Autism and Fragile X: Is There a Neurochemical Link?

Nagwa A. Meguid1, Hazem M. Atta2, Laila A. Rashed3, Amr S. Gouda4, Rehab O. Khalil1, Adel F. Hashish1†

1Department of Research on Children with Special Needs, National Research Center, Cairo, Egypt; 2Clinical Biochemistry Department, King Abdulaziz University, Rabigh Branch, Jeddah, 21589, Kingdom of Saudi Arabia, and 3Unit of Biochemistry and Molecular Biology, Medical Biochemistry Department, Faculty of Medicine, Cairo University, Kasr El Aini, Cairo 11562, Egypt; 4Unit of Biochemistry and Molecular Biology, Medical Biochemistry Department, Faculty of Medicine, Cairo University, Kasr El Aini, Cairo 11562, Egypt; 5Division, Elbehoos street Dokki, GIZA 12622, Egypt.

Key words: Autism; Fragile X Syndrome; Neurotransmitters; Serotonin; GABA; Glutamate.

Correspondence: Dr. Adel Ferig Hashish, National Research Centre, Children with Special Needs, Medical Division, Elbehoos street Dokki, GIZA 12622, Egypt. Phone: +202/105955294; Fax: +202/7844922; E-Mail: adelferig@yahoo.com

Citation: Meguid NA, Atta HM, Rashid LA, Gouda AS, Khalil RO, Hashish AF. Autism and Fragile X: Is There a Neurochemical Link? Maced J Med Sci. 2014 Dec; 15(4):551-556. http://dx.doi.org/10.3889/MJMS.1857-5773.2014.0420.

BACKGROUND: Autism and Fragile X syndrome are intertwined. This study aimed at assessing Serotonin, Glutamate, and Gama Amino Butyric Acid (GABA) in autism and Fragile X syndrome patients and to detect possible neurochemical similarities between the 2 disorders that can be used as metabolic biomarkers.

DESIGN AND METHODS: Eighty subjects divided into four groups, two diseased groups (20 male patients with Autism and 20 males with Fragile X syndrome) and two control groups (20 neurotypical male controls and 20 Down syndrome male patients) were included. Estimation of Serotonin, Glutamate and GABA were done using Enzyme linked Immunosorbent Assay (ELISA), Tandem Mass Spectrometry and high-pressure liquid chromatography (HPLC), respectively.

RESULTS: Serotonin was, exclusively, significantly low in autistic children. GABA was significantly high in both autistic and Fragile X children only, but not in Down syndrome children. Glutamate was significantly high in children with autism, Fragile X and Down syndrome children.

CONCLUSIONS: Autism and Fragile X syndrome share some neurochemical similarities with regards of high Glutamate and GABA levels while Serotonin was significantly different in the 2 disorders and may be used as a unique biomarker for autism.
Basic Science

1:291, respectively) in the total population. The prevalence of female carriers of the premutation in the normal population was 34.4 (95% CI: 8.9–60.3) per 10,000, or 1:291 (Hunter et al., 2014) [6].

Fragile X and autism are closely related. Two to seven percent of children have a mutation in Fragile X Mental Retardation 1 (FMR1) gene [7]. In the mean time, about 15-30% of Fragile X children have autistic features [8]. Fragile X mental retardation protein (FMRP) regulates the translation of neuroligins, neurorexins, SHANK, PTEN, CYFIP, and PSD95 proteins, which are associated with autism [7]. FMR1 gene regulates the expression of other genes and affect synaptic formation and plasticity [9]. It is a neuronal and gonadal protein with key roles in neuroplasticity and neuronal translation (Soden et al., 2010) [10]. Therefore, decline or absence of FMRP, may be associated with significant alterations indifferent proteins that affect brain development, ending in developmental disorders, particularly autism (Darnell, 2011) [11].

Several studies have determined different neurotransmitters in children with autism; however, only one study in 1984 has estimated serotonin level in Fragile X syndrome patients with and without autism. Here, we evaluate 3 neurotransmitters in the 2 intertwined disorders, autism and Fragile X Syndrome. To our knowledge, this is the first study that estimates Glutamate and GABA levels in Fragile X compared to autistic, Down Syndrome (DS) and neurotypical children.

