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Metabotropic glutamate receptor 5 upregulation in children with autism is associated with underexpression of both Fragile X mental retardation protein and $GABA_A$ receptor beta 3 in adults with autism

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Abstract

Recent work has demonstrated the impact of dysfunction of the GABAergic signaling system in brain and the resultant behavioral pathologies in subjects with autism. In animal models, altered expression of Fragile X mental retardation protein (FMRP) has been linked to downregulation of GABA receptors. Interestingly, the autistic phenotype is also observed in individuals with Fragile X syndrome. This study was undertaken to test previous theories relating abnormalities in levels of FMRP to $GABA_A$ receptor underexpression. We observed a significant reduction in levels of FMRP in the vermis of adults with autism. Additionally, we found that levels of metabotropic glutamate receptor 5 (mGluR5) protein were significantly increased in vermis of children with autism vs. age and postmortem interval (PMI) matched controls. There was also a significant decrease in level of $GABA_A$ receptor beta 3 (GABRβ3) protein in vermis of adult subjects with autism. Finally, we found significant increases in glial fibrillary acidic protein (GFAP) in vermis of both children and adults with autism when compared with controls. Taken together, our results provide further evidence that altered FMRP expression and increased mGluR5 protein production potentially leads to altered expression of $GABA_A$ receptors.

Keywords

FMRP; mGluR5; GABRβ3; autism; vermis

Introduction

Autism is a neurodevelopmental disorder first systematically described by Leo Kanner based purely on behavioral observations (Kanner, 1943; Realmuto and Azeem, 2008). It is characterized by ritualized or stereotyped behavior, deficits in communication, and abnormalities in social interaction (APA, 1994). Recent evidence indicates an increased

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incidence of autism of unknown origin (Fombonne et al., 2006). A resurgence of interest in identifying the biologic underpinnings of this debilitating disease has been helped by the availability of a well characterized set of postmortem brains belonging to subjects with autism, matched against a cohort of normal control brains, resulting in a number of important, well replicated findings (Blatt et al., 2001, Fatemi and Halt 2001; Perry et al., 2001; Purcell et al., 2001; Fatemi et al., 2002a, 2005, 2009a,b, 2010; Araghi-Niknam and Fatemi, 2003; Palmen et al., 2004; Laurence and Fatemi, 2005; Yip et al., 2007; Oblak et al., 2009).

One of the important recent findings in autism deals with the abnormality of GABAergic neurotransmission. Our laboratory has shown a number of salient findings describing a major deficit with the GABAergic system in autism over the past decade including: significant downregulation of glutamic acid decarboxylase 65 and 67 kDa (GAD65 and GAD67) proteins, the rate limiting enzymes that convert glutamate to GABA (Fatemi et al., 2002a); reduction in blood and brain levels of Reelin glycoprotein (Fatemi et al., 2002b, 2005); and global reductions in $GABA_A$ and $GABA_B$ receptors in three brain areas in autism (Fatemi et al., 2009a,b, 2010). These abnormalities have now been replicated by other laboratories showing the validity of these observations (Blatt, 2005; Yip et al., 2007; Garbett et al., 2008; Oblak et al., 2009).

The autistic phenotype is also seen to occur in a number of other disorders including tuberous sclerosis, Asperger syndrome, and specifically, Fragile X syndrome (FXS). FXS is one of the most common causes of inherited mental retardation with a prevalence of 1:4,000 in males and 1:8,000 in females (Crawford et al., 1999; Oostra and Willemsen, 2009). FXS is caused primarily by expansion of a CGG repeat in the 5’ untranslated region of the FMR1 gene, which is located on the X chromosome (Oostra and Willemsen, 2009). Indeed, FXS may be considered the first example of a trinucleotide repeat expansion mutation (Oostra and Willemsen, 2009).

The Fragile X mental retardation protein (FMRP) is an RNA-binding protein (De Rubeis and Bagni, 2010). FMRP shuttles between the nucleus and cytoplasm and is involved in regulation of multiple steps in post-transcriptional events such as splicing, nuclear export, stability, and localization and translation of various RNAs (Keene, 2007; De Rubeis and Bagni, 2010). Any major abnormality in function of FMRP may profoundly affect control of multiple downstream genes leading to a group of pathologies. For example, expansion of the trinucleotide repeat in the 5’ untranslated region of the FMR1 gene leads to transcriptional silencing of this gene and loss of FMRP expression leading to Fragile X syndrome which is well characterized by mental retardation and autistic behavior. A variant form of abnormalities in FMRP expression is also associated with Fragile X associated tremor-ataxia syndrome (FXTAS), which is characterized by progressive ataxia and intention tremor (De Rubeis and Bagni, 2010). FMRP is highly expressed in the brain and mainly localized to the cytoplasm and, at lower levels, to the nucleus of the neuron (Feng et al., 1997). Additionally, FMRP can be observed not only in neuronal soma but localized along the dendrites and the base of synaptic spines and in axonal growth cones as well as in mature axons (Antar et al., 2004; Centonze et al., 2008; De Rubeis and Bagni, 2010). During the passage of FMRP from neuronal soma to synapse, it partners with a number of proteins, various mRNAs and even non-coding RNAs giving rise to a large complex known as the messenger ribonucleoprotein particle (mRNP) which upon reaching the synapse is translationally unrepressed and released via neuronal stimulation leading to change in spine morphology and ultimately affecting synaptic plasticity (Dölen and Bear, 2008).

Several recent intriguing publications have reