

Chapter

Glutamate Dysregulation in Cingulated Cortices Is Associated with Autism Spectrum Disorder Traits

*Carmen Jimenez-Espinoza, Francisco Marcano Serrano
and José González-Mora*

Abstract

Autism spectrum disorder (ASD) is a severe developmental syndrome that arises largely as a disorder of the neural systems. Despite unclear etiology, one of the most studied causes is the increase in the excitation/inhibition relationship in the sensory and social systems which may explain certain phenotypic expressions in ASD. Glutamate (Glu) is the most important excitatory neurotransmitter in mammals, and the excessive activation of once N-methyl-D-aspartate (NMDA) receptors leads to neuronal death. Crucially, in this study, the finding of elevated Glu concentration [12.10 ± 3.92 (mM) * $p = 0.02$] by ^1H -MRS in the anterior cingulate cortices (ACC) provides strong empirical support for increased arousal in ASD. The imbalance of Glu in cingulated cortices was correlated to dysfunction of social skills, attention switching/tolerance to change, attention to detail, communication, and imagination, (the five deficits present in ASD described in the Autism Quotient test), suggesting new therapeutic avenues.

Keywords: autism spectrum disorders, excitotoxicity, cingulated cortices, spectroscopy resonance magnetic, social skills, attention switching/tolerance to change, attention to detail, imagination, communication

1. Introduction

Autism spectrum disorder (ASD) is a severe developmental syndrome that arises largely as a disorder of neural systems in early childhood. Currently, the etiology remains unclear, and therapeutic options for ASD are limited; however, one of the most studied causes currently is the imbalance in the excitation/inhibition relationship in the sensory systems that can explain the extensive phenotypic variations in ASD, which are generally characterized by deficits in social reciprocity, communication, imagination, and restrictions interests and behaviors [1, 2]. Moreover, it might present secondary medical symptoms that include sleep and eating disorders, anxiety, depression, attention problems, aggressive behavior toward others or themselves,

epilepsy, and gastrointestinal problems. Given this scenario, treating a person with ASD is a serious health problem for specialized medical care, not to mention the high costs they generate.

Through the different magnetic resonance techniques applied in research, many studies have been carried out to observe the involvement the glutamate (Glu) in people with ASD. However, currently, data referring to adults with ASD are scarce, given that most studies have been carried out in childhood, increasingly aggravating the lack of specific pharmacotherapies for adults. The specific pharmacotherapeutic approach in ASD is one of the main objectives of research studies, hence the need to delve deeper into the search for biomarkers that lead to the development of novel drugs for therapeutic application in the clinic. In this sense, the evidence suggests that an imbalance in glutamatergic metabolism and its products is linked to pathophysiological changes in ASD [3–12]. The interest in the different resonance magnetic modalities highlights specific brain areas such as the involvement of the ACC, and posterior cingulate cortex (PCC) in ASD symptoms, specifically magnetic resonance spectroscopy which relates cingulated cortices dysfunction to deficits in joint attention and social skills in ASD [13–21]. Increasing of these, neuropathology advances [22–27], structural MRI [28–32], fMRI [33–40], PET [41, 42], and SPECT [43, 44], including EEG-evoked potentials [45–48] indicate the cingulated cortices as one of the most affected areas of the brain responsible for the symptoms of the autism triad mainly [49].

Taking into account the plentiful evidence mentioned above, our objective is to study the role of Glu metabolism in the ACC and PCC, and its correlation with the five phenotypical characteristics as domains of interest (social, communication, imagination, attention to detail, and attention shifting/change tolerance), evaluated in the psychometric Autism Spectrum Quotient (AQ) test for adults, which we will describe later [2]. Here, we report an independent study of glutamate metabolism in the cingulated cortices in adults with ASD. A deeper follow-up investigation leads to larger when related to highlighted symptoms and/or severity within the AQ test for adults with ASD.

2. The brain metabolism

From the physiological point of view, the metabolism of the brain is made up of a wide variety of molecules, including peptides, neurotransmitters, enzymes, etc., interacting with water. All these molecules, due to their functions and activity, can maintain the physiological balance necessary for the healthy functioning of the brain. A very controlled balance, which represents 80% of the mass of the brain and (2–3) % of the body weight that receives 15% of the blood flow at rest (quote), it is also one of the organs with greater energy demand, which consumes 20% of the oxygen and 25% of the glucose ingested by the body [50]. All this neurometabolic exchange must be rapid and effective, precisely because the brain has few energy reserves and only receives them through the cerebral vascular system, which implies a correct supply of cerebral circulation through the circle of Willis [51, 52].

There is a group of biomolecules in the brain that are detected by proton magnetic resonance spectroscopy, we refer to neurotransmitters, defined as endogenous substances that act as chemical messengers transmitting signals, and that are normally released by neurons into the synaptic space where they exert their function on other neurons or other target cells through a synapse. Although neurotransmitters are

related to their overall excitatory or inhibitory activity, some neurotransmitters can exert both excitatory and inhibitory effects depending on the type of receptors that are present.

There are other molecules that can be released from the same axon terminals as neurotransmitters, and they are known as endogenous neuromodulators [53] of the central nervous system (CNS), also known as neuropeptides, which can increase, prolong, inhibit, or limit the effect of the main neurotransmitter on the postsynaptic membrane, which acts through a system of second messengers [54]. It has also been pointed out that defects in the synthesis, release, or degradation of some neurotransmitters are involved in the pathogenesis of many neurological, muscular, and psychiatric diseases [55]. Evaluating the dynamics of neurotransmitters and neuromodulators in both health and neurological diseases is a challenge, precisely due to the lack of *in vivo* tools to monitor them with high spatiotemporal resolution and thus have a clear understanding of their functions within the nervous system.

2.1 Glutamate

Glutamate is an amino acid that has the four basic criteria to be considered a neurotransmitter. It is one of the most important neurotransmitters in our nervous system and it has a very high concentration. Recognized for functions such as a mediator of memory formation, the management of attention, or the regulation of emotions, in addition to intervening in 80% of all synapses necessary in processes such as neuroplasticity, learning, or movement. The physiological role of glutamate and its dysfunction has gained importance in neurology and psychiatry to the extent that knowledge has deepened about its metabolism, types of receptors, transporters, and homeostasis mechanisms, whose dysfunction can lead to neuronal death.

Additionally, glutamate and aspartate are the brain's dominant excitatory amino acids and constitute the main transmitters of pyramidal cells, the dominant neurons of the cortex where developmental changes occur capable of carrying out transient steps in the visual cortex and the hippocampus, especially during critical periods [56].

The glutamatergic system is distributed throughout the CNS, unlike other neurotransmission systems with more discrete metabolic pathways, which is why Glu is considered a general activator of the CNS [57, 58]. Under physiological conditions, endogenous Glu is one of the most abundant amino acids in our body and is the main excitatory neurotransmitter whose main purpose is to provide energy to the brain. However, exogenous Glu can be dangerous for our brain health in excessive concentrations, causing neurotoxicity. From a functional point of view, Glu acts as an "on switch" in nerve pathways and requires the neurotransmitter g-aminobutyric acid (GABA) as an "off switch" providing the necessary balance between these two neurotransmitters for at proper functioning of the CNS and essential for regulating cognition, learning, memory, and emotional behaviors. The imbalance between Glu excitation and GABA inhibition leads to hyperarousal of CNS related to ASD symptoms [59, 60].

2.1.1 Compartmentalization of glutamate in nervous system cells

One of the functional characteristics of glutamate is the establishment of compartmentalization in neurons and astrocytes. Within glutamatergic transmission, the release of gliotransmitters is an event that occurs by astrocytes through calcium waves [61, 62], where the elevation of intracellular calcium upon physiological neuronal

stimulation is initiated by the activation of the mGluR5 receptor. This fact makes the difference in the vision of astrocytes as neuronal support cells to cells actively participating in neurotransmission and therefore in the processes mediated by neurotransmission [63, 64], glutamate itself being one of the gliotransmitters released [65–67].