This study aimed at detection of neurobiological similarities between Autism and FXS so targeted treatment currently being studied in FXS can be applied to autistic patients. Also assessment of these neurotransmitters could be used as metabolic biomarkers for Autism or FXS.

Subjects and Methods

The present study included 80 subjects divided into 4 groups, 2 diseased groups (20 male patients with Autism and 20 male with FXS) and 2 control groups (20 neurotypical male controls and 20 Down syndrome male patients). All the children enrolled in the present study aged from 3-11 years old. Autism, FXS and DS patients were selected from The Autism Disorders Clinic, Medical Research Center of Excellence, National Research Center (NRC), Cairo, Egypt. The ethical approval of the NRC ethical committee was obtained prior to the study and a written informed consent was provided by the parents of autistic children.

All patients were subjected to careful pedigree construction and complete diagnostic workup including medical, psychiatric, psychological and neurological evaluation. Autistic children were clinically diagnosed with Autism Diagnostic Interview Revised (ADI-R) [12] and degree of severity was assessed by Childhood autism rating scale (CARS) [13]. Nine autistic patients out of 20 were classified as having severe autism, while the other 11 were having mild to moderate autism. Fragile X syndrome patients were diagnosed clinically by Hagerman checklist [14] and in the laboratory by DNA Fragile X test. Fragile X patients were classified according to the presence of autistic features into 6 with autistic features and 14 without autistic features. Down syndrome patients were subjected to karyotyping. Exclusion criteria included neurological, metabolic, endocrine, cardiovascular, pulmonary, liver, kidney or other medical disease.

Metabolic biomarkers as Serotonin, GABA and Glutamate were estimated in the 4 groups. The aim of including DS as a control group was to see if these metabolic biomarkers are specific to certain disease as Fragile X disease and autism or it is present in other disorders associated with mental retardation as DS.Three ml of venous blood samples were taken from the children on EDTA (Ethylene Diamine Tetra Acetate) vacuum tubes. Serotonin assay was done using Serotonin ELISA kit (DIA source Immunoassy S.A. Rue de Industrie 8, 1400 Nivelles, Belgium). Calibration curve was obtained by plotting the absorbance readings against the corresponding standard concentrations. Plasma GABA levels were measured by high-performance liquid chromatography-electrochemical method [15]. Calibration curve was drawn using four standard solutions of GABA. Glutamate was assayed from dried blood spots using Mass Spectrometry/ Mass Spectrometry (MS/MS) The detector monitors the ion current, amplifies it and the signal is then transmitted to the data system where it is recorded in the form of mass spectra [16].

Analysis of results was performed using statistical package for social science (SPSS) software(SPPS Inc., Chicago, IL, USA) , version 15 for Microsoft Windows. Multivariate analysis was used to compare between the different groups [17].

Results

Autistic children had a significantly very low serotonin level when compared to the 3 other groups (Fragile X Syndrome, DS and neurotypical children) (Table 1). On the contrary, FXS syndrome showed significantly high levels when compared to patients with autism, DS and normal healthy children. Down syndrome cases showed lower serotonin levels compared to neurotypical children but this difference was not statistically significant.

The second neurotransmitter assayed was Glutamate (Table 2). Autism cases showed
significantly high levels of Glutamate compared to other groups (FXS, DS, and normal controls). Meanwhile, cases with FXS demonstrated significantly higher glutamate levels compared to DS patients and normal healthy controls. On the other hand, when the 2 control groups were compared to each other, DS children had significantly higher Glutamate levels compared to neurotypical children.

| Neurotransmitters | Mild-Moderate Autism | Severe Autism | P Value |
|-------------------|---------------------|--------------|---------|
| Serotonin (ng/ml) | 73.11 ± 40.87       | 85.54 ± 55.18| P<0.05 |
| Glutamate (µmol/L)| 1.48 ± 43.94        | 1.76 ± 13.21 | P<0.05 |
| GABA (ng/ml)      | 1.35 ± 24.94        | 1.03 ± 32.84 | P<0.05 |

Table 2: Glutamate levels in the different studied groups (µmol/L).

