

Chapter

In Vivo, Magnetic Resonance Spectroscopy (1-HRM)-Derived Autism-Related Neurotransmitter Variations

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Abstract

Autism spectrum disorder (ASD) is a severe developmental syndrome of unclear etiology, arising largely as a disorder of neural systems. One of the most studied causes is the increased excitation-inhibition in sensory and social systems, which may explain certain phenotypic expressions in ASD related to different neurotransmitters and their imbalances (NT). This study aims to assess neurotransmitter levels in the anterior cingulate (ACC) and posterior cingulate (PCC) cortices in subjects with ASD using *in vivo* proton magnetic resonance spectroscopy (1H-MRS), considered a robust tool. The finding of an imbalance in the neurotransmitters myoinositol (mI), choline (Cho), N-acetyl aspartil glutamate (NAA + NAAG), and glutamate (Glu) in cingulated cortices sparked our interest as an important cause of their multiple etiology, providing strong empirical support for increased arousal in ASD. This metabolic imbalance between ACC and PCC was correlated using the autism quotient (AQ) test, suggesting potential therapeutic interventions.

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 is related to the triad of social, communicative, imaginative, and behavioral limitations described by Wing and Gould, 1979 [1]. However, recent evidence shows that autism presents a great phenotypic variation that gives it the identity of a multifactorial disease. This has led to the latest revision of the Diagnostic and Statistical Manual of Mental Disorders DSM-V [2], where autism has been renamed autism spectrum disorder (ASD) as a single category. Although the new rename of autism has increased the diagnostic accuracy with more accuracy, using more accurate procedures and instruments, that has improved the knowledge and training of professionals, the real

increase in the incidence of this disorder may be due to the increased global population. In this sense, the development of new diagnostic methods to quantify and study the evolution of the spectrum of autism in each patient could help us better understand the neurometabolic processes involved more reliably. From the physiological point of view, brain metabolism comprises a wide variety of molecules, including peptides, neurotransmitters, enzymes, all interacting with water. These molecules can maintain the physiological balance necessary for the healthy functioning of the brain due to their functions and activity. Furthermore, the brain is one of the organs with the highest energy demand, consuming 20% of the oxygen and 25% of the glucose ingested by the body [3, 4]. Considering that, all this neurometabolic exchange must be continued, fast, and effective, precisely because the brain has few energy reserves and only receives them through the cerebral vascular system, which involves a correct supply of cerebral circulation through the circle of Willis.¹

The Proton Magnetic Resonance Spectroscopy (1H-MRS) is a technique available in radiological practice that provides a biochemical, metabolic, and functional assessment of tissues considering that all the mechanisms and pathways that accompany synapses in the central nervous system (CNS) occur in a very short time scale (milliseconds). It is used principally in the evaluation of brain tumors and now has spread to the valuation of other diseases such as metabolic disorders, studies of dementia, vascular disorders, and assessment of some psychiatric disorders, and more recently in heart disease, liver, breast, and prostate assessment. Despite the consideration of autism as a multifactorial disease and the wide application of spectroscopy in some psychiatric disorders, there is still no consensus that allows the use of this tool for the assessment of ASD. Recent studies have indicated the existence of some defects in the synthesis, release, or degradation of some neurotransmitters involved in the pathogenesis of many neurological, muscular, and psychiatric diseases [5], directing our objective to the study, using 1H-MRS, of possible variations of NT in the brain tissue of people with ASD.

2. Neurotransmitter's characteristics and functions

A group of biomolecules in the brain can be detected using proton magnetic resonance spectroscopy. We are referring to neurotransmitters, defined as endogenous sub-signals that act as chemical messengers that transmit signals and are normally released by neurons into the synaptic space, where they exert their function on other neurons or other target cells through a synapse. In summary, at least 100 neurotransmitters (NT) have been described already, and many others have yet to be discovered. Neurotransmitters can transmit one of three possible actions: excitatory, inhibitory, and modulatory messages, depending on the NT function. The better-known categories and neurotransmitter examples and their functions are included in **Table 1**. Some are grouped into types according to their chemical nature and are used as markers in 1H-MRS.

Neurotransmitters are generally classified into two main categories based on their size: (a) small molecule neurotransmitters and (b) neuropeptides, related to their overall excitatory or inhibitory activity. Another group of biomolecules involved in

¹ Circle of Willis: Is a junction of several important arteries at the bottom part of the brain. It helps blood flow from both the front and back sections of the brain. <https://www.medicalnewstoday.com/articles/circle-of-willis>

Metabolite	Abbreviation	Cellular location	Function	Decrease	Increase
N-Acetyl-aspartate (6,7,8,9)	NAA	Different types of neurons, oligodendrocytes.	Neuronal marker. Amino acid precursor of NAAG together with glutamate. Its concentration varies in different types of neurons.	Loss of neurons or axons. Dementia, epilepsy, multiple sclerosis, ischemia, tumors. Manic states.	Canavan disease.
N-acetyl-aspartyl glutamate (8,9)	NAAG	Neurons, microglia, astrocyte, and oligodendrocyte.	Synapse-modulating peptide. Responsible, along with glial cells, for synaptic plasticity.	Damage to neurons in the motor system.	Neuropro-tection by reduces excess of glutamate.
Glutamate ⁽¹⁰⁾	Glu	Presynaptic vesicles. Neurons, glia, and non-synaptic activities (cellular metabolism)	Excitatory-inhibitory neurotransmitter of the synapse. Glial marker. It shares with glutamine an intercellular cycle between neurons and glia.	Concentrate trouble mental exhaustion. Insomnia. Low energy.	Extra or intra cellular glial edema, tissue damage.
Glutamine ⁽¹⁰⁾	Gln	Astrocytes	Glial marker. Acts as an inhibitory neurotransmitter at the brain level. Precursor glutathione.	Decreased γ -aminobutyrate and the enzyme glutamine synthetase.	Hyperactivity. Toxicity by cumulative ammonia.
Creatine (11,12,13)	Cr	Cytosol of muscles and neurons	Homeostasis of cellular bioenergetics (indicator of ATP formation).	Hyper-metabolic states. (Brain tumor)	Hypo-metabolism states
Choline (11,12,13)	Cho	Membrane of nerve and muscle cells.	Precursor of the neurotransmitter Acetylcholine. Muscular control and memory. Synthesis and destruction of the cell membrane (cell- turnover)	Deficient exchange of cell membranes. States of mania and depression.	Myelin- destruction, tumor proliferation gliomas, meningiomas
Myoinositol (11,12,13)	ml	Glia, Astrocytes	Glial marker. Cell signal transduction. Useful in the assessment of brain maturation.	Non-glial tumors. Manic states	Gliosis and reactive astrocytosis Alzheimer's
Glutathione 14,15,16,17	GSH	Cytoplasm of glial cells and neurons (higher concentration in neurons than in glia). Extracellular space	Cellular toxicity marker. Neurohormone and modulators. Cellular and mitochondrial protectors from oxidative damage and peroxidation. Cellular homeostasis.	coronary and autoimmune diseases, cancer, dysfunction mitochondrial, muscle weakness and fatigue.	High oxidative stress, immune deficiency, detoxification xenobiotics

Table 1.