This is an indication of the biochemical participation of glial cells in the glutamatergic system, also called the glutamate-glutamine cycle. Here, astrocytes express two key enzymes in glutamate metabolism that are not expressed in neurons and one enzyme that is not expressed in astrocytes but is expressed in neurons. One of the enzymes expressed in astrocytes but not in neurons is glutamate dehydrogenase (GDH) [68], indicative of glutamate compartmentalization in the CNS. Thus, the glia-neuron glutamate-glutamine coupling mechanism demonstrates that the expression of the enzyme glutamine synthetase (GS) is glia-specific [69].

There is also another important compartmentalization establishment for Glu, we are referring to the synthesis of the neuromodulator *N*-acetyl-aspartyl-glutamate (NAAG), which has been the target of study in recent decades. This dipeptide is synthesized from *N*-acetyl-aspartate (NAA) and Glu by the enzyme NAA-synthase following the anabolic pathway, forming a reservoir of Glu that cannot be metabolized, and the NAAG produced is hydrolyzed, under the catabolic pathway by the NAAG enzyme-peptidase, which releases glutamate by activating the mGluR3 receptor, where its metabolic activity occurs by interconnecting neurons, astrocytes, and oligodendrocytes.

This dipeptide derivative of NAA and L-glutamate acts as a neurotransmitter and neuromodulator and is widely distributed in the CNS and peripheral in millimolar [mM] concentrations [70, 71]. Here, the establishment compartmentalization of Glu involves the participation of neurons, oligodendrocytes, and astrocytes where development an important metabolic activity develops known as the tri-cellular metabolism system [72]. This is the only metabolic cycle of amino acids in the brain known yet, that requires the three types of nervous cells. One of the main functions of NAAG is precisely the activation of glutamate (mGluRs) receptors that act as selective agonists of group II metabotropic receptors (mGluR, mGluR3) and involved the potential importance of metabotropic glutamate receptors, showing the neuromodulator NAAG a good candidate for elucidate the glutamatergic pathway ASD targeting. Considering that NAAG is distributed together with different neurotransmitters including Glu and GABA.

2.2 Autism spectrum quotient test (AQ)

The AQ is an instrument to measure the degree to which an adult with normal intelligence has the traits associated with the autism spectrum. Baron-Cohen and colleagues have described the adult test as a new self-assessment screening instrument to measure the degree to which an individual of normal intelligence displays autistic traits and was translated into Spanish by Betty Trabal, Editorial Amat, S.L., Barcelona, [73] with reasonable construct validity in that item that is intended to measure each one of the five domains of interest (social, communication, imagination, attention to detail, and attention shifting/change tolerance) showing moderate to high alpha coefficients.

To carry out this study, the AQ test was previously validated in an aleatory sample of adults with Spanish as their mother tongue [74], the Spanish version of the AQ has shown satisfactory levels of internal consistency and supports the use of the Spanish version of the AQ for the evaluation of ASD. In this sense, is considered a valuable

instrument to quickly quantify where a given individual falls on the continuum from autism to normality.

3. Materials and methods

To further *understand* the role of the glutamatergic imbalance in the cingulated cortices and their relationship with the development of ASD symptoms related to psychometric test AQ scores, we conducted a clinical study of proton magnetic resonance spectroscopy (1H-MRS) to explore the cerebral glutamate levels in ACC and PCC, and determine its correlation with the five undergo change characteristics in ASD development (social skills, attention to detail, attention shifting/tolerance of change, communication, and imagination) compared to control subjects.

3.1 Population recruitment, demographic, and behavioral evaluations

3.1.1 Participants

We recruited 61 adult participants: Nineteen subjects with ASD (3 females; mean $\pm$ age SD: 20.58 $\pm$ 0.71 years, range: 17.8–30.9 years) and 42 typical developmental (TD) control subjects (25 females; 23.2 $\pm$ 0.71 years, 18.4–31.5 years) free of psychiatric or developmental disorders participated (see **Table 1**). The 61 participants were further divided into four subtypes (AQ1, AQ2, AQ3, and AQ4) according to the AQ test for adult's cause met the AQ test score cut-off criteria in all five characteristics domains. All participants with ASD were recruited through the research program through the faculty of Health Sciences. Dept. of Basic Medical Sciences of University of La Laguna (ULL), Tenerife, Spain. Potential participants were excluded if they had a comorbidity, psychiatric, or medical disorder that affects brain development (e.g., schizophrenia or psychosis), a history of head injury, or a genetic disorder associated with ASD, for example, tuberous sclerosis or fragile X syndrome [75]. The participants with ASD who suffered from anxiety or depressive disorders, gastrointestinal disorders, and muscular hypotonia were not excluded, given the high frequency of these comorbidities in ASD. In addition, based on participants' self-report, all participants were without previous medication at the time of the examination.

Informed written consent was obtained from all participants or from their legal guardians, as well as ethical approval for this study provided by the ethical standards and the Helsinki Declaration of 1964, revised in 2000 and approved by our local ethics committee. This study was approved by the Research Ethics and Animal Welfare Committee (CEIBA) (registration number: CEIBA2013–0056) of the University of La Laguna.

Additionally, participants were assessed and stratified into four subgroups according to established categories, empirically derived from Baron-Cohen & collaborators, AQ test scores, where the subgroup algorithm, which combines scores on the five domains of the AQ: Social skills, attention shifting/tolerance to change, attention to detail, communication, and imagination defined the cut-off threshold for producing reliable ASD subgroups [73]. This approach is consistent with previous publications on this sample, providing a description of participant characteristics [74].

The AQ test score for adult, included the five symptom domains and was divided into four subtypes, each with the following cutoff and meaning: AQ1 (0 to 10 points) = below average; AQ2 (11–21 points) = average values of the normal

Demographic characteristics	ASD (n = 19) mean (S.D.)	(TD) (n = 42) mean (S.D.)	Statistics p value
Gender (male/female)	16/3	16/25	P = 0.016
Age (years)	20.58 /0.71)	23.19 (0.71)	P = 0.049
AQ (0–50) points	33.84 (6.36)	11.67 (7.07)	P < 0.0001
Social skills	5.92 (2.54)	1.22 (0.61)	P < 0.0001
Attention switching/tolerance to change	6.81 (1.41)	3.49 (2.06)	P < 0.0001
Attention to detail	4.3 (1.91)	5.03 (2.3)	P < 0.0001
Communication	7.5 (1.75)	2.15 (1.63)	P < 0.0001
Imagination	5.9 (1.82)	2.22 (1.59)	P < 0.0001
Muscular hypotonia	14	0	P < 0.0001
Gastrointestinal disorders	17	0	P < 0.0001
Epilepsy	5	0	P < 0.0015
Familial hypothyroidism	15	0	P < 0.0001
Special education/transition to adult life	6	0	P < 0.0008
Elementary school	13	41	P < 0.0002
Middle school	6	41	P < 0.0008
High school	3	41	P < 0.008

ASD (autism spectrum disorder), TD (Typical development). *p, 0.05 vs. controls; values for age and AQ are group mean $\pm$ standard deviation (range). AQ = Autism Quotient.

Table 1.
Demographic data, neuropsychological, and physical measures.

population (female mean is 15 and male mean is 17); AQ3 (22–31 points) = above average; AQ4 (32–50 points) = very high index of autistic characteristics (Asperger syndrome or high-functioning autism has an average score of 35). Due to the differences in the results of the AQ test domains, we carried out a follow-up to evaluate the neurometabolic pattern of the four subtypes (AQ1, AQ2, AQ3, and AQ4) in the studied population, according to the implication of autistic characteristics. Considering the AQ1 subtype as a typical development control group (TD).