GABA level in autism was significantly higher (117.90 ± 33.13) when compared to the neurotypical children (78.75 ± 21.63) and DS (66.40 ± 27.47) (Table 3). Also, GABA in FXS children was also significantly higher (125.35 ± 49.19) when compared to neurotypical children and DS children. Interestingly, FXS children had higher GABA levels than children with autism; however, this difference was not statistically significant. When the 2 control groups were compared to each other, DS children showed lower GABA levels (66.40 ± 27.47) than control group (78.75 ± 21.63). However, this difference was not statistically significant.

Table 3: GABAA levels in the different studied groups (ng/ml).

Table 4: Neurotransmitters in mild-moderate and severe autism.

Discussion

The present study evaluated 3 neurotransmitters in autism compared to Fragile X Syndrome to assess the possibility of neurochemical similarities between the 2 disorders. Autism and Fragile X syndrome have an overlap at the molecular level [18]. Proteins that regulate the balance of activity in brain glutamate and GABA systems, including PSD95 and Arc were found to be defective. These proteins are directly regulated by FMRP [18].

Previous studies have estimated different neurotransmitters in autism compared either to their relatives or normal healthy children. To our knowledge, this is the first study that evaluated Serotonin, Glutamate and GABA in autism compared to Fragile X syndrome; also it is first study to evaluate Glutamate and GABA in Fragile X Syndrome.

The most studied neurotransmitter in autism is Serotonin which is related to many human behaviors including mood, sleep, appetite and memory. An early study in 1961 showed an increased levels of endogenous 5-HT in whole blood in 26% of autistic children [19]. These results have also been confirmed by other studies [20].

In the present study, significantly low levels of plasma serotonin were detected in autism when compared to the other 2 groups (Fragile X Syndrome, and neurotypical children). These results are in agreement with those of Spivak et al., [21] who estimated plasma serotonin in 10 autistic children compared to 12 neurotypical children and concluded...
that low plasma serotonin levels may have a role in the pathophysiology and symptomatology of autism. However another study in Saudi Arabia [22] detected high plasma serotonin levels in 16 Saudi autistic children compared to 16 neurotypical controls and concluded that high lead level is associated with remarkable high levels of serotonin.

The detection of low plasma serotonin levels in our study can be explained by the possibility of enhanced serotonin accumulation in platelet [23] that subsequently associated with increased platelet serotonin levels and parallel-diminished free plasma serotonin levels. Also low cerebrospinal fluid levels of 5- hydroxyindoleaceticacid [24] and low serotonin urinary excretion rate in autism [25] supports our findings.

High significant levels of serotonin in Fragile X were detected in present study compared to autism, neurotypical children and D.S. Another study reported normal serotonin levels in 12 Fragile autistic children out of 13 and only one had low serotonin levels [26].

High serum glutamate levels were reported in autism compared to normal controls [27, 28]. Plasma amino acid analyses were carried out on a recent cohort of 138 autistic children and 138 normal controls using reverse-phase HPLC, it concluded that elevated levels of excitatory amino acids (glutamate and asparagine), decreased essential amino acids (phenylalanine, tryptophan and methionine) and decreased precursors of neurotransmitters (tyrosine and tryptophan) are the distinct characteristics of plasma amino acid profile of autistic children (Naushad et al., 2013) [29].

Another study also reported high levels of glutamate and homocystein in autism while the levels of glutamine and tryptophan are decreased (Ghanizadeh, 2013) [30].

Our study replicated these results and could explain some clinical symptoms accompanied with autism as seizures and Electroencephalogram (EEG) abnormalities. Valproic acid is a mood stabilizer and was found to be effective intreating autistic patients with or without clinical seizures but with epileptiform abnormalities [31].This explains the high rates of seizure disorders in autism that are due to high glutamate levels.

Increased glutamate levels in autism could be explained by presence of low levels of Pyridoxal Phosphate (PLP) in children with autism due to very low activity of pyridoxal kinase leading to blockage of glutamate utilization [32]. Also, alterations in the glutamate transporter or in glutaminase or glutamine synthetase activity would potentially affect glutamate levels [33].