A summary of the main metabolites detectable in the human brain, their functions, and the consequence of their imbalance is referenced in the following works [6–17].

brain metabolism are the proteins that constitute a group of “receptors” and “transporters” necessary for the metabolism of neurotransmitters. Receptors are proteins that allow certain neurotransmitters to interact with metabolic processes in the cell, or in the nerve ending that captures a stimulus and transmits it to produce a response, and are divided into two groups: (a) ligand-opened ion channels (Ionotropic) and (b) receptors coupled to G proteins (Metabotropic). Their effect can be excitatory if they tend to depolarize the membrane or inhibitory if they repolarize it, where after acting they are degraded or recaptured by the presynaptic cell quickly [18].

Likewise, neurotransmitter transporters are classified into two groups: (a) the reuptake transporter, located in presynaptic neurons, which pumps NT from the extracellular space into the cell; and (b) the transporter located in the vesicle membranes and concentrates NT in them for later exocytosis. Furthermore, another group of transporters, which have mostly been cloned and are grouped into two large families based on their molecular characteristics and electrogenic properties, called axolemmal is known. Those dependent on Na^+/Cl^- which include transporters for GABA, norepinephrine, dopamine, serotonin, choline, proline, betaine, glycine, and taurine, and those dependent on Na^+/K^+ include transporters for glutamate as well as alanine, serine, and cysteine [19].

Also, existing other molecules can be released from the same axon terminals as neurotransmitters, and are known as neuromodulators [20] and they can increase, prolong, inhibit, or limit the effect of the main neurotransmitter on the postsynaptic membrane, acting through a system of second messengers [21]. Among the endogenous neuromodulators also known as neuropeptides of the central nervous system, oxytocin acts by modulating social behaviors and its decrease has recently been linked to the appearance of autism [22].

Functionally, inhibitory properties of neurotransmitters are characterized by preventing or blocking chemical messages, decreasing the stimulation of nerve cells in the brain. For example, glutamate (Glu) acts as an “on switch” in neuronal pathways and requires the neurotransmitter GABA as an “off switch” providing the necessary balance between these two neurotransmitters for the correct functioning of the CNS, essentially in the regulation of cognition, learning, memory and emotional behaviors [23–25]. Thus, the imbalance between Glu-excitation and GABA-inhibition leads to hyperactivation of the CNS that has been linked to ASD symptoms.

3. Materials and methods

Neurotransmitter metabolism in cingulate cortices has aroused great interest in recent research on autism spectrum disorders (ASD). In this chapter, we present a study carried out in the cingulate cortices of autistic adults, one of the areas that make up the limbic system, because it is related to memory, attention, and behavior in humans, qualities that are affected in people with ASD. In both regions of the cingulate cortices, anterior (ACC) and posterior (PCC), we applied the proton magnetic resonance spectroscopy (^1H -MRS) technique, as a powerful tool that provides biochemical, functional, and metabolic information that complements obtained from conventional imaging studies. Here, changes in the concentration levels of different brain metabolites, as markers of neuronal density, glial density, cell membrane processes, and energy metabolism, are evaluated between the anterior and posterior cingulate cortex of subjects with ASD.

3.1 Sample size and strength

This study was carried out under ethical standards, and the latest Declaration of Helsinki (revised in 2000) was approved by the University of La Laguna. This study aims to detect the levels of neurotransmitters present in brain-cingulated cortices in patients with ASD. For this purpose, an adult population was assessed with 1H-MRS between 17 and 23 (mean, 20.8 ± 1.8) years. The sample of subjects with ASD ($n = 19$) and subjects with typical development (TD) ($n = 41$) were chosen to make the comparisons. Previously, a small number of adults who met the criteria for ASD ($n = 5$) and TD ($n = 1$) were excluded from the analyses because they had epilepsy.

3.2 Sample recruitment, sample characteristics, and adherence to the study

The adults were selected and recruited by the respective psychiatry and psychology service of the Hospital Universitario de Canarias (HUC), Tenerife, Spain. They were diagnosed with ASD in childhood and during their growth by applying the tests used to evaluate and measure early childhood development [26–31] without any variation in diagnosis during growth according to clinical history. The Autism Diagnostic Interview-Revised (ADI-R), or the Autism Diagnostic Observation Schedule (ADOS), is the standardized diagnostic test for assessing autism spectrum disorder which, not applied independently by us in this study, considers that there was a high degree of concordance between clinical and research diagnoses [32], thus avoiding overdiagnosis. Despite this lack of diagnosis can be seen as a limitation of the methodology, we considered applying the Spanish version of the AQ (Autism-Spectrum Quotient) test (Baron-Cohen S, 2005) [33] to all participants for a significant differentiation between the groups in the study, namely, subjects with autism group (ASD) and subjects with typical development (TD) used as control (see **Table 2**).

3.3 1H-MRS acquisition data

All patients underwent MRI and 1H-MRS on a Signa HD 3 T MRI scanner (GE Healthcare, Waukesha, WI, USA). 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° , 256 acquisitions using the point-resolved spectroscopy (PRESS) technique. During the acquisitions, voxels ($20 \times 20 \times 20 \text{ mm}^3$) were placed in ACC and PCC (see **Figure 1**), by the same experienced neuroradiologist blinded to the clinical data. We used TE-23 ms because myo-inositol is known

Characteristics	ASD ($n = 19$)	TD ($n = 41$)	<i>p</i> Value
Gender (male/female)	16/3	16/25	$p = 0.016$
Age (years)	20.58 (0.71)	23.19 (0.71)	$p = 0.049$
Epilepsy	5		$p < 0.0015$
Gastrointestinal disorders	17		$p < 0.0001$
Muscular hypotonia	14		$p < 0.0001$
Autism Quotient (0–50) points	33.84 (6.36)	11.67 (7.07)	$p < 0.0001$

Table 2.
Demographic and psychometric summary of participants.

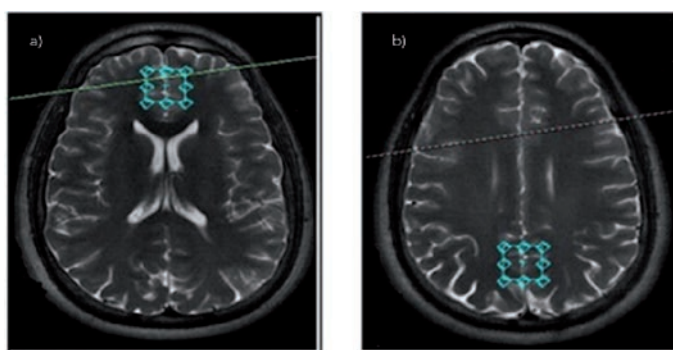


Figure 1.

Locations of the volume studied in the (a) anterior and (b) posterior cingulate 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°, 256 acquisitions with point-resolved spectroscopy (PRESS) technique. While we obtained acquisitions, the same experienced neuroradiologist who was blind to the clinical data, placed voxels (20 x 20 x 20 mm³) at ACC and PCC.

to be easily detected in the short TE 1H-MRS spectra in the brain due to its high concentration of 4–8 mM [34]. Each voxel was carefully positioned in the study area in each subject to exclude signal contamination from the skull and subcutaneous fat. Furthermore, the morphological examination excluded other pathologies, such as congenital anomalies, cerebral palsy lesions, tumors, and hydrocephalus in this study.