3.2 Proton magnetic resonance spectroscopy (1H-MRS) data acquisition

Proton magnetic resonance spectroscopy (1H-MRS) is a non-invasive imaging method that provides spectroscopic information that allows us to infer the metabolic cellular activity of the individual studied. 1H-MRS data were acquired using a 3 T Signa-HD MR scanner (GE Healthcare, Waukesha, WI, USA). T2-weighted images were used for positioning the volumes of interest (VOIs). The single voxel acquisition used a spin-echo sequence recorded within the following parameters: TE = 23 ms, TR = 1070 ms, NEX = 2, flip angle = 90°, and 256 acquisitions with the point-resolved spectroscopy (PRESS) technique. During data acquisition, the same experienced neuroradiologist, blind to the clinical data, placed the voxels (2 × 2 × 2) cm³ at the ACC and PCC (See **Figure 1**) so careful to exclude contamination of signal from the

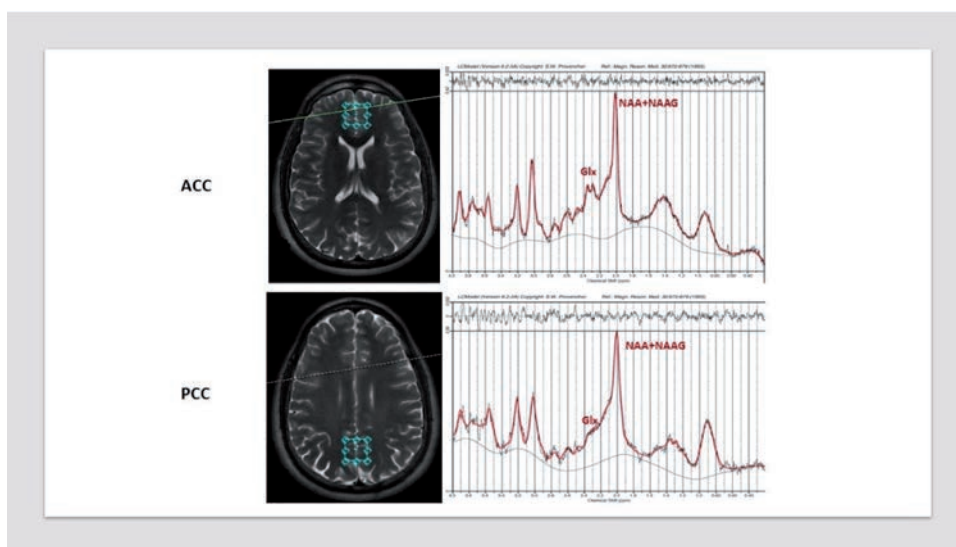


Figure 1.

Locations of the volume studied in the anterior (ACC) and posterior (PCC) cingulated cortices. The single voxel acquisition used a spin-echo sequence recorded within the following parameters: Echo time (TE) = 23 ms, repetition time (TR) = 1070 ms, 2 NEX, flip angle = 90, and 256 acquisitions with the point-resolved spectroscopy (PRESS) technique. During data acquisition, the same experienced neuroradiologist blinded the clinical data to place the voxels at interesting brain areas.

skull and subcutaneous fat. The 1H-MRS data sets collected showed the quantification of the absolute concentrations of brain metabolites, expressed in millimoles per kilogram of wet weight, involving the correction of many factors, such as the tissue composition of the voxel (relative amounts of cerebrospinal fluid and gray and white matter), the T1 and T2 relaxation times of the metabolites in the patient, the location of the voxels and their relationship with the electromagnetic properties of the coil, and any temporary variation in the scanner [76].

3.2.1 Automatic quantitation of localized in vivo 1H spectra

LCModel is automatic (non-interactive) software version 6–1–0 (Stephen Provencher Incorporated, Oakville, Canada) [77] with no subjective input. Approximately maximum-likelihood estimates of the metabolite concentrations, phases, referencing shift, line shape, baseline, etc., and their uncertainties in the concentrations (Cramér-Rao lower bounds) are obtained [78]. The main metabolite resonances were limited for NAA, creatine and phosphocreatine, together abbreviated (Cr), choline-containing compounds phosphocholine, glycerophosphocholine, choline proper, and acetylcholine, together abbreviated (Cho), myo-inositol (mI), glutamate (Glu), glutamine (Gln), and the peak, “Glx,” was for the sum of glutamate and glutamine [79]. One possible weakness of our method is the reliance on accurate suppression of the NAAG signal in the NAA scan, particularly the NAA signal in the NAAG scan (due to the higher concentration of NAA) [80]. An intense peak at 2 ppm is generally assigned to NAA (which is responsible for the greater part of the signal), but in this work, it was assumed to correspond to NAA + NAAG. Considered also, any small N-acetyl molecules in the brain will contribute to the peak, and moreover, small contributions from other N-acetyl species (e.g., N-acetyl-glutamate) could result in

overestimation of the NAA and NAAG concentrations. Notwithstanding, the paradigm used here allowed us to resolve the NAAG peak with a %SD of <20% based on the reliability indicators or lower levels of Cramér–Rao. Each spectrum was reviewed to ensure an adequate signal-to-noise ratio, as well as the absence of artifacts which allowed us to define previously which were the best times TE/TR for the spectra obtained to show the highest number of metabolites.

The different metabolites were resolved using Cr as an internal reference according to LCModel, following standard clinical practice, because it is considered the most stable metabolite in cell tissue, [78]. Nor can the ratios of a metabolite represent all possible differences, if a single specific denominator is used (as is the case for creatine), if the signals recorded in all subjects were obtained in the same scanner unit and using the same protocol. Therefore, the excellent reproducibility for each metabolite allows us to quantify their absolute concentration in the ACC and PCC (see **Table 2**), and consequently to observe the different alterations in ASD (see **Figure 2**).

Anteriorly, studies have also used the LCModel in Ref. to disorders in epilepsy, [81–83] multiple sclerosis, [84–86] tumors, [87, 88] Alzheimer’s disease, [89, 90] and other pathologies, [91–95] including the identification and quantitation of unusual metabolites, increasing the robustness of the results.

Brain area [mM]	ASD (n = 19) mean (S.D.)	TD (n = 42) mean (S.D.)	Statistics	P value
Anterior cingulate cortex				
NAA + NAAG	9.78 (0.49)	10.44 (0.29)		*p = 0.02
NAA	9.37 (1.36)	9.91 (0.68)	n.s.	
Glx(Glu+Gln)	16.10 (6.87)	15.19 (9.02)	n.s.	
Glu	12.10 (3.92)	10.54 (5.64)		*p = 0.02
GPC+PCh	2.08 (0.14)	2.08 (0.13)	n.s.	
Cr+PCr	6.98 (1.56)	7.40 (1.87)	n.s.	
mI	5.40 (0.78)	5.25 (0.27)	n.s.	
Posterior cingulate cortex				
NAA + NAAG	10.80 (0.86)	11.02 (0.68)	n.s.	
NAA	10.47 (1.39)	10.68 (0.20)	n.s.	
Glx(Glu+Gln)	13.87 (4.09)	14.08 (2.15)	n.s.	
Glu	10.22 (3.19)	10.71 (2.06)	n.s.	
GPC+PCh	1.55 (0.44)	1.61 (0.38)	n.s.	
Cr+PCr	6.72 (0.90)	6.99 (0.42)	n.s.	
mI	4.98 (0.68)	5.13 (1.94)	n.s.	

*The different metabolites concentrations detectable in ASD vs. TD, N-acetyl-aspartate (NAA), N-acetyl-aspartate + N-acetylasparyl-glutamate (NAA + NAAG), glutamate+ glutamine Glx = (Glu + Gln), glutamate (Glu), creatine (PCr), choline (PCG + PCh), and myo-inositol (mI). Brain areas = anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC). values for metabolites’ absolute concentration are group mean ± standard deviation (range). *p < 0.05 considered significantly different, while n.s. represents non-significant results.*

Table 2.
Absolute metabolic concentrations detected by 1H-MRS.