Few studies had been conducted to assess the Glutamate in Fragile X syndrome, one of these studies was by Gruss and Braun [34] who reported unaltered Glutamate in all regions of brain in both ages (Juvenile and Adult). The present study reported high significant levels of Glutamate in autism compared to neurotypical children and to D.S.

The final neurotransmitter that was assessed in this study was GABA which is known to play a crucial role in synaptic tuning and neuronal wiring in late pre and early postnatal days (Ben Ari et al., 2012) [35]. Studies from animal models of ASDs indicate that a dysfunction in GABAergic signaling within particular neuronal circuits may account for most of the clinical symptoms found in autistic patients. The high co-morbidity of ASDs with epilepsy (30% of cases) further confirms this issue (Frye et al., 2013) [36].

Plasma GABA was significantly high in autistic children compared to neurotypical and Down syndrome. Our results were in concordant with Dhossche et al. [37]. However, they detected significantly higher plasma GABA levels in 9 youngsters with autism than in 9 youngsters with Attention Deficit Hyperactivity Disorder (ADHD). Several studies also supported the high GABA levels in autism. A study had been conducted in Saudi Arabia showed high levels of lead and GABA in plasma [22]. Increased plasma GABA levels were found to be associated with reduced numbers of neurons expressing GABA and low brain GABA levels [38]. Divalproex is effective in treating autistic children who showed improved behavior due to elevation of brain GABA by inhibiting catabolic enzyme of GABA (GABA Transaminase) [39].

Some clinical observations have been added to support the role of GABA in autism [37]. There is increasing evidence that GABA neuronal dysfunction is implicated in various psychiatric disorders including schizophrenia, mood disorders, and anxiety disorders [40]. In some disorders, GABA dysfunction may occur in conjunction with abnormalities in reelin, a glycoprotein involved in the developmental regulation of GABAergic transmission Reductions of reelin in the cerebellar cortex of people with autism have also been reported [41]. Abnormalities on the long arm of chromosome15 have been found in a small proportion of autistic people. A cluster of genes coding for GABA A receptor subunits have been identified in that location (chromosome15q11-13). The GABA receptor beta-3 subunit gene has been implicated as an autism susceptibility locus in previous genetic studies, although the evidence is far from conclusive [42]. It is possible that in a subgroup of autistic people, GABA dysfunction is present in some brain areas owing to abnormalities of the assembly of GABA A receptor subunits into the GABA receptor complex. Thirdly, a few reports have suggested that benzodiazepines, i.e., positive modulators of GABA metabolism, have a negative and even paradoxical effect in autistic people.
Finally, the present study detected high significant levels of GABA in Fragile X children compared to normal healthy children and to D.S. Previous study [34] reported that GABA was significantly higher in the brainstem of FMR1 knockout mice than in wild type.

The involvement of GABAA receptors in ASDs was provided by genetic studies that have revealed submicroscopic abnormalities known as “copy-number variations” in chromosomal loci 15q11–q13, which contains a number of genes encoding for GABAA receptor subunits Coghlan et al., 2012 [43].

It became more evident that autism and Fragile X Syndrome share a lot on the molecular and biochemical levels, so treatments that are used for FXS can also be beneficial for some autistic patients. There are large list of genes implicated in autism which can be mapped onto specific brain pathways [18]. Fragile X syndrome and autism are intertwined. Up regulation of mGlu R 5 pathways was reported in Fragile X children and nowadays treatment are aiming at reversing these problems [44]. These treatment can also be very useful for some children who are having autism.

In conclusion, the present study showed that low serotonin levels was exclusively and unique to autistic patients and it can be consider as a metabolic marker for autism. Also we found that autism and FXS share neurobiological similarities as GABA was significantly high in both disorders than neurotypical children, meanwhile it was specific to Autism and FXS as GABA level was not high in D.S. On the contrary DS showed lower (non significant) GABA levels than neurotypical children. With regards Glutamate, it was high in the 3 disorders but with the highest levels in Autism followed by FXS and DS, respectively.