3.4 Post-processing of the spectra

The data acquired by the magnetic resonance scanner was sent to the General Electric workstation (GE Workstation) in our image processing laboratory. There, the LC Model program [35] was installed on a Linux workstation dedicated to the automatic quantification of the spectra obtained. Also, the LC Mgui graphical interface, available to the user, was used to run the LC Model program more comfortably. Thus, the results of the processing with LC Model are obtained in a two-page printable file (postScript), for each voxel studied (see **Figure 2**) of results, where the concentration of each metabolite is summarized with its respective Cramér-Rao limit² [36]. In addition, before looking at the concentrations of the metabolites, it is important to first look at the standard deviation (expressed as a percentage, % SD) in the input column, since these have been estimated according to the lower limits of Cramér-Rao concentrations, and although they are only the lower limits, they are still the most useful reliability indicators. The reliability indicator represented by % SD ≤ 20 is subjectively considered as a very approximate criterion for acceptable reliability estimates.

The statistical package used to make the graphical representations and statistical analysis in this thesis was the GraphPad Prism v 10.4.0 program (GraphPad Software, inc. La Jolla, San Diego. USA).

3.5 Neurometabolite post-processing and quantification

1H-MRS datasets were collected using the SAGE and LCMODEL 6.3 software platforms considering that the calculation of absolute concentrations (e.g., in molar

² Cramér-Rao lower bounds. These are approximate maximum likelihood estimates and their uncertainties. Provencher SW. 1993

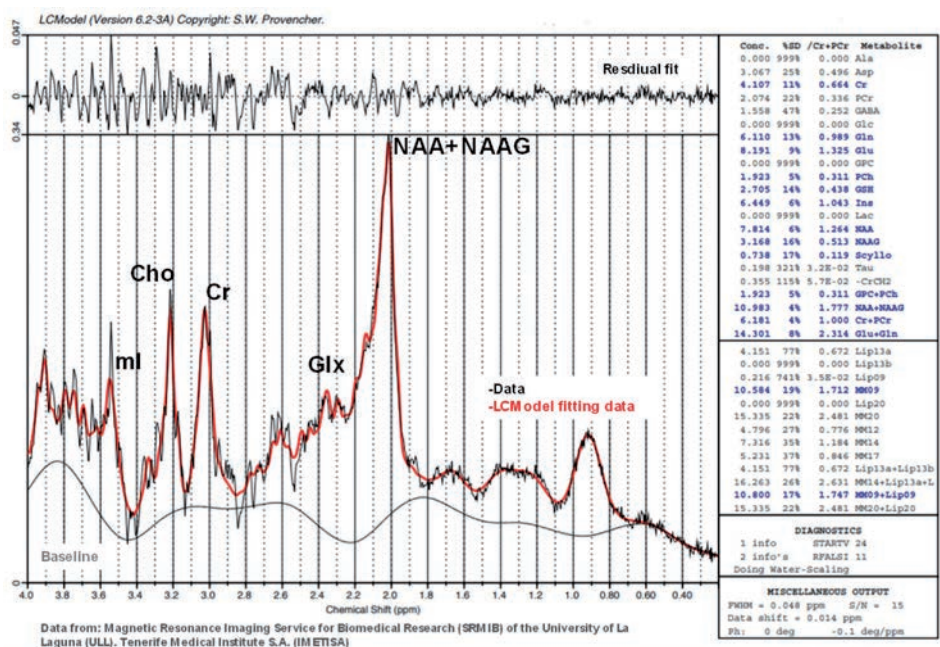


Figure 2.
Post-processed spectrum in a single voxel, obtained by LCMODEL. MR spectrum acquired showing the prominent resonances of the brain metabolites' N-acetyl aspartate+N-acetyl aspartyl glutamate (NAA + NAAG), Glutamate+ Glutamine (Glx), creatine (Cr), Choline (Cho), myo-inositol (ml). The black line, looks like noise one is the actually data, and the red line is the LCMODEL fitting data. The noisy trace at the top (black) is the residual fit. The numbers on the right quantification results, including error estimates (%SD) in Cramér-Rao lower bounds (CRLBs).

units) involves correction for many factors, including 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 subject under study, the location of the voxels and their relationship to the electromagnetic properties of the coil, and any temporal variations in the scanner. However, the assessment of neurometabolite proportion with creatine is generally the fastest and most widely used analysis method for clinical 1H-MRS. Furthermore, ratios account for all non-metabolite-specific differences, therefore can be reasonably compared across all participants scanned at the same institution with the same protocol.

4. Results and discussion

4.1 Neurometabolite variability between anterior (ACC) and posterior (PCC) cingulated cortices in subjects with and without ASD. Proton spectroscopy (1H-MRS)

The resonance signals in the spectra of the detected neurometabolites are fitted by the curvature under the area of each peak shown within the voxel (see **Figures 1** and **2**). The absolute concentrations [mM] of the neurotransmitters present (see **Table 3**) were calculated using creatine as an internal reference because it is the most stable metabolite under the applied magnetic field [37]. Spectroscopy

Metabolites absolute concentration	ACC	PCC	P
ASD	[mM]	[mM]	
NAA + NAAG	10.29	10.87	n.s
NAA	9.70	10.53	n.s
Glx	16.84	13.92	n.s
Glu	12.60	10.29	*p = 0.04
Cho	2.15	1.56	*p = 0.0008
Cr	7.20	6.76	n.s
mI	5.24	5.03	n.s
GSH	3.08	3.43	n.s
TD			
NAA + NAAG	10.63	10.73	n.s
NAA	9.95	10.40	n.s
Glx	9.55	8.40	n.s
Glu	9.98	9.83	n.s
Cho	1.53	1.07	*p = 0.02
Cr	7.43	6.73	n.s
mI	5.55	4.94	*p = 0.03
GSH	3.75	3.31	n.s

Case-control analysis of metabolites values according to cerebral regions in ASD vs. TD, for N-acetyl aspartate (NAA), N-acetyl aspartate+N-acetyl aspartil glutamate (NAA + NAAG), Glutamate+Glutamine (Glx), Glutamate (Glu), creatine (Cr), Choline (Cho), myoinositol (mI), and Glutathione (GSH). Absolute concentration group means. Regions = Anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC). P < 0.05 significantly differences. No significant differences = n.s.

Table 3.
Absolute concentrations [mM] of the main metabolites detected in ACC and PCC, within each group ASD and TD.

studies were performed on subjects in the ASD and TD groups, calculating the averages of the absolute concentrations of the metabolites detected in the voxel positioned in ACC and PCC. The resulting neurometabolic report is shown in **Table 4**.

When observing the pattern of neurometabolites obtained in the TD group and the variation in absolute concentrations in ACC and PCC, significant differences were observed in the metabolites myoinositol ($p = 0.03$) and choline ($p = 0.02$) between ACC and PCC (see **Table 3**). This describes the metabolic needs in each, related to the function of each area. On the one hand, myoinositol is considered a glial marker responsible for cellular signal transduction and useful for assessing brain maturation, and on the other hand, choline is responsible for maintaining the structural integrity of membranes and acts as a modulator of cholinergic neurotransmission. Furthermore, the function of choline is critical during fetal development, influencing the risk of neural tube defects and lifelong memory function [38]. We then considered this metabolic pattern present in healthy subjects with typical development as a control for comparing them with the results obtained in the group with ASD.

