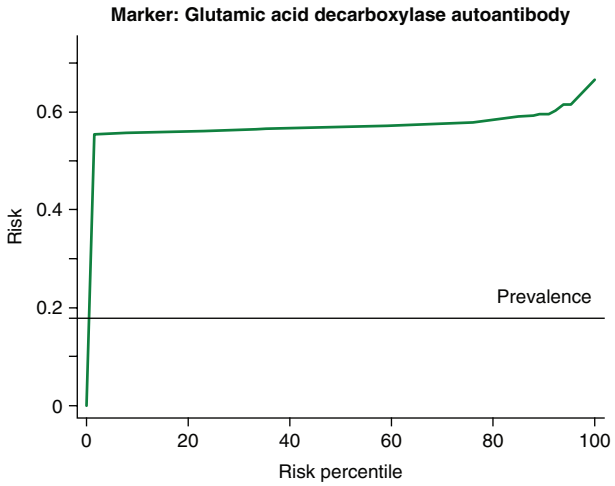
The finding in the present study is consistent with a study performed by Bernstein *et al.*,[68] who concluded that the GS density in different brain regions in schizophrenic patients who had attempted suicide was higher than that in healthy controls. A reduced glutamate/glutamine ratio has been previously reported in a study using proton magnetic resonance spectroscopy to investigate adult individuals with autism, and this reduced ratio may be related to the increase in the GS enzymes in brain areas of autistic individuals.[69] The valproic acid rodent model of autism supports the role of astroglial abnormalities in autism.[70] The prenatal administration of valproic acid induces neuronal death by an indirect effect due to astrocytic morphological changes,[71] which are accompanied by the release of the pro-inflammatory cytokine TNF-α.[72] The significantly increased GS concentration in the plasma

Table 2. Individual and combined ROC for the three measured markers in the autistic group

					95%CI	
Group	Area under the ROC	Cut-off value	Sensitivity %	Specificity %	Lower bound	Upper bound
GS	0.804	9.476	89.5%	60.0%	0.698	0.911
GLS1, mitochondrial	0.762	0.030	79.5%	57.6%	0.653	0.871
GAD-ab	0.570	0.070	100.0%	21.4%	0.425	0.715
Combined	0.833	—	82.9%	71.4%	0.732	0.933

CI, confidence interval; GAD-ab, glutamic acid decarboxylase autoantibody; GLS1, kidney-type glutaminase; GS, glutamine synthetase; ROC, receiver–operator curve.





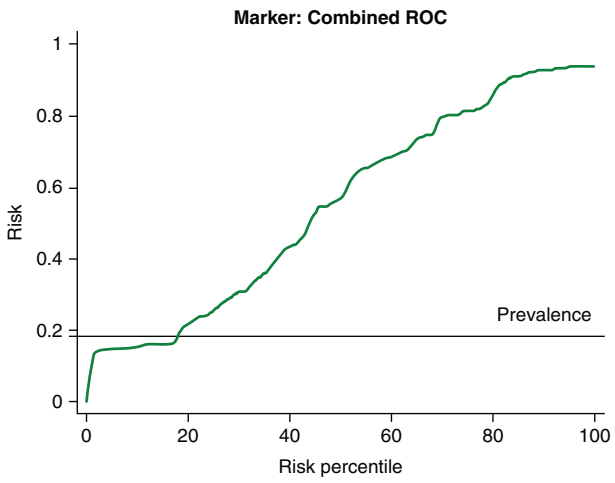
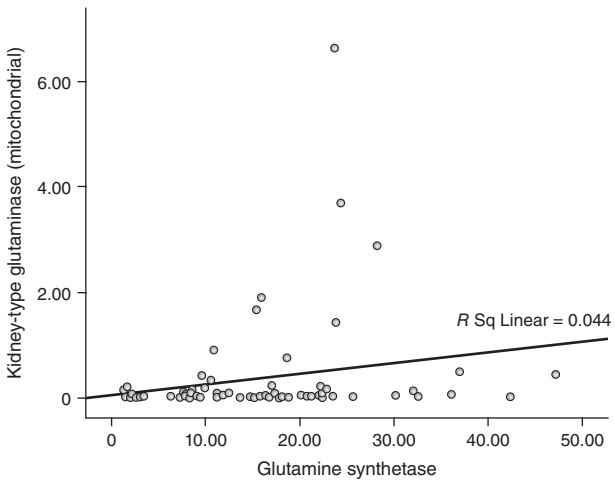