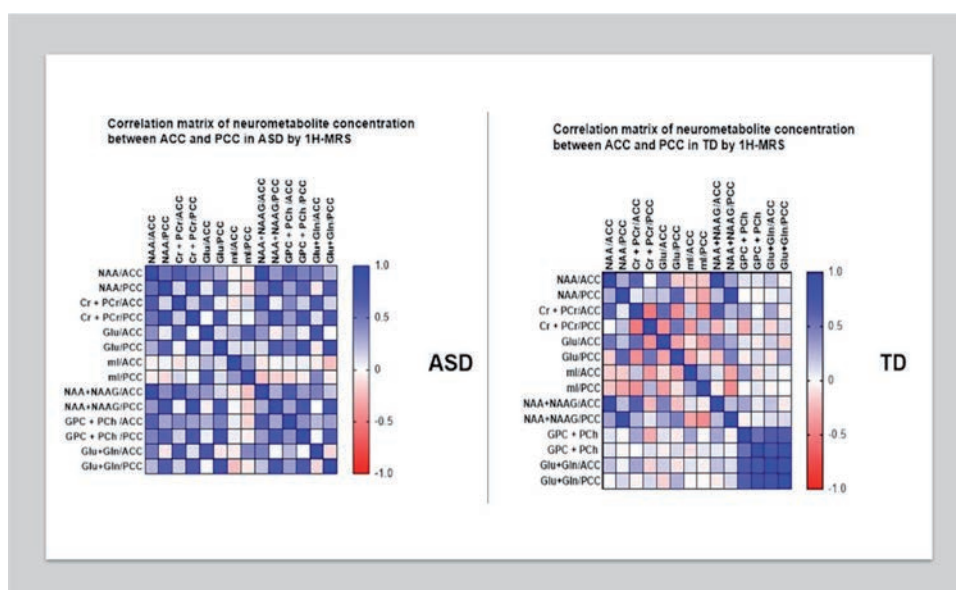


Figure 2. The correlation matrix was used as a statistical technique to evaluate the relationship between two variables in metabolites' absolute concentrations set present in ACC and PCC, represented by values for N-acetyl aspartyl-glutamate (NAA + NAAG), glutamate + glutamine (Glx = Glu + Gln), glutamate (Glu), creatine (Cr + PCr), N-acetyl-aspartate (NAA), choline (GPC + PCh), and myo-inositol (mI) in ASD (n = 19) vs. TD (42). In this sense, we can summarize a large amount of data to identify patterns. Every cell contains a correlation coefficient, where 1 is considered a strong relationship between variables, 0 is a neutral relationship, and -1 is a not strong relationship. Above, the observed metabolic pattern is evidence of differences between subjects with or without autism. (* $p < 0.05$) considers statistical significance.

4. Results

4.1 Demographic characteristics data

Following our objective of characterizing the glutamate dysfunctions in adult subjects with ASD by quantifying (1) glutamate levels in the anterior and posterior cingulate cortex using proton magnetic resonance spectroscopy and, (2) its correlation with the AQ test on their different domains, of characteristics of ASD compared to the TD group presented in materials and method section. The ASD group differed significantly from the TD in the total AQ, which prompted us to use this psychometric test to correlate the Glu concentrations with each domain evaluated in the test.

4.2 Cingulate cortices neuro metabolites pattern in ASD

The 1H-MRS results showed an overall increase in Glu concentration in adults with ASD, which was only observed in ACC. This suggests that functional changes in Glu concentration could reflect an adaptation to previous glutamatergic dysfunctions rather than being key to the pathophysiology of ASD (see **Figure 3**).

However, when we diversified the population using the AQ test, a pattern was observed in the variation of Glu concentration linked to the severity of autistic

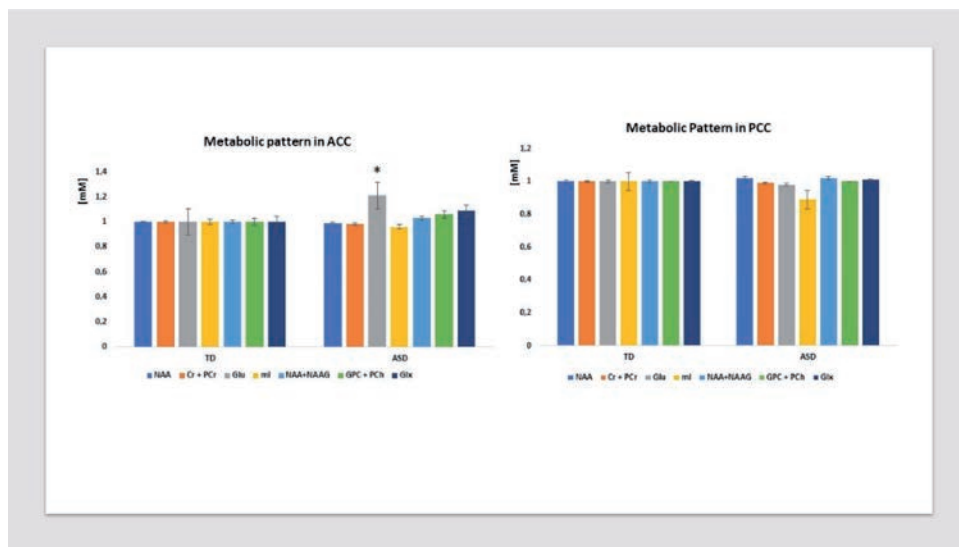


Figure 3. Normalized data metabolic variability of the different metabolites present in ACC and PCC in the ASD group ($n = 19$) and the TD group ($n = 42$), allows bias problems to be minimized. Glutamate is significantly elevated in the ACC, revealing a dysfunction pattern in excitatory/inhibitory metabolism in ACC patients with autism. ($^*p < 0.05$) considers statistical significance.

characteristics, evidencing the commitment of this neurotransmitter in the cingulate cortices. The compartmentalization of Glu with NAAG (see the introduction section) is also observed in ACC indicating a marked difference with PCC, which marks an important finding linked to ASD syndrome. (see **Figure 4**).

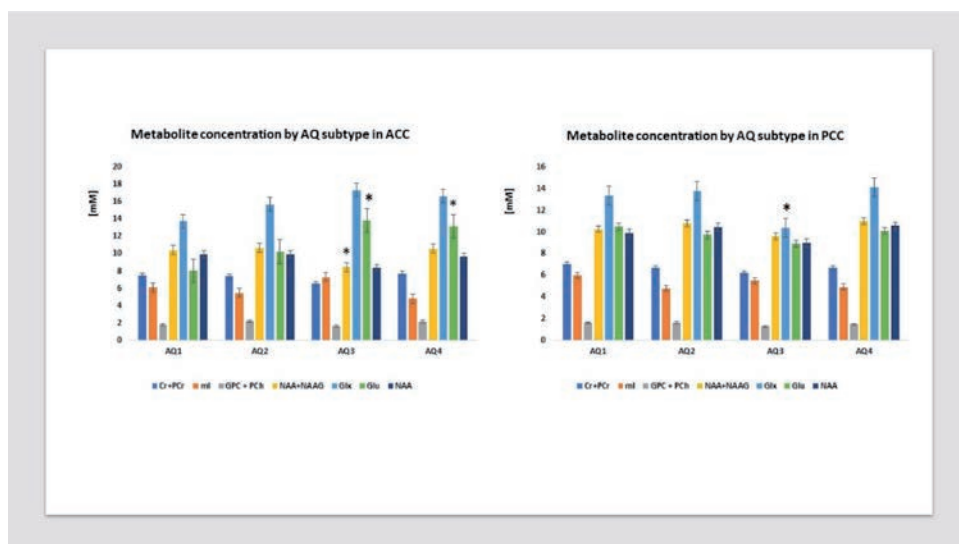


Figure 4. Pattern of variability of neurometabolites present in the cingulate cortices, when the ASD and TD groups were stratified into the AQ groups (AQ1, AQ2, AQ3, and AQ4), according to autistic characteristics. Graphic representation of the metabolic differentiation pattern and symptoms' severity between ACC and PCC. ($^*p < 0.05$) considered significantly different.