Contrary to the result of the TD group, the neurometabolite pattern obtained in the ASD group showed a significant increase in absolute glutamate (Glu) concentration (12.85; $p = 0.04$) in the ACC, indicating an imbalance of glutamatergic

Metabolite [mM] $\pm$ SD	AQ1 Score = (0–10)	AQ2 Score = (11–22)	AQ3 Score = (23–31)	AQ4 Score = (32–50)
ACC				
Cr + PCr	7.48 $\pm$ 0.81	7.42 $\pm$ 1.86	6.53 $\pm$ 0.98	7.72 $\pm$ 1.77
mI	6.12 $\pm$ 0.81	5.47 $\pm$ 1.42	7.29 $\pm$ 3.61	4.83 $\pm$ 1.93
GPC + PCho	1.83 $\pm$ 0.49	2.26 $\pm$ 0.59	1.63 $\pm$ 0.70	2.18 $\pm$ 0.63
NAA + NAAG	10.41 $\pm$ 0.79	10.66 $\pm$ 2.73	8.46 $\pm$ 0.49	10.56 $\pm$ 2.57
Glu + Gln	13.74 $\pm$ 4.06	15.67 $\pm$ 4.13	17.34 $\pm$ 1.43	16.65 $\pm$ 7.24
GSH	3.51 $\pm$ 0.87	3.96 $\pm$ 1.32	2.21 $\pm$ 0.84	3.36 $\pm$ 0.60
Glu	8.04 $\pm$ 1.62	10.25 $\pm$ 2.70	13.87 $\pm$ 2.13	13.14 $\pm$ 4.14
NAA	9.96 $\pm$ 1.27	9.95 $\pm$ 2.38	8.41 $\pm$ 0.38	9.71 $\pm$ 1.78
PCC				
Cr + PCr	7.05 $\pm$ 0.45	6.69 $\pm$ 1.38	6.21 $\pm$ 0.69	6.68 $\pm$ 0.51
mI	5.99 $\pm$ 1.79	4.80 $\pm$ 0.71	5.51 $\pm$ 0.99	4.91 $\pm$ 0.83
GPC + PCho	1.63 $\pm$ 0.39	1.66 $\pm$ 0.41	1.29 $\pm$ 0.36	1.48 $\pm$ 0.32
NAA + NAAG	10.28 $\pm$ 2.60	10.79 $\pm$ 2.08	9.60 $\pm$ 0.95	10.98 $\pm$ 1.24
Glu + Gln	13.37 $\pm$ 2.11	13.76 $\pm$ 0.83	10.39 $\pm$ 0.37	14.14 $\pm$ 5.45
GSH	3.08 $\pm$ 1.07	3.36 $\pm$ 0.91	2.50 $\pm$ 1.11	3.44 $\pm$ 0.94
Glu	10.51 $\pm$ 2.48	9.74 $\pm$ 2.15	8.94 $\pm$ 2.66	10.10 $\pm$ 2.20
NAA	9.89 $\pm$ 2.16	10.47 $\pm$ 2.04	8.98 $\pm$ 1.21	10.57 $\pm$ 1.56

Table 4.
Metabolite concentrations are grouped by AQ score (AQ1, AQ2, AQ3, and AQ4) in ACC and PCC regions.
Mean $\pm$ SD.

metabolism (see **Table 3**), and confirming a large body of research results proposing this glutamate imbalance as one of the many etiologies of ASD. In this regard, a hyperglutamatergic state has been previously reported in ASD subjects, in late childhood and adolescence, specifically in the anterior cingulate cortex [39] further supporting our results. It is important to consider that glutamatergic gliotransmission is controlled by gliotransmitters released by oligodendrocytes and microglia [40–42], being the main responsible for excitotoxicity in the CNS; hence, it is related to a deregulation of the excitatory/inhibitory response in the brain as proposed by the new models of neurodevelopmental deficits in ASD. Glutamate, together with aspartate, constitutes the main transmitter of the dominant neurons of the cortex (called pyramidal cells), where developmental changes occur in the transitional stages of the visual cortex, affecting the nature of spatial and temporal integration in neurons that contribute to the maturation of visual functions [43]. Recently, the effects of excess glutamate in the anterior cingulate cortex have been directly correlated with attention shifting/shift tolerance in subjects with ASD [44].

However, although glutamate contributes to the development of the nervous system and its synaptic plasticity, important physiological failure within the human brain is excitotoxicity, attributed precisely to an accumulation of glutamate that contributes to a large number of disorders such as ischemia, epilepsy, AIDS-associated dementia, and the appearance of neurodegenerative pathologies such as Alzheimer's

disease (AD) [45], Huntington's disease [46], Parkinson's disease [47], among others [48] highlighting the importance of adequate glutamatergic metabolism at the brain level. It should be noted that glutamate is considered the main and most important excitatory neurotransmitter within the CNS, which shares with glutamine an intercellular cycle between neurons and glia [49, 50].

Highlighting, the choline (Cho) absolute concentration differences between ACC and PCC are very significant ($p = 0.0008$) in comparison with the TD group suggesting hypermetabolism in membrane turnover, also associated with myelin destruction [51, 52], related to a metabolic imbalance between these two brain areas (see **Figure 3**). Some characterized choline functions include sustained attention, sleep/wake regulation, and learning and memory within the CNS [51], which are related to principal ASD symptoms. Cholinergic innervation of the human cerebral cortex arises in the basal forebrain and reaches its highest density in the limbic system components where they branch off into the limbic cortex formed by the cingulate

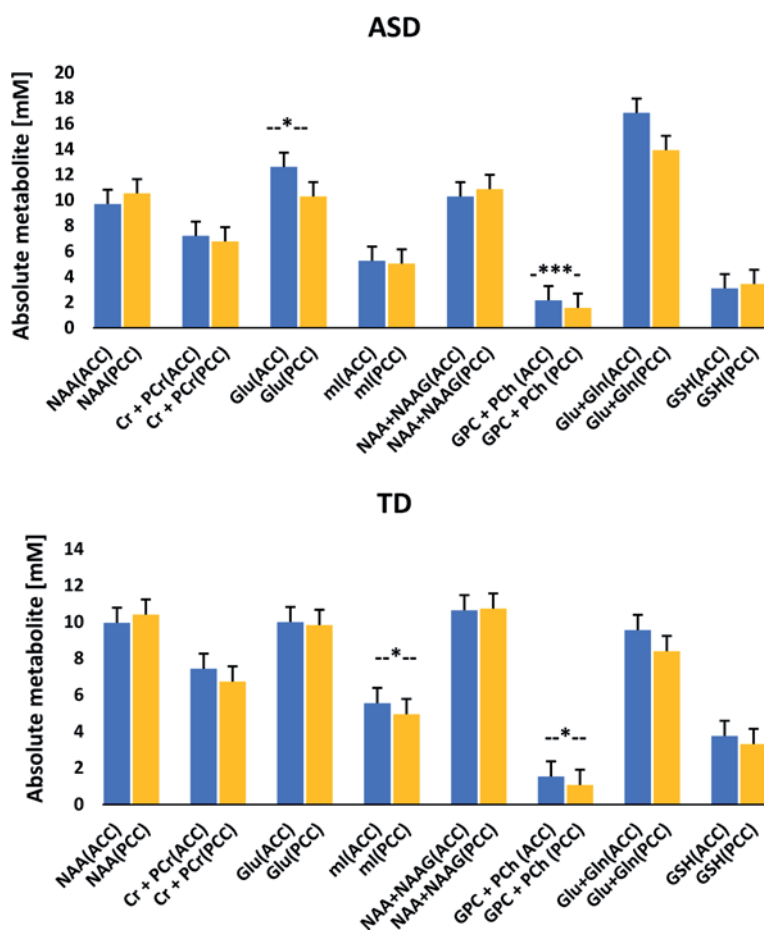


Figure 3. Metabolic pattern in cingulated cortices ACC and PCC by ASD and TD groups. Mean absolute concentrations of the metabolites N-acetyl aspartate (NAA), N-acetyl aspartate+N-acetyl aspartyl glutamate (NAA + NAAG), Glutamate+Glutamine (Glx), Glutamate (Glu), creatine (Cr), Choline (Cho), myoinositol (mI), and Glutathione (GSH) in each ASD group ($n = 19$) and TD ($n = 41$). Comparison between the regions of the anterior and posterior cingulate cortex. Mann-Whitney test of Absolute NT. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$.

gyrus. The behavioral affiliations of cortical cholinergic pathways span the domains of attention and memory [52], both of which are deficient in ASD [53].