References

1. American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders. 4th ed. Arlington, VA: American Psychiatric Publishing, 2000.

2. Developmental Disabilities Monitoring Network Surveillance Year 2010 Principal Investigators; Centers for Disease Control and Prevention (CDC). Prevalence of autism spectrum disorder among children aged 8 years - autism and developmental disabilities monitoring network, 11 sites, United States, 2010. MMWR Surveill Summ. 2014;63(2):1-21.

3. Hallmayer J, Cleveland S, Torres A et al. Genetic heritability and shared environmental factors among twin pairs with autism. Arch Gen Psychiatry. 2011; 68:1095–102.

4. Novarino G, El-Fishawy P, Kayserili H et al. Mutations in BCKD-kinase lead to a potentially treatable form of autism with epilepsy. Science. 2012; 338:394-7.

5. McLennan, Y., Polussa, J., Tassone, F. et al. Fragile X syndrome. Curr Genomics. 2011; 12: 216–224.

6. Hunter J, Rivero-Arias O, Angelov A, et al. Epidemiology of fragile X syndrome: A systematic review and meta-analysis. Am J Med Genet. 2014; 164A:1648–1658.

7. Hagerman RJ, Rivera SM, Hagerman PJ, Hagerman, Fragile X and autism: Intertwined at the molecular level leading to targeted treatments. Molecular Autism. 2010; 1:12.

8. Harris SW, Hessl D, Goodlin-Jones B. Autism profiles of males with fragile X syndrome, Am J Mental Retardation. 2008; 113:427–38.

9. Bassell GJ, Warren ST. Fragile X syndrome: loss of local mRNA regulation alters synaptic development and function. Neuron. 2008; 2:201–14.

10. Soden, M.E., and Chen, L. Fragile X protein FMRP is required for homeostatic plasticity and regulation of synaptic strength by retinoic acid. J Neurosci. 2010; 30:16910–16921.

11. Dalnelli, J.C., Van Driesche, S.J., Zhang, C, et al. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. Cell. 2011; 146, 247–261.

12. Rutter M, Couteur A, Lord C, eds. Autism Diagnostic Interview Revised (ADI-R), 2003.

13. Schopler E, Reichler RJ, Renner BR. Childhood Autism Rating Scale. Los Angeles, CA: Western Psychological Services, 1999.

14. Hagerman, R. J., Amiri, K. and Cronister, A. Fragile X checklist. American Journal of Medical Genetics. 1991;38: 283–287.

15. de Freitas Silva DM, Ferraz VP et al. J Neurosci Methods. 2009;2:289-93.

16. Zoppa M, Gallo L, Zacchello F et al., Method for the quantification of underivatized amino acids on dry blood spots from newborn screening by HPLC ESI-MS/MS. Journal of Chromatography B. 2006; 831:267-273.

17. David HA, Gunnink, Jason L. The Paired t Test under Artificial Pairing", The American Statistician. 1997; 51:9–12.

18. Wang LW, Berry-Kravis E, Hagerman RJ, Fragile X. leading the way for targeted treatments in autism. Neurotherapeutics. 2010; 3:264-74.

19. Schain R, Freedman D. Studies of Hydroyxindole metabolism in autistic and other mentally retarded children. J Pediatr. 1981;98:315-320.

20. Hrnčířová D, Bujas-Petkovic Z, Vragovic R et al. Hyperserotonemia in adults with autistic disorder. J Autism Dev Disord. 2007;37:1934-40.

21. Spivak B, Golubchik P, Mozes T et al. Low Platelet-Poor Plasma Levels of Serotonin in Adult Autistic Patients. Neuropsychobiology. 2004; 50:157-160.

22. El-Ansary AK, Bacha AB, Ayahdi LY. Relationship between chronic lead toxicity and plasma neurotransmitters in autistic patients from Saudi Arabia. Clin Biochem. 2011; 44:116-20.

23. Reichelt KL, Knivsberg AM. Can the pathophysiology of autism be explained by the nature of the discovered urine peptides? Nutr Neurosci. 2003; 6:19–28.