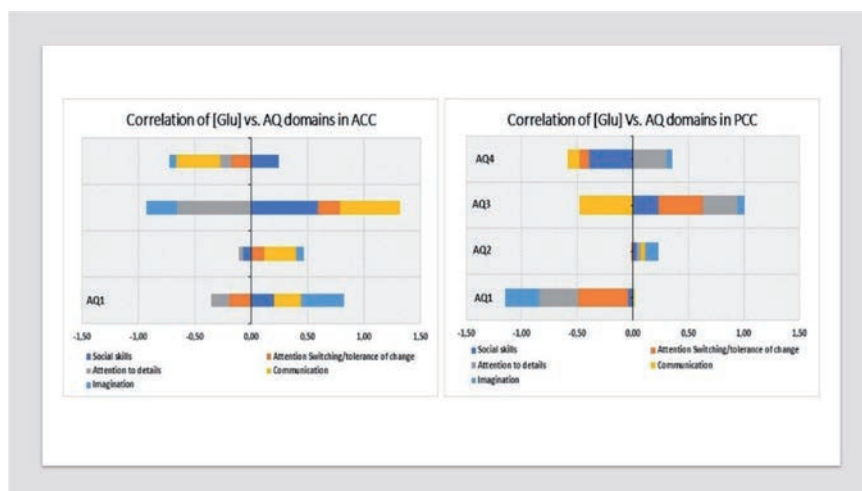


Figure 5. Graph of correlation pattern Pearson's coefficient of [Glu] concentration present in ACC and PCC, and the different autistic characteristics represented by AQ2, AQ3, and AQ4 groups with the AQ1 group taken as a reference or control of healthy AQ index, which can be used as a marker of the severity of symptoms in ACC and PCC. * $p < 0.05$ considered significantly different.

4.3 Correlation of Glu with the autistic characteristics evaluated within the AQ

Here, interesting results were observed regarding Glu's commitment to each one of the five domains of interest (social, communication, imagination, attention to detail, and attention shifting/tolerance to change), suggesting how much it affects TD and the ASD group. Observing a direct correlation between Glu concentration and social skills, communication, and imagination in the AQ1 group (autistic characteristics below average). In contrast to the AQ4 group (very high index of autistic characteristics), where this direct correlation was observed with social skills only. However, the AQ3 group (above average), showed a direct correlation to social skills, attention switching/tolerance of change, and communication in ACC (see **Figure 5**). Suggest a broad interpretation of the effect caused by Glu dysfunction on the development of autistic triad mainly characteristics.

However, the results obtained in the PCC reflect that [Glu] concentration is directly correlated only with communication in the AQ1 group. Compared to the AQ4 group, which correlates directly with attention to detail and imagination. However, the AQ3 group shows a direct correlation with social skills, imagination, attention to detail, and attention shifting/tolerance to change, highlighting the biggest compromising of Glu dysfunction in this group.

One of the biggest concerns in children with autism is the development of speech or communication. This leads us to consider the importance of Glu dysfunction in PCC for groups AQ3 and AQ4 where autistic characteristics are more exacerbated, and its correlation with the development of communication.

5. Discussion

Herein, we presented evidence demonstrating a potential connection of ASD with glutamatergic dysfunction. We focused specifically on biochemical links, between ACC and PCC, and its functional connectivity.

The glutamatergic pathways in the brain are extensive. Glutamate is excreted into the synaptic cleft by the process of exocytosis or glutamatergic neurotransmission which involves processes of glial reuptake, presynaptic reuptake, AMPA agonism, NMDA agonism, and Kainate and Quisqualate receptor agonism.

In this 1H-MRS study, the finding of a significant increase in Glu concentration in the ACC, observed in adults with ASD, is in line with previous studies that reflect an imbalance of excitation/inhibition in the children and young brain [96–98], and support the hypothesis of excitatory/inhibitory imbalance in ASD.

The excitotoxicity induced by an increase in the level of Glu in the brain can have pathological consequences, due to the deregulation of intracellular Ca^{2+} concentrations. This delicate balance between the mechanisms that allow the entry of physiological Ca^{2+} and those that can limit the intracellular excess to avoid neurodegenerative processes are the ones that determine the loss of neuronal viability [99]. This statement allows us to conjecture, that this significant increase in glutamate levels in the ACC would be a possible cause of disorder within the autism spectrum, either due to deregulation of intracellular Ca^{2+} and/or, due to neurotoxicity due to excessive activation of NMDA receptors. Since they are the most permeable to calcium Ca^{2+} , and act cooperatively with AMPA-kainate-type receptors, fundamentally permeable to sodium [100].

Other studies have found atypical undergrowth of auditory and visual networks which was associated with the severity of autistic core socio-communication symptoms, that of the visual network was correlated with the severity of restricted and repetitive behaviors in ASD adults [101].

In this sense, in deregulation due to excess glutamate, there is a risk of losing cognitive capacity and even cell death, even in adults. Therefore, due to the role that glutamate has in various neurodegenerative pathologies, it results in an important – although also complex – pharmacological target. In this study, it was observed how an excess of glutamate in the cingulate cortex intervenes in the cognitive development of adult subjects with autism from childhood, when comparing them with neurotypical subjects. From the cognitive impairment that affects the functioning of functions such as attention, imagination, communication, and detail, as well as tolerance to change, to the main and most studied to date in the autistic condition - social skills - as seen in **Figure 5**.

An important milestone is the finding presented here of the metabolic deregulation of Glu, reported in the cingulate cortices, that justifies the hypofunction of the principal networks: The salience network (SAN), the default network (DMN), and the frontotemporal visual networks; as well as the motor skills which confirm the functional and neurochemical differences between ACC and PCC in subjects with ASD [102]. In addition to this, the dendritic complexity of the PCC is much lower than that present in the ACC, as other authors have shown [103], which would explain the metabolic differences between both regions in ASD and that are balanced in neurotypical subjects.

6. Conclusions

- The effects of excess glutamate in the **ACC** in subjects with ASD are directly correlated with the three central characteristics within the autism spectrum.
- The study of Glu receptors is ambitious in allowing greater knowledge of the functioning of the nervous system in people with ASD, which will open the doors for the development of more effective therapeutic strategies based on the regulation of glutamatergic neurotransmission.

- Currently, numerous NMDA receptor antagonists have been synthesized to reduce the entry of Ca^{2+} through the targets of the AMPA-Kainate and NMDA receptors and thus reduce the excitotoxic effect of glutamate in the brain, but their clinical development is limited and has been hampered by the appearance of important adverse effects that would make its implementation in therapeutics difficult.
- Not surprisingly, drugs developed through animal models for ASD have not had enough success in human trials to justify their use in the clinic, and therefore there may be little enthusiasm for investing in Glu-regulating drugs as a possible therapeutic approach. Perhaps it is time to consider the investments that will bring this knowledge to the clinic.

Acknowledgements

Mainly we want to thank the Roviralta Foundation (Barcelona, Spain) for financing this pioneering project in the Canary Islands. As well as all the people who voluntarily participated in this study, technical and medical staff from the Canary Islands University Hospital (HUC), and the Magnetic Resonance Service for Biomedical Research (SEGAI). To the associations (APANATE, ASPERTEN, and ALDIS) that represent and serve the population with ASD in Tenerife, Canary Islands, Spain; for giving me your trust and support and for making all the years of effort worth it. I would like to add it under acknowledgments as funding (“Examination of GABA/ glutamate neurotransmitters and functional connectivity in cognitive inhibition tasks. PID2021-126172NB-I00”).

Conflict of interest

The authors declare no conflict of interest.

Author details

Carmen Jimenez-Espinoza*, Francisco Marcano Serrano and José González-Mora
Laboratory Neurochemistry and Neuroimages, Physiology Section, Faculty of Health Sciences, Department of Basic Medical Sciences, University of La Laguna, Tenerife, Spain

*Address all correspondence to: carmen.jimenez.87@ull.edu.es

IntechOpen

© 2024 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/3.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited. 