Another important result in the ASD group was the significantly reduced concentration of myoinositol (mI) in ACC compared to the TD group. Neurochemical changes of mI in the anterior cerebral cortex have previously been associated with circadian disruption and depression in young adults [54] in agreement with our results. In more severe cases within ASD, the subjects are found to be disrupted to sleep during the night, increasing stress and inflammation in the brain. Myoinositol is found in glial cells and astrocytes controlling gliosis and reactive astrocytosis [55, 56]. Both cells interact with synapses that play important physiological roles in learning and memory [57] so a deficiency of mI in the ACC of subjects with ASD would be the cause of a lower performance in both functions [58].

Given the evidence, knowing the neurochemical substrate of both cingulate cortices, specifically, the PCC, which has a cerebral blood flow and metabolic rate of approximately 40% above the average of the entire brain [59], places it in the focus of our research, for understanding the relationship of activity that exists between both nodes (see **Figure 4**).

Taking into account ACC is the region that receives projections primarily from the amygdala and together with the dorsolateral prefrontal cortex, it intervenes in the regulation of behavior and plays an important role in shifting attention during the operation of working memory. Consequently, lesions in the anterior cingulate interfere with selective attention, monitoring of competing responses, and self-initiation of behavior, all skills that are deficient in subjects with ASD. In contrast to the ACC, the posterior cingulate cortex (PCC) receives most of the projections from the hippocampus, forming the emotional-social component of the memory system [60]. In this way, we present these two brain regions as substantially important for the investigation of ASD etiology.

On the other hand, ASD has been linked to a high vulnerability to oxidative stress and low capacity of methylation reaction with ASD symptoms in children [61, 62]. However, recent studies reveal that oxidative stress is related to a decrease in GSH concentration seen in peripheral blood mononuclear cells, plasma, lymphoblastoid cell lines, brain tissue, and mitochondria in individuals with ASD [63, 64] and that support

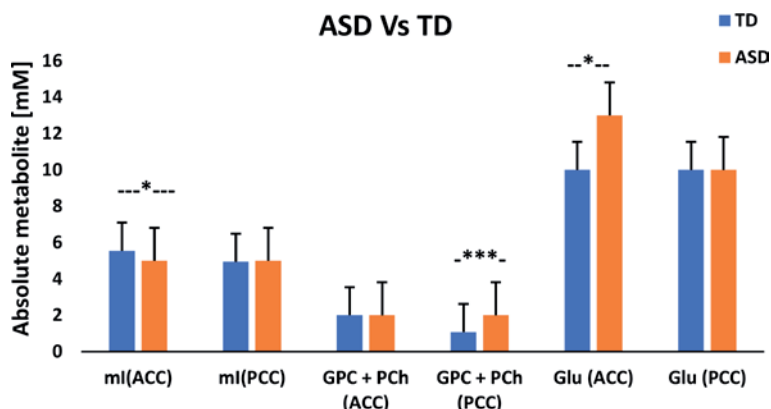


Figure 4. Neurochemistry metabolite variation of absolute concentrations of Glutamate (Glu), Choline (Cho), and myoinositol (mI) in ASD group ($n = 19$) compared to TD ($n = 41$). Comparison between the regions of the anterior and posterior cingulate cortex. Mann-Whitney test of Absolute NT. * $p < 0.05$; ** $p < 0.001$; *** $p < 0.0001$.

the results in this study, identifying glutathione as a biomarker of oxidative stress caused by an alteration in its metabolism, key in the etiology of ASD [14, 16, 64–66].

4.2 Neuroplasticity³ in ACC and PCC is associated with the intensity of autism features according to the AQ-test score and the corresponding neurometabolic pattern

Our results suggest the usefulness of the AQ-Test in characterizing subjects within the broad spectrum of autism. The subjects were then grouped according to the range of the AQ-Test autism coefficient described by Baron-Cohen [67] which provides a broader view of the variations within the autism spectrum. Within the broad social spectrum in which autism traits are reflected, and their distribution within the population as a whole, the autism quotient (AQ)⁴ represents the position an individual occupies within that broad spectrum or social continuum. Likewise, the author of the AQ Test attributes two essential uses to it:

1. In clinical practice as a screening tool, allowing physicians or psychologists to identify individuals potentially affected by ASD for subsequent referral for a more precise diagnosis; and
2. In research, in comparative studies, to separate “affected” from “non-affected” individuals in the search for the point that best separates the categories.

The psychometric background that supports the AQ-Test defines it as a psychometric test characterized by being an instrument used in psychology for the measurement of psychological attributes, standardized, composed of selected and organized items, conceived to produce in the individual the recordable reactions for which it was designed. The AQ-Test questionnaire consists of 50 questions, divided into five areas or subscales, and each subscale consists of 10 items, carefully selected from the behavioral characteristics of Wing’s “Triad” [1] of autism symptoms (social skills, attention switching, attention to detail, communication, and imagination).

The test result is a number between zero and 50, with the latter value being the end of the scale with the highest burden of autistic characteristics, where the most adverse levels of affect are located. The test has proposed four ranges or cutoff points for autistic characteristics:

AQ1 (0–10) points = Autistic characteristics below the population average.

AQ2 (11–22) points = Average values of autistic characteristics in a normotypical population (the female average is 15 and the male average is 17).

AQ3 (23–31) points = Autistic characteristics above the population average.

AQ4 (32–50) points = Very high level of autistic characteristics. (Asperger’s syndrome or high-functioning autism has an average score of 35).

The study population was grouped according to the resulting score when applying the AQ test, into four groups or phenotypes AQ1 = 28.33%, AQ2 = 43.33%, AQ3 = 10.00%, and AQ4 = 18.33%. When comparing the results of the

³ Phenotypic plasticity has been defined as the capacity of the human brain to change its morphological patterns, interactions in its sensory modalities, and patterns of neurotransmitter secretion. These changes arise by virtue of external and internal factors and represent a phenomenon called neuroplasticity [68, 69].

⁴ As the author himself points out, the term quotient is not used here in the arithmetic sense of dividing one quantity by another, but rather derives from the Latin word “quotients,” meaning “how much.”

Correlation (r) ACC vs. PCC	NAA + NAAG r; p	NAA r; p	Glu + Gln r; p	Glu r; p	GPC + PCh r; p	Cr + PCr r; p	mI r; p	GSH r; p
AQ1	0.14; n.s	0.18; n.s	-0.13; n.s	-0.11; n.s	0.11; n.s	-0.40; n.s	0.26; n.s	0.10; n.s
AQ2	0.14; n.s	0.16; n.s	-0.08; n.s	-0.02; n.s	0.07; n.s	-0.45; 0.02	0.23; n.s	0.05; n.s
AQ3	0.82; 0.05	0.70; n.s	-0.65; n.s	0.03; n.s	-0.54; n.s	0.51; n.s	0.57; n.s	0.41; n.s
AQ4	0.06; n.s	0.25; n.s	0.03; n.s	0.13; n.s	0.58; n.s	0.04; n.s	0.31; n.s	-0.04; n.s

Table 5.