Figure 2. Predictiveness curves of the three measured parameters independently and combined. ROC, receiver–operator curve.

Table 3. Pearson's correlations among the three measured parameters

Parameters	*R* (Pearson correlation)	Sig.	
GS – GLS1, mitochondrial	0.260*	0.034	$P^{\dagger}$
GS – GAD-ab	0.254*	0.043	$P^{\dagger}$

*Correlation is significant at the 0.05 level.
†Positive correlation.
GAD-ab, glutamic acid decarboxylase autoantibody; GLS1, kidney-type glutaminase; GS, glutamine synthetase.



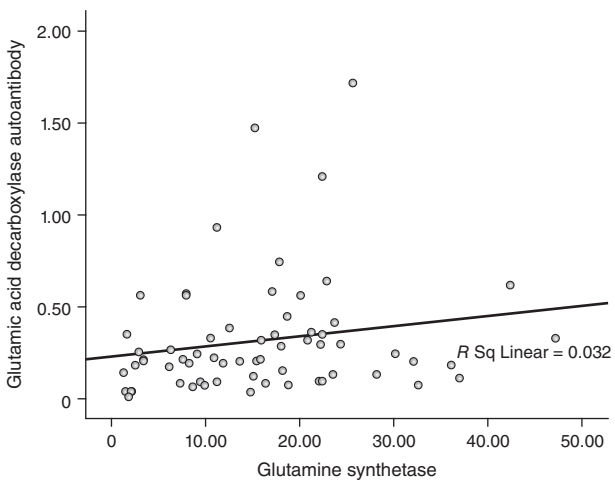


Figure 3. Correlation between glutamine synthetase and different parameters.

from autistic patients presented in Table 1 can be easily related to the oxidative stress and disrupted BBB etiological mechanisms repeatedly observed in autism. Moreover, our results can be explained by the disrupted BBB, which can result in the release of glutamate and related enzymes through an active efflux pump mechanism.[73]

In neurodegenerative diseases, the forkhead box transcription factor FOXO3a is well known to mediate the cellular response to oxidative stress by regulating the expression of selective genes, including GS. However, while the levels of GS may increase, its activity may be compromised by the increasing levels of oxidative stressors, including glutamate, resulting in dysfunctional enzyme activity, neuronal excitotoxic death, and cognitive impairment.[74] The proposed relation between glutamate excitotoxicity and GS upregulation is supported by an *in vitro* study conducted by Lehmann *et al.*,[75] who demonstrated that high extracellular glutamate levels stimulate the expression of astrocyte GS. In general, the impairment in the normal metabolism of glutamate and ammonium overload are two biochemical features,[76,77] and both can be related to the GS overexpression repeatedly reported and result in the neurotransmission imbalance causatively linked to mental and cognitive impairments.[32,78]