References

- [1] Roehr B. American psychiatric association explains DSM-5. *BMJ*. 2013;**6**:346
- [2] Baron-Cohen S, Wheelwright S, Skinner R, Martin J, Clubley E. The autism-spectrum quotient (AQ): Evidence from Asperger syndrome/high-functioning autism, males and females, scientists, and mathematicians. *Journal of Autism and Developmental Disorders*. 2001;**31**:603
- [3] Rubenstein JL, Merzenich MM. Model of autism: Increased ratio of excitation/inhibition in key neural systems. *Genes, Brain and Behavior*. 2003;**2**(5):255-267
- [4] Oblak A, Gibbs TT, Blatt G. Decreased GABAA receptors and benzodiazepine binding sites in the anterior cingulate cortex in autism. *Autism Research*. 2009;**2**(4):205-219
- [5] Pizzarelli R, Cherubini E. Alterations of GABAergic signaling in autism spectrum disorders. *Neural Plasticity*. 2011;**2011**:297153
- [6] Galineau L, Arlicot N, Dupont AC, Briend F, Houy-Durand E, Tauber C, et al. Glutamatergic synapse in autism: A complex story for a complex disorder. *Molecular Psychiatry*. 2023;**28**(2):801-809
- [7] Briend F, Barantin L, Cléry H, Cottier JP, Bonnet-Brilhault F, Houy-Durand E, et al. Glutamate levels of the right and left anterior cingulate cortex in autistics adults. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. 2023;**30**(126):110801
- [8] Libero LE, Reid MA, White DM, Salibi N, Lahti AC, Kana RK. Biochemistry of the cingulate cortex in autism: An MR spectroscopy study. *Autism Research*. 2016;**9**(6):643-657
- [9] Montanari M, Martella G, Bonsi P, Meringolo M. Autism spectrum disorder: Focus on glutamatergic neurotransmission. *International Journal of Molecular Sciences*. 2022;**23**(7):3861
- [10] Chakraborty P, Dey A, Gopalakrishnan AV, Swati K, Ojha S, Prakash A, et al. Glutamatergic neurotransmission: A potential pharmacotherapeutic target for the treatment of cognitive disorders. *Ageing Research Reviews*. 2023;**5**:101838
- [11] Oya M, Matsuoka K, Kubota M, Fujino J, Tei S, Takahata K, et al. Increased glutamate and glutamine levels and their relationship to astrocytes and dopaminergic transmissions in the brains of adults with autism. *Scientific Reports*. 2023;**13**(1):11655
- [12] Varfolomeev S, Bykov V, Tsybenova S. Kinetic model of the glutamate neuron-astrocytic system. N-acetylaspartylglutamate and glutamate carboxypeptidase. *ChemRxiv*. 2023. DOI: 10.26434/chemrxiv-2023-7r957
- [13] Fujii E, Mori K, Miyazaki M, Hashimoto T, Harada M, Kagami S. Function of the frontal lobe in autistic individuals: A proton magnetic resonance spectroscopic study. *The Journal of Medical Investigation*. 2010;**57**(1, 2):35-44
- [14] Levitt JG, O'Neill J, Blanton RE, Smalley S, Fadale D, McCracken JT, et al. Proton magnetic resonance spectroscopic imaging of the brain in childhood autism. *Biological Psychiatry*. 2003;**54**(12):1355-1366

- [15] Oner O, Devrimci-Ozguven H, Oktem F, Yagmurlu B, Baskak B, Munir KM. Proton MR spectroscopy: Higher right anterior cingulate N-acetylaspartate/choline ratio in Asperger syndrome compared with healthy controls. *American Journal of Neuroradiology*. 2007;**28**(8):1494-1498
- [16] Bernardi S, Anagnostou E, Shen J, Kolevzon A, Buxbaum JD, Hollander E, et al. *In vivo* 1H-magnetic resonance spectroscopy study of the attentional networks in autism. *Brain Research*. 2011;**22**(1380):198-205
- [17] Gabis L, Huang W, Azizian A, DeVincent C, Tudorica A, Kesner-Baruch Y, et al. 1H-magnetic resonance spectroscopy markers of cognitive and language ability in clinical subtypes of autism spectrum disorders. *Journal of Child Neurology*. 2008;**23**(7):766-774
- [18] Mundy P. Annotation: The neural basis of social impairments in autism: The role of the dorsal medial-frontal cortex and anterior cingulate system. *Journal of Child Psychology and Psychiatry*. 2003;**44**(6):793-809
- [19] Ito H, Mori K, Harada M, Hisaoka S, Toda Y, Mori T, et al. A proton magnetic resonance spectroscopic study in autism spectrum disorder using a 3-tesla clinical magnetic resonance imaging (MRI) system: The anterior cingulate cortex and the left cerebellum. *Journal of Child Neurology*. 2017;**32**(8):731-739
- [20] Du Y, Chen L, Yan MC, Wang YL, Zhong XL, Xv CX, et al. Neurometabolite levels in the brains of patients with autism spectrum disorders: A meta-analysis of proton magnetic resonance spectroscopy studies (N= 1501). *Molecular Psychiatry*. 2023;**28**:1-2
- [21] Gudmundson AT, Koo A, Virovka A, Amirault AL, Soo M, Cho JH, et al. Meta-analysis and open-source database for *in vivo* brain magnetic resonance spectroscopy in health and disease. *Analytical Biochemistry*. 2023;**1**(676):115227
- [22] Vogt BA. Regions and subregions of the cingulate cortex. In: *Cingulate Neurobiology and Disease*. Vol. 4 (1). Oxford: Oxford Academic; 2009. p. 31
- [23] Hadders-Algra M. Emerging signs of autism spectrum disorder in infancy: Putative neural substrate. *Developmental Medicine & Child Neurology*. 2022;**64**(11):1344-1350
- [24] Xiong Y, Chen J, Li Y. Microglia and astrocytes underlie neuroinflammation and synaptic susceptibility in autism spectrum disorder. *Frontiers in Neuroscience*. 2023;**20**(17):1125428
- [25] Ecker C, Schmeisser MJ, Loth E, Murphy DG. Neuroanatomy and neuropathology of autism spectrum disorder in humans. *Translational Anatomy and Cell Biology of Autism Spectrum Disorder*. 2017;**224**:27-48
- [26] Yenkovyan K, Grigoryan A, Fereshetyan K, Yepremyan D. Advances in understanding the pathophysiology of autism spectrum disorders. *Behavioural Brain Research*. 2017;**28**(331):92-101
- [27] Fetit R, Hillary RF, Price DJ, Lawrie SM. The neuropathology of autism: A systematic review of post-mortem studies of autism and related disorders. *Neuroscience & Biobehavioral Reviews*. 2021;**1**(129):35-62
- [28] Pagnozzi AM, Conti E, Calderoni S, Fripp J, Rose SE. A systematic review of structural MRI biomarkers in autism spectrum disorder: A machine learning perspective. *International Journal of Developmental Neuroscience*. 2018;**1**(71):68-82