Metabolite Pearson correlation by AQ score (AQ1, AQ2, AQ3, and AQ4) in ACC vs. PCC regions for N-acetyl aspartate (NAA), N-acetyl aspartate + N-acetyl aspartil glutamate (NAA + NAAG), Glutamate + Glutamine (Glx), Glutamate (Glu), creatine (Cr + PCr), Choline (GPC + PCh), myoinositol (mI), and Glutathione (GSH). Regions = Anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC). $p < 0.05$ significantly differences. $P < 0.05$ significant correlation. No significant correlation = n.s. Pearson correlation = r .

spectroscopies reported in point 4.1 where the subjects were grouped according to their neuropsychiatric reports as ASD and TD, with the results obtained by grouping them according to the AQ-test score, significant differences were observed in the absolute concentrations of the neurometabolites between the four groups AQ1, AQ2, AQ3, and AQ4 suggesting a pattern of neuroplasticity [70, 71] characteristic of these disorders.

The results show the absolute concentrations (mM) of the metabolites detected in ACC of the AQ2 group (11–22, mean values of the neurotypical population), AQ3 (23–31, above average) and AQ4 (32–50, very high rate of autistic characteristics) groups compared to the AQ1 group (0–10, below the mean), as well as in the PCC, revealing a specific neurometabolic secretion pattern present in each of the AQ1, AQ2, AQ3, and AQ4 groups (see **Table 4**). Likewise, these results suggest a better fit, when referring to the entire broad spectrum of autism, which could explain the diversity of developmental characteristics in people with this disorder and its complication when making a diagnosis.

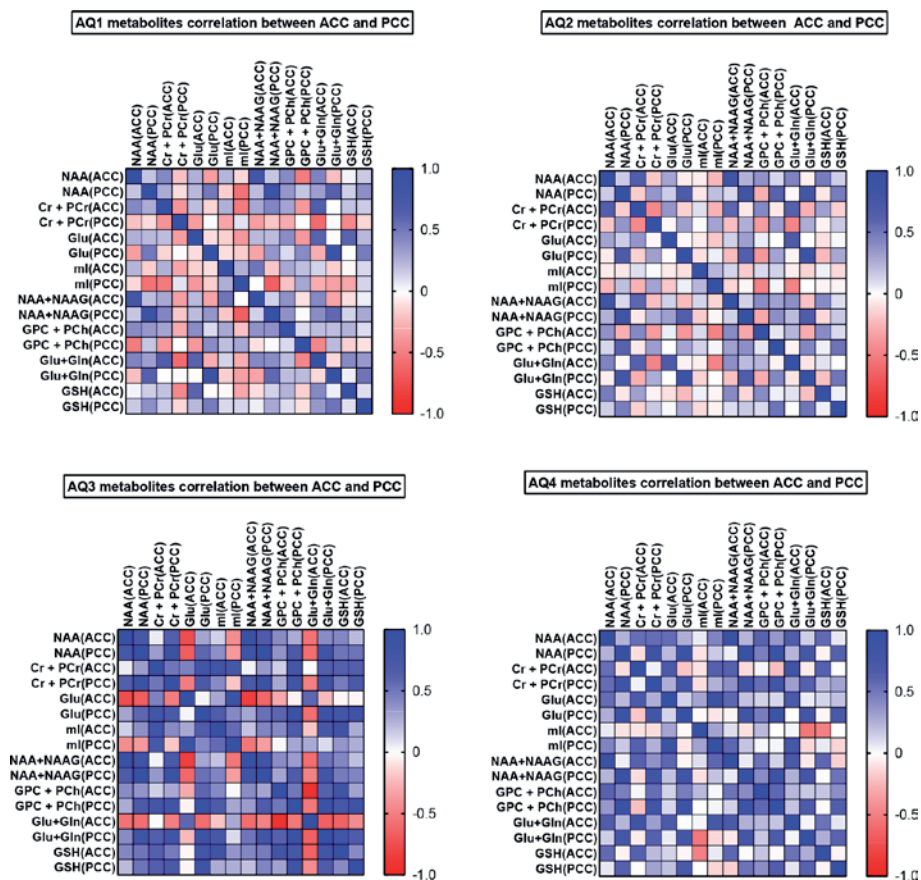


Figure 5. Heatmap correlation phenotypic neuroplasticity in ACC and PCC within the autism spectrum according to the AQ. Absolute concentrations (mM) of the detected metabolites, for N-acetyl aspartate (NAA), N-acetyl aspartate +N-acetyl aspartil glutamate (NAA + NAAG), Glutamate+Glutamine (Glx), Glutamate (Glu), creatine (Cr + PCr), Choline (GPC + PCh), myoinositol (ml) and Glutathione (GSH). Regions = Anterior cingulate cortex (ACC), and posterior cingulate cortex (PCC). $P < 0.05$ significantly differences.

By correlating the absolute concentrations of metabolites in ACC vs. PCC, a significant negative correlation was observed for (Cr + PCr) in the AQ2 ($r = -0.45$; $p = 0.02$) group. However, AQ3 highlights that the concentration of (NAA + NAAG) has shown a significant positive correlation in ACC vs. PCC ($r = 0.82$; $p = 0.05$), showing a new promising marker for the detection of ASD by spectroscopy (see **Table 5**).

With these results, we graphed the neurometabolic pattern corresponding to metabolite correlation in ACC vs. PCC in adults, characterized according to the AQ-Test, which made us suggest the great influence due to metabolic dysfunction within the broad spectrum of autism and its relationship with the diversity of characteristics observed in subjects who develop this disorder (see **Figure 5**).

It is necessary to remember that NAAG is distributed together with different neurotransmitters, including glutamate (Glu), known to be a neurotransmitter with excitatory and inhibitory activity in the central nervous system, and gamma-aminobutyric acid (GABA), known as the most common inhibitory neurotransmitter in the CNS. Furthermore, imbalanced (NAAG) metabolism has been described before in some neurological conditions such as schizophrenia, amyotrophic lateral sclerosis, traumatic brain injury, and now in ASD [68, 69, 72].

It caught our attention that although the AQ2 group is made up of subjects with a range of AQ-Test scores (11–22) that are the average values of neurotypical population according to the work carried out by Barón-Cohén; however, neurometabolically, variabilities were observed in the concentrations of the metabolites that make us suggest a dysfunction between ACC and PCC in these subjects without them having the diagnosis of ASD. This does not mean that they do not have some of the characteristics that would identify them as ASD, but that at the neurometabolic and functional level they would show some cognitive deficiencies compared to control group AQ1.

Furthermore, dysregulation of metabolism (NAA + NAAG) between ACC and PCC may also affect the visual system in ASD, highlighting that the posterior cingulate cortex is one of the cortical structures involved in visual attention [73].

5. Conclusions

The pattern we observed in the Heatmap reveals an abnormal neuroplasticity process within the broad spectrum of autism caused by a malfunction of the signaling between neurons in ACC vs. PCC, when observing the neurometabolic correlation pattern of each neurometabolite (see **Figure 5**). The onset of a metabolic change in AQ2, AQ3, and AQ4 is finely detectable when compared to the group of subjects below the average of autistic characteristics AQ1. This study is carried out in adult subjects, which leads us to think that the aberrant neuroplasticity observed in children [74] with ASD continues with them into adulthood.