Despite the fact that neurons are the predominant cell type expressing glutamate, the regulatory mechanisms remain unclear. Ye *et al.*[79] studied neuronal glutaminase and observed the upregulation of GLS in primary human neurons following treatment with two inflammatory cytokines (IL-1β and TNF-α), supporting the potential involvement of GLS in neurotoxicity during inflammation. This finding suggests that glutamate neurotoxicity is related to the observed 10-fold elevation in GLS in the autistic patients compared to the control participants (Table 1).[6] In a recent review written and analyzed by Dickinson *et al.*,[80] compared to control individuals, a glutamate/GABA imbalance was present in autistic patients. Most reviewed studies have demonstrated that inhibitory neurotransmission was decreased due to the reduced numbers of GABA receptors,[12–14,81] and only one study reported that excitation decreased due to either a lower AMPA-type glutamate receptor density or decreased GLS levels in autistic individuals.[82] Therefore, glutamate excitotoxicity is a confirmed etiological mechanism of autism.[6] Because the level of GLS is increased and GLS is an enzyme that catalyzes the conversion of glutamine to glutamate, excitotoxicity-induced neuronal cell death can lead to the clinical presentation of autism. This hypothesis is supported by an early study performed by Newcomb *et al.*,[83] who showed that neuronal death is followed by increased extracellular glutamate levels via mitochondrial glutaminase.

Glutamate formed as a product of GLS in GABAergic neurons is converted to the inhibitory neurotransmitter GABA by GAD enzymes. In GABAergic neurons, the released GABA is taken up by astrocytes and presynaptic neurons. In astrocytes, GABA is metabolized to succinate in a two-step reaction, followed by its further conversion to α-ketoglutarate during the Krebs cycle and then back to glutamate. Because glutamate is a substrate for both GS and GAD, resulting in the formation of glutamine and GABA, respectively, it was interesting to compare the Km values of these two enzymes competing for the same substrate (i.e., glutamate). Early studies revealed values of 3.5 mM and 1.2 mM for human brain GS and GAD, respectively.[84,85] Because higher Km values indicate a lower affinity of an enzyme to a substrate, the higher Km values of GS to glutamate compared to those of GAD to glutamate indicate that GS alone cannot effectively lower the extracellular glutamate concentration, unless it is present in great excess. This hypothesis may explain the finding in the present study in which GS expression in response to glutamate excitotoxicity was enhanced, while no significant changes in GAD were observed. Although the etiology of autism is unknown, autoimmune dysfunction is believed to play a critical role in the etiopathogenesis. The present study shows non-significant differences in the autoantibody positivity against GAD in the plasma from autistic patients. This finding is inconsistent with previous studies performed by Rout *et al.*,[41] who observed high levels of GAD antibodies in serum from autistic individuals and negative results in control participants. Kalra *et al.*[86] performed a comparative study involving autistic individuals, developmentally delayed children, and healthy controls and concluded that 98% of all children participating in this study showed seronegativity against GAD-ab, which supports the current finding regarding the GAD antibodies. A similar finding has been reported in a recent study[49] in which the authors concluded that there was no seropositivity of antineuronal antibodies in autistic children and control children, and no evidence was provided supporting

the association between autism and the antibody positivity of GAD-ab. This discrepancy among GAD-ab results requires more studies to clarify the role of anti-GAD autoantibodies in the etiology of autism. The small number of participants is a main limitation in the present study. More autistic patients should be recruited to support the obtained data. Future studies investigating the gene expression of the studied enzymes might confirm the hypotheses of the present study.

Conclusion

The obtained data suggest that glutamate excitotoxicity is a biochemical mechanism in autism that is likely due to an impairment in the glutamate/glutamine cycle rather than the impaired biosynthesis of GABA as an inhibitory neurotransmitter. However, the E/I imbalance still contributes as an etiological mechanism by increasing the glutamate concentration and decreasing the number of GABA receptors. The understanding of the E/I imbalance has important implications for identifying an autism biomarker profile and developing effective drugs that ameliorate autistic behaviors.

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DISCLOSURE STATEMENT

None of the authors has any conflicts of interest to disclose.

AUTHOR CONTRIBUTIONS

N.H.: Data acquisition. L.A.: Diagnosis and provision of samples. M.O., A.E.: Co-drafting of the manuscript. H.Q., N.M.: Data acquisition. A.E.: Suggestion of the work and drafting of the manuscript.

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