24. Cohen DJ, Shaywitz BA, Johnson WT et al. Biogenic amines in autistic and atypical children. Arch Gen Psychiatry. 1974; 31:845–853.

25. Narayan M, Srinath S, Anderson GM et al. Cerebrospinal fluid levels of homovanillic acid and 5-hydroxyindoleacetic acid in autism. Biol Psychiatry. 1993; 33:630-5.

26. Jackson A3rd, Hogerman R, Levitas A. Serotonin levels in fragile X autistic patients. J Autism Dev Disord. 1984;4:451-2.

27. Moreno-Fuenmayor H, Borjas L, Arrieta A et al. Plasma excitatory amino acids in autism. Invest Clin. 1996; 2:113-28.

28. Shinohe A, Hashimoto K, Nakamura K et al. Increased serum levels of glutamate in adult patients with autism. Prog Neuropsychopharmacol Biol Psychiatry. 2006; 30:1472-7.
29. Naushad SM, Jain JM, Prasad CK et al. Autistic children exhibit distinct plasma amino acid profile. Indian J Biochem Biophys. 2013;50(5):474-8.

30. Ghanizadeh A. Increased glutamate and homocysteine and decreased glutamine levels in autism: a review and strategies for future studies of amino acids in autism. Dis Markers. 2013;35(5):281-6.

31. Tuchman R, Rapin I. Epilepsy in autism. Lancet Neurol. 2002; 1:352–5.

32. Adams JB, George F, Audhya T. Abnormally high plasma levels of vitamin B6 in children with autism not taking supplements compared to controls not taking supplements. J Altern Complement Med. 2006;12(1):59-63.

33. Aldred S, Moore KM, Fitzgerald M et al., Plasma amino acid levels in children with autism and their families. J Autism Dev Disord. 2003;33:93–7.

34. Gruss M, Braun K. Alterations of amino acids and monoamine metabolism in male Fmr1 knockout mice: a putative animal model of the human fragile X mental retardation syndrome. Neural Plast. 2001; 8:285-98.

35. Ben-Ari Y, Woodin MA, Sernagor E et al. Refuting the challenges of the developmental shift of polarity of GABA actions: GABA more exciting than ever! Front Cell Neurosci. 2012; 6:35.

36. Frye RE, Rossignol D, Casanova MF et al. A review of traditional and novel treatments for seizures in autism spectrum disorder: findings from a systematic review and expert panel. Front Public Health. 2013; 1:31.

37. Dhosche D, Applegate H, Abraham A et al. Elevated plasma gamma-aminobutyric acid (GABA) levels in autistic youngsters: stimulus for a GABA hypothesis of autism. Med Sci Monit. 2002;8:1-6.

38. Schmitz C, van Kooten I, Hof PR et al. Autism: neuropathology, alterations of the GABAergic system, and animal models. Int Rev Neurobiol. 2005; 71:1-26.

39. Hollander E, Dolgoff-Kaspar R, Cartwright C, Rawitt R, Novotny S. An open trial of divalproex sodium in autism spectrum disorders. J Clin Psychiatry. 2001;62(7):530-4.

40. Goddard A, Mason G, Almai A. Reductions in occipital cortex GABA levels in panic disorder detected with 1H-magnetic resonance spectroscopy. Arch Gen Psychiatry. 2001; 58:556-61.

41. Fatemi S, Earle J, McMenomy T. Reduction in Reelin immunoreactivity in hippocampus of subjects with schizophrenia, bipolar disorder and major depression. Molecular Psychiatry. 2000; 5:654-63.

42. Martin E, Menold M, Wolpert C et al. Analysis of linkage disequilibrium in gamma-aminobutyric acid receptors subunit genes in autistic disorder. Am J Med Genet. 2000; 96:43-8.

43. Coghill C, Horder J, Inkster B, et al. GABA system dysfunction in autism and related disorders: from synapse to symptoms. Neurosci Biobehav Rev. 2012; 36:2044–55.

44. Berry-Kravis E, Knox A, Hervey C. Targeted treatments for fragile X syndrome. J NeurodevDisord. 2011; 3:193-210.