Thus, the need to perform a correct neurometabolic diagnosis and subsequent treatment in children with ASD is increasingly imminent, recognizing that the same neurometabolites that are treated in the different diseases mentioned throughout this chapter are also altered in ASD. Namely the amino acids and neuropeptides with their derivatives involved in this neuroplasticity process, including glutamate (Glu), glycine (Gly), γ -aminobutyric acid (GABA), and N-Acetyl aspartyl glutamate (NAAG) among others (see **Table 4**). 1H-MRS studies in ASD can be expected to continue to gain importance. Overall, the knowledge gained about neuroplasticity

can be applied in clinical and educational areas where all developmental disabilities from childhood onwards underline. Increased knowledge on the neurochemical alteration of autism could lead to a completely new approach to the pharmacological management of autism and to the identification of biomarkers with greater specificity and sensitivity.

Author contributions

C.J.-E. contributed to writing—review and editing, writing—original draft, and research. F.M.S. contributed to writing—review and editing and software. J.L.G-M. contributed to review and editing, supervision, methodology, and conceptualization.

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Institutional review board statement

This study was carried out in accordance with the recommendations and guidelines set by the European Union (86/609/EU) and in accordance with the current legal regulation of Spain Law 32/2007, RD 53/2013, Law 9/2003, and RD 178/2004. The protocol research was approved by the Ethics Committee of La Laguna University and Ministry of Science and Innovation (Spain), with approval number CEIBA2013–0056. This study was carried out in accordance with the recommendations of the ethical principles for medical research involving human, and law 14/2007 for biomedical research, with written information from all subjects. The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Board.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Conflict of interest

The authors declare no conflict of interest.

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References

- [1] Wing L, Gould J. Severe impairments of social interaction and associated abnormalities in children: Epidemiology and classification. *Journal of Autism and Developmental Disorders*. 1979;**9**(1):11-29
- [2] American Psychiatric Association et al. Diagnostic and statistical manual of mental disorders. Text Revision. 2000
- [3] Clarke PHD, Dudley D, Sokoloff L. Circulation and Energy Metabolism in the Brain/Donald D. Clarke and Louis Sokoloff; 1999
- [4] Siesjö BK. Brain energy metabolism and catecholaminergic activity in hypoxia, hypercapnia and ischemia. *Journal of Neural Transmission. Supplementum*. 1978;**14**:17-22
- [5] Teleanu RI et al. Neurotransmitters—Key factors in neurological and neurodegenerative disorders of the central nervous system. *International Journal of Molecular Sciences*. 2022;**23**(11):5954
- [6] Hetherington HP, Gadian DG, Ng TC. Magnetic resonance spectroscopy in epilepsy: Technical issues. *Epilepsia (Series 4)*. 2002;**43**
- [7] Beckwith-Hall BM et al. Nuclear magnetic resonance spectroscopic and principal components analysis investigations into biochemical effects of three model hepatotoxins. *Chemical Research in Toxicology*. 1998;**11**(4):260-272
- [8] Baslow MH. Functions of N-acetyl-L-aspartate and N-acetyl-L-aspartylglutamate in the vertebrate brain: Role in glial cell-specific signaling. *Journal of Neurochemistry*. 2000;**75**(2):453-459
- [9] Pioro EP et al. Detection of cortical neuron loss in motor neuron disease by proton magnetic resonance spectroscopic imaging in vivo. *Neurology*. 1994;**44**(10):1933-1933
- [10] Courvoisie H et al. Neurometabolic functioning and neuropsychological correlates in children with ADHD-H: Preliminary findings. *The Journal of Neuropsychiatry and Clinical Neurosciences*. 2004;**16**(1):63-69
- [11] Kreis R, Ernst T, Ross BD. Cuantificación absoluta de agua y metabolitos en el cerebro humano. II. Concentraciones de metabolitos. *Journal of Magnetic Resonance, Serie B*. 1993;**102**(1):9-19
- [12] Castillo M, Smith JK, Kwok L. Correlation of myo-inositol levels and grading of cerebral astrocytomas. *American Journal of Neuroradiology*. 2000;**21**(9):1645-1649
- [13] Govindaraju V, Young K, Maudsley AA. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR in Biomedicine: An International Journal Devoted to the Development and Application of Magnetic Resonance In Vivo*. 2000;**13**(3):129-153
- [14] Frye RE et al. Redox metabolism abnormalities in autistic children associated with mitochondrial disease. *Translational Psychiatry*. 2013;**3**(6):e273-e273
- [15] Janáky R et al. Glutathione in the nervous system: Roles in neural function and health and implications for neurological disease. In: *Handbook of Neurochemistry and Molecular Neurobiology: Amino Acids and Peptides*

in the Nervous System. Springer US; 2007. pp. 347-399

[16] Rossignol DA, Frye RE. Mitochondrial dysfunction in autism spectrum disorders: A systematic review and meta-analysis. *Molecular Psychiatry*. 2012;17(3):290-314

[17] Pastore A et al. Analysis of glutathione: Implication in redox and detoxification. *Clinica Chimica Acta*. 2003;333(1):19-39

[18] Darnell J, Lodish H, Baltimore D. *Molecular Biology of the Cell*. 1990

[19] Garcia-Lopez M. Axolemmal transporters for neurotransmitter uptake. *Revista de Neurologia*. 1999;29(11):1056-1063

[20] Nadim F, Bucher D. Neuromodulation of neurons and synapses. *Current Opinion in Neurobiology*. 2014;29:48-56

[21] Nan F et al. Dual function glutamate-related ligands: Discovery of a novel, potent inhibitor of glutamate carboxypeptidase II possessing mGluR3 agonist activity. *Journal of Medicinal Chemistry*. 2000;43(5):772-774

[22] Yamasue H, Domes G. Oxytocin and autism spectrum disorders. *Behavioral Pharmacology of Neuropeptides: Oxytocin*. 2018:449-465

[23] Kim D, Lee J-S. Neurotransmitter-induced excitatory and inhibitory functions in artificial synapses. *Advanced Functional Materials*. 2022;32(21):2200497

[24] Chao H-T et al. Dysfunction in GABA signalling mediates autism-like stereotypies and Rett syndrome phenotypes. *Nature*. 2010;468(7321):263-269

[25] Cornell-Bell AH et al. Glutamate induces calcium waves in cultured astrocytes: Long-range glial signaling. *Science*. 1990;247(4941):470-473

[26] Kaufman AS, Raiford SE, Coalson DL. *Intelligent Testing with the WISC-V*. John Wiley & Sons; 2015

[27] Kaufman NL, Kaufman AS. Comparison of normal and minimally brain dysfunctions children on the McCarthy scales of children's abilities. *Journal of Clinical Psychology*. 1974;30(1)

[28] Bracken BA et al. Universal nonverbal intelligence test (UNIT). *Alternative Assessments With Gifted and Talented Students*. 2021

[29] Ling W et al. Construction of CPM scale for leadership behavior assessment. *Acta Psychologica Sinica*. 1987;19(02):89

[30] Baron-Cohen S et al. Early identification of autism by the checklist for autism in toddlers (CHAT). *Journal of the Royal Society of Medicine*. 2000;93(10):521-525

[31] Mazefsky CA, Oswald DP. The discriminative ability and diagnostic utility of the ADOS-G, ADI-R, and GARS for children in a clinical setting. *Autism*. 2006;10(6):533-549

[32] Rutter M, Le Couteur A, Lord C. ADI-R. *Autism Diagnostic Interview Revised. Manual*. Los Angeles: Western Psychological Services; 2003