- [29] Stigler KA, McDonald BC, Anand A, Saykin AJ, McDougle CJ. Structural and functional magnetic resonance imaging of autism spectrum disorders. *Brain Research*. 2011;22(1380):146-161
- [30] Katuwal GJ, Cahill ND, Baum SA, Michael AM. The predictive power of structural MRI in autism diagnosis. In: 2015 37th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC). Milan, Italy: IEEE; 2015. pp. 4270-4273. DOI: 10.1109/EMBC.2015.7319338
- [31] Ali MT, ElNakieb Y, Elnakib A, Shalaby A, Mahmoud A, Ghazal M, et al. The role of structure MRI in diagnosing autism. *Diagnostics*. 2022;12(1):165
- [32] Dekhil O, Ali M, Haweel R, Elnakib Y, Ghazal M, Hajjdiab H, et al. A comprehensive framework for differentiating autism spectrum disorder from neurotypicals by fusing structural MRI and resting state functional MRI. *Seminars in Pediatric Neurology*. 2020;34:100805
- [33] Philip RC, Dauvermann MR, Whalley HC, Baynham K, Lawrie SM, Stanfield AC. A systematic review and meta-analysis of the fMRI investigation of autism spectrum disorders. *Neuroscience & Biobehavioral Reviews*. 2012;36(2):901-942
- [34] Silani G, Bird G, Brindley R, Singer T, Frith C, Frith U. Levels of emotional awareness and autism: An fMRI study. *Social Neuroscience*. 2008;3(2):97-112
- [35] Koshino H, Carpenter PA, Minshew NJ, Cherkassky VL, Keller TA, Just MA. Functional connectivity in an fMRI working memory task in high-functioning autism. *NeuroImage*. 2005;24(3):810-821
- [36] Pierce K. Early functional brain development in autism and the promise of sleep fMRI. *Brain Research*. 2011;22(1380):162-174
- [37] Hull JV, Dokovna LB, Jacokes ZJ, Torgerson CM, Irimia A, Van Horn JD. Resting-state functional connectivity in autism spectrum disorders: A review. *Frontiers in Psychiatry*. 2017;4(7):205
- [38] Liu M, Li B, Hu D. Autism spectrum disorder studies using fMRI data and machine learning: A review. *Frontiers in Neuroscience*. 2021;15(15):697870
- [39] Koshino H, Kana RK, Keller TA, Cherkassky VL, Minshew NJ, Just MA. fMRI investigation of working memory for faces in autism: Visual coding and underconnectivity with frontal areas. *Cerebral Cortex*. 2008;18(2):289-300
- [40] Karavallil Achuthan S, Coburn KL, Beckerson ME, Kana RK. Amplitude of low frequency fluctuations during resting state fMRI in autistic children. *Autism Research*. 2023;16(1):84-98
- [41] Nakamura K, Sekine Y, Ouchi Y, Tsujii M, Yoshikawa E, Futatsubashi M, et al. Brain serotonin and dopamine transporter bindings in adults with high-functioning autism. *Archives of General Psychiatry*. 2010;67(1):59-68
- [42] Andersson M, Tangen Ä, Farde L, Bölte S, Halldin C, Borg J, et al. Serotonin transporter availability in adults with autism—A positron emission tomography study. *Molecular Psychiatry*. 2021;26(5):1647-1658
- [43] Makkonen I, Riikonen R, Kokki H, Airaksinen MM, Kuikka JT. Serotonin and dopamine transporter binding in children with autism determined by SPECT. *Developmental Medicine & Child Neurology*. 2008;50(8):593-597
- [44] Oblak A, Gibbs TT, Blatt GJ. Reduced serotonin receptor subtypes in a

limbic and a neocortical region in autism. *Autism Research*. 2013;**6**(6):571-583

[45] Brittenham C, Gordon J, Zemon VM, Siper PM. Objective frequency analysis of transient visual evoked potentials in autistic children. *Autism Research*. 2022;**15**(3):464-480

[46] Trenado C, González-Ramírez A, Lizárraga-Cortés V, Pedroarena Leal N, Manjarrez E, Ruge D. The potential of trial-by-trial variabilities of ongoing-EEG, evoked potentials, event related potentials and fMRI as diagnostic markers for neuropsychiatric disorders. *Frontiers in Neuroscience*. 2019;**17**(12):850

[47] Black MH, Chen NT, Iyer KK, Lipp OV, Bölte S, Falkmer M, et al. Mechanisms of facial emotion recognition in autism spectrum disorders: Insights from eye tracking and electroencephalography. *Neuroscience & Biobehavioral Reviews*. 2017;**1**(80):488-515

[48] Aykan S, Gürses E, Tokgöz-Yılmaz S, Kalaycıoğlu C. Auditory processing differences correlate with autistic traits in males. *Frontiers in Human Neuroscience*. 2020;**7**(14):584704

[49] Cashin A, Barker P. The triad of impairment in autism revisited. *Journal of Child and Adolescent Psychiatric Nursing*. 2009;**22**(4):189-193

[50] Guyton AC, Hall JE. *Tratado de fisiología médica*. 12ª ed. Madrid-España: El Seiver; 2011. p. 432

[51] Vrselja Z, Brkic H, Mrdenovic S, Radic R, Curic G. Function of circle of Willis. *Journal of Cerebral Blood Flow & Metabolism*. 2014;**34**(4):578-584

[52] Rosner J, Reddy V, Lui F. *Neuroanatomy, Circle of Willis*. Treasure Island (FL): StatPearls Publishing; 2018

[53] Curtis H. *Invitación a la Biología*. Editorial Médica Panamericana; San Juan, Puerto Rico; 2006

[54] Snell RS. Los núcleos de los nervios craneales, sus conexiones centrales y su distribución. In: *Neuroanatomía Clínica*. 6ª ed. Buenos Aires: Médica Panamericana; 2007. pp. 357-369

[55] Brennenstuhl H, Jung-Klawitter S, Assmann B, Opladen T. Inherited disorders of neurotransmitters: Classification and practical approaches for diagnosis and treatment. *Neuropediatrics*. 2019;**50**(1):2-14

[56] Cavanagh ME, Parnavelas JG. Neurotransmitter differentiation in cortical neurons. In: *The Making of the Nervous System*. London: Oxford Univ. Press; 1988. pp. 435-453

[57] Eroglu C, Barres BA. Regulation of synaptic connectivity by glia. *Nature*. 2010;**468**(7321):223-231

[58] Hassel B, Dingledine R. Glutamate and glutamate receptors. In: *Basic Neurochemistry*. Amsterdam, Netherlands: Academic Press; 2012. pp. 342-366

[59] Coghlan S, Horder J, Inkster B, Mendez MA, Murphy DG, Nutt DJ. GABA system dysfunction in autism and related disorders: From synapse to symptoms. *Neuroscience and Biobehavioral Reviews*. 2012;**36**:2044-2055

[60] Chao HT, Chen H, Samaco RC, Xue M, Chahrour M, Yoo J, et al. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature*. 2010;**468**:263-269

[61] Cornell-Bell AH, Finkbeiner SM, Cooper MS, Smith SJ. Glutamate induces calcium waves in cultured astrocytes:

Long-range glial signaling. *Science*. 1990;**247**(4941):470-473

[62] Charles AC, Merrill JE, Dirksen ER, Sandersont MJ. Intercellular signaling in glial cells: Calcium waves and oscillations in response to mechanical stimulation and glutamate. *Neuron*. 1991;**6**(6):983-992

[63] Nedergaard M. Direct signaling from astrocytes to neurons in cultures of mammalian brain cells. *Science*. 1994;**263**(5154):1768-1771

[64] Smith SJ. Neural signalling: Neuromodulatory astrocytes. *Current Biology*. 1994;**4**(9):807-810

[65] Panatier A, Vallée J, Haber M, Murai KK, Lacaille JC, Robitaille R. Astrocytes are endogenous regulators of basal transmission at central synapses. *Cell*. 2011;**146**(5):785-798

[66] Gordon GR, Iremonger KJ, Kantevari S, Ellis-Davies GC, MacVicar BA, Bains JS. Astrocyte-mediated distributed plasticity at hypothalamic glutamate synapses. *Neuron*. 2009;**64**(3):391-403

[67] Rosenegger DG, Gordon GR. A slow or modulatory role of astrocytes in neurovascular coupling. *Microcirculation*. 2015;**22**(3):197-203

[68] Spanaki C, Kotzamani D, Petraki Z, Drakos E, Plaitakis A. Heterogeneous cellular distribution of glutamate dehydrogenase in brain and in non-neural tissues. *Neurochemical Research*. 2014;**39**:500-515

[69] Tasker JG, Oliet SH, Bains JS, Brown CH, Stern JE. Glial regulation of neuronal function: From synapse to systems physiology. *Journal of Neuroendocrinology*. 2012;**24**(4):566-576

[70] Morland C, Nordengen K. N-acetyl-aspartyl-glutamate in brain health

and disease. *International Journal of Molecular Sciences*. 2022;**23**(3):1268

[71] Shave E, Pliss L, Lawrance ML, Fitz Gibbon T, Stastny F, Balcar VJ. Regional distribution and pharmacological characteristics of [3H] N-acetyl-aspartyl-glutamate (NAAG) binding sites in rat brain. *Neurochemistry International*. 2001;**38**(1):53-62