[33] Baron-Cohen S. *La gran diferencia: Cómo son realmente los cerebros de hombres y mujeres*. Editorial AMAT. 2005

[34] Graaf D, Robin A. *In Vivo NMR Spectroscopy: Principles and Techniques*. John Wiley & Sons; 2019

- [35] Provencher SW. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine*. 1993;**30**(6):672-679
- [36] Cramér H. *Mathematical Methods of Statistics*. Princeton University Press; 1999
- [37] 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
- [38] Ross RG et al. Perinatal choline effects on neonatal pathophysiology related to later schizophrenia risk. *American Journal of Psychiatry*. 2013;**170**(3):290-298
- [39] Bejjani A et al. Elevated Glutamatergic Compounds in Pregenual Anterior Cingulate in Pediatric Autism Spectrum Disorder Demonstrated by ¹H MRS and ¹H MRSI. 2012
- [40] Santello M, Bezzi P, Volterra A. TNF α controls glutamatergic gliotransmission in the hippocampal dentate gyrus. *Neuron*. 2011;**69**(5):988-1001
- [41] Petrelli F, Pucci L, Bezzi P. Astrocytes and microglia and their potential link with autism spectrum disorders. *Frontiers in Cellular Neuroscience*. 2016;**10**:21
- [42] Habbas S et al. Neuroinflammatory TNF α impairs memory via astrocyte signaling. *Cell*. 2015;**163**(7):1730-1741
- [43] Murphy KM et al. Development of human visual cortex: A balance between excitatory and inhibitory plasticity mechanisms. *Developmental Psychobiology: The Journal of the International Society for Developmental Psychobiology*. 2005;**46**(3):209-221
- [44] Jimenez-Espinoza C, Serrano FM, González-Mora J. Glutamate Dysregulation in Cingulated Cortices Is Associated with Autism Spectrum Disorder Traits. 2024
- [45] Hynd MR, Scott HL, Dodd PR. Glutamate-mediated excitotoxicity and neurodegeneration in Alzheimer's disease. *Neurochemistry International*. 2004;**45**(5):583-595
- [46] Estrada-Sánchez AM et al. Glutamate toxicity in the striatum of the R6/2 Huntington's disease transgenic mice is age-dependent and correlates with decreased levels of glutamate transporters. *Neurobiology of Disease*. 2009;**34**(1):78-86
- [47] Caudle WM, Zhang J. Glutamate, excitotoxicity, and programmed cell death in Parkinson disease. *Experimental Neurology*. 2009;**220**(2):230-233
- [48] Young KW et al. Different pathways lead to mitochondrial fragmentation during apoptotic and excitotoxic cell death in primary neurons. *Journal of Biochemical and Molecular Toxicology*. 2010;**24**(5):335-341
- [49] Murphy DGM et al. Asperger syndrome: A proton magnetic resonance spectroscopy study of brain. *Archives of General Psychiatry*. 2002;**59**(10):885-891
- [50] Cacciaguerra L et al. Neuroimaging features in inflammatory myelopathies: A review. *Frontiers in Neurology*. 2022;**13**:993645
- [51] Bertrand D, Wallace TL. A review of the cholinergic system and therapeutic approaches to treat brain disorders. *Behavioral Pharmacology of the Cholinergic System*. 2020:1-28

- [52] Mesulam M-M. The cholinergic innervation of the human cerebral cortex. *Progress in Brain Research*. 2004;**145**:67-78
- [53] Chan AS et al. Disordered connectivity associated with memory deficits in children with autism spectrum disorders. *Research in Autism Spectrum Disorders*. 2011;**5**(1):237-245
- [54] Robillard R et al. Lower in vivo myo-inositol in the anterior cingulate cortex correlates with delayed melatonin rhythms in young persons with depression. *Frontiers in Neuroscience*. 2017;**11**:336
- [55] Pettem KL et al. Interaction between autism-linked MDGAs and neuroligins suppresses inhibitory synapse development. *Journal of Cell Biology*. 2013;**200**(3):321-336
- [56] Sani A, Fathiah N, et al. Gene expression profile in different age groups and its association with cognitive function in healthy Malay adults in Malaysia. *Cells*. 2021;**10**(7):1611
- [57] Tay TL et al. Microglia across the lifespan: From origin to function in brain development, plasticity and cognition. *The Journal of Physiology*. 2017;**595**(6):1929-1945
- [58] Southwick JS et al. Memory functioning in children and adolescents with autism. *Neuropsychology*. 2011;**25**(6):702
- [59] Leech R, Braga R, Sharp DJ. Echoes of the brain within the posterior cingulate cortex. *Journal of Neuroscience*. 2012;**32**(1):215-222
- [60] Mazengenya P, Bhikha R. An analysis of the structural and functional anatomy of the spine as viewed by Avicenna (980—1037 AD). *Research Journal of Medical Sciences*. 2016;**1**:676-682
- [61] James SJ et al. Metabolic biomarkers of increased oxidative stress and impaired methylation capacity in children with autism. *The American Journal of Clinical Nutrition*. 2004;**80**(6):1611-1617
- [62] Melnyk S et al. Metabolic imbalance associated with methylation dysregulation and oxidative damage in children with autism. *Journal of Autism and Developmental Disorders*. 2012;**42**:367-377
- [63] Rose S et al. Evidence of oxidative damage and inflammation associated with low glutathione redox status in the autism brain. *Translational Psychiatry*. 2012;**2**(7):e134-e134
- [64] Jiménez-Espinoza C, Serrano FM, González-Mora J. Imbalance Glutathione Biosynthesis in ASD: A Kinetic Pattern “in Vivo”. 2018
- [65] Rossignol DA, FRYE, Richard E. Evidence linking oxidative stress, mitochondrial dysfunction, and inflammation in the brain of individuals with autism. *Frontiers in Physiology*. 2014;**5**:150
- [66] Rossignol DA, Genuis SJ, Frye RE. Environmental toxicants and autism spectrum disorders: A systematic review. *Translational Psychiatry*. 2014;**4**(2):e360-e360
- [67] Baron-Cohen S et al. 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**:5-17
- [68] Neale JH et al. The neurotransmitter N-acetylaspartylglutamate in models of pain, ALS, diabetic neuropathy, CNS injury and schizophrenia.

Trends in Pharmacological Sciences.
2005;**26**(9):477-484

[69] Tsai S-J. Central N-acetyl
aspartylglutamate deficit: A possible
pathogenesis of schizophrenia.
Medical Science Monitor.
2005;**11**(9):HY39-HY45

[70] Buonomano DV, Merzenich MM.
Cortical plasticity: From synapses to
maps. Annual Review of Neuroscience.
1998;**21**(1):149-186

[71] Debanne D et al. Brain plasticity and
ion channels. Journal of Physiology-Paris.
2003;**97**(4-6):403-414

[72] Jiménez-Espinoza C, Marcano
Serrano F, González-Mora JL.
N-acetylaspartyl-glutamate metabolism
in the cingulated cortices as a biomarker
of the etiology in ASD: A 1H-MRS model.
Molecules. 2021;**26**(3):675

[73] Rademacher J et al. Human cerebral
cortex: Localization, parcellation,
and morphometry with magnetic
resonance imaging. Journal of Cognitive
Neuroscience. 1992;**4**(4):352-374

[74] Chen Z et al. Neuroplasticity of
children in autism spectrum disorder.
Frontiers in Psychiatry. 2024;**15**:1362288