[72] Baslow MH. Evidence that the tri-cellular metabolism of N-acetylaspartate functions as the brain's "operating system": How NAA metabolism supports meaningful intercellular frequency-encoded communications. *Amino Acids*. 2010;**39**:1139-1145

[73] Baron-Cohen, S. *La Gran Diferencia: Cómo son Realmente los Cerebros de Hombres y Mujeres* 2005; Editorial AMAT: Barcelona, Spain; 2005

[74] Jiménez-Espinoza C, Rodríguez B, González M, Garrote M, González-Mora JL. Autism-Spectrum quotient (AQ): A preliminary study of its diagnostic validity in a clinical Spanish sample, more than a psychometric test? In: *Proceedings of the International Meeting for Autism Research (IMFAR)*, Salt Lake City, UT, USA. ResearchGate Community, website and search engine. Berlín, Alemania. 13-16 May 2015

[75] Khachadourian V, Mahjani B, Sandin S, Kolevzon A, Buxbaum JD, Reichenberg A, et al. Comorbidities in autism spectrum disorder and their etiologies. *Translational Psychiatry*. 2023;**13**(1):71

[76] Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR in Biomedicine*. 2000;**13**:129-153

[77] Provencher SW. Automatic quantitation of localized *in vivo*

- 1H spectra with LCModel. NMR in Biomedicine: An International Journal Devoted to the Development and Application of Magnetic Resonance *In Vivo*. 2001;**14**(4):260-264
- [78] Cramér H. A contribution to the theory of statistical estimation. Scandinavian Actuarial Journal. 1946;**1946**:85-94
- [79] De Graaf RA. *In Vivo* NMR Spectroscopy: Principles and Techniques. Hoboken, NJ, USA: John Wiley and Sons; 2013
- [80] Edden RA, Pomper MG, Barker PB. *In vivo* differentiation of N-acetyl aspartyl glutamate from N-acetyl aspartate at 3 tesla. Magnetic Resonance in Medicine. 2007;**57**:977-982
- [81] Savic I, Lekvall A, Greitz D, Helms G. MR spectroscopy shows reduced frontal lobe concentrations of N-acetyl aspartate in patients with juvenile myoclonic epilepsy. Epilepsia. 2000;**41**:290-296
- [82] Haki C, Gümüştaş OG, Bora I, Gümüştaş AU, Parlak M. Proton magnetic resonance spectroscopy study of bilateral thalamus in juvenile myoclonic epilepsy. Seizure. 2007;**16**(4):287-295
- [83] Lin K, Carrete H Jr, Lin J, Peruchi MM, De Araújo Filho GM, Guaranha MS, et al. Magnetic resonance spectroscopy reveals an epileptic network in juvenile myoclonic epilepsy. Epilepsia. 2009;**50**(5):1191-1200
- [84] Helms G, Stawiarz L, KKP K, Link H. Regression analysis of metabolite concentrations estimated from localized proton MR spectra of active and chronic multiple sclerosis lesions. Magnetic Resonance in Medicine. 2000;**43**:102-110
- [85] Brief EE, Vavasour IM, Laule C, Li DK, Mackay AL. Proton MRS of large multiple sclerosis lesions reveals subtle changes in metabolite T1 and area. NMR in Biomedicine. 2010;**23**(9):1033-1037
- [86] Kirov II, Liu S, Tal A, Wu WE, Davitz MS, Babb JS, et al. Proton MR spectroscopy of lesion evolution in multiple sclerosis: Steady-state metabolism and its relationship to conventional imaging. Human Brain Mapping. 2017;**38**(8):4047-4063
- [87] Chong VFH, Rumpel H, Aw Y-S, Ho G-L, Fan Y-F, Chua E-J. Temporal lobe necrosis following radiation therapy for nasopharyngeal carcinoma: 1H MR spectroscopic findings. International Journal of Radiation Oncology, Biology, Physics. 1999;**45**:699-705
- [88] Chen WS, Li JJ, Zhang JH, Hong L, Xing ZB, Wang F, et al. Magnetic resonance spectroscopic imaging of brain injury after nasopharyngeal cancer radiation in early delayed reaction. Genetics and Molecular Research. 2014;**13**(3):6848-6854
- [89] Rose SE, de Zubicaray GI, Wang D, Galloway GJ, Chalk JB, Eagle SC, et al. A 1H MRS study of probable Alzheimer's disease and normal aging: Implications for longitudinal monitoring of dementia progression. Magnetic resonance imaging. 1999;**17**:291-299
- [90] Wang H, Tan L, Wang HF, Liu Y, Yin RH, Wang WY, et al. Magnetic resonance spectroscopy in Alzheimer's disease: Systematic review and meta-analysis. Journal of Alzheimer's Disease. 2015;**46**(4):1049-1070
- [91] Pioro EP, Majors AW, Mitsumoto H, Nelson DR, Ng TC. 1H-MRSevidence of neurodegeneration and excess glutamate+glutamine in ALS medulla. Neurology. 1999;**53**:71-79

- [92] Targosz-Gajniak MG, Siuda JS, Wicher MM, Banasik TJ, Bujak MA, Augusciak-Duma AM, et al. Magnetic resonance spectroscopy as a predictor of conversion of mild cognitive impairment to dementia. *Journal of the Neurological Sciences*. 2013;**335**(1-2):58-63
- [93] Zand DJ, Simon EM, Pulitzer SB, Wang DJ, Wang ZJ, Rorke LB, et al. *In vivo* pyruvate detected by MR spectroscopy in neonatal pyruvate dehydrogenase deficiency. *American Journal of Neuroradiology*. 2003;**24**(7):1471-1474
- [94] Wilichowski E, Pouwels PJW, Frahm J, Hanefeld F. Quantitative proton magnetic resonance spectroscopy of cerebral metabolic disturbances in patients with MELAS. *Neuropediatrics*. 1999;**30**:256-263
- [95] Wang R, Hu B, Sun C, Geng D, Lin J, Li Y. Metabolic abnormality in acute stroke-like lesion and its relationship with focal cerebral blood flow in patients with MELAS: Evidence from proton MR spectroscopy and arterial spin labeling. *Mitochondrion*. 2021;**1**(59):276-282
- [96] Bernardino I, Dionísio A, Violante IR, Monteiro R, Castelo-Branco M. Motor cortex excitation/inhibition imbalance in young adults with autism spectrum disorder: A MRS-TMS approach. *Frontiers in Psychiatry*. 2022;**14**(13):860448
- [97] Bejjani A, O'Neill J, Kim JA, Frew AJ, Yee VW, Ly R, et al. Elevated glutamatergic compounds in pregenual anterior cingulate in pediatric autism spectrum disorder demonstrated by ¹H MRS and ¹H MRSI. *PLoS One*. 2012;**7**(7):e38786
- [98] Tebartz van Elst L, Maier S, Fangmeier T, Endres D, Mueller GT, Nickel K, et al. Disturbed cingulate glutamate metabolism in adults with high-functioning autism spectrum disorder: Evidence in support of the excitatory/inhibitory imbalance hypothesis. *Molecular Psychiatry*. 2014;**19**(12):1314-1325
- [99] Foster TC. Calcium homeostasis and modulation of synaptic plasticity in the aged brain. *Aging Cell*. 2007;**6**(3):319-325
- [100] Masuda F, Nakajima S, Miyazaki T, Yoshida K, Tsugawa S, Wada M, et al. Motor cortex excitability and inhibitory imbalance in autism spectrum disorder assessed with transcranial magnetic stimulation: A systematic review. *Translational Psychiatry*. 2019;**9**(1):110
- [101] Watanabe T, Rees G. Anatomical imbalance between cortical networks in autism. *Scientific Reports*. 2016;**6**(1):31114
- [102] Uddin LQ. *Salience Network of the Human Brain*. Amsterdam, Netherlands: Academic Press; 2016
- [103] Schüz A, Miller R, editors. *Cortical Areas: Unity and Diversity*. Vol. 5. London, UK: CRC Press; 30 May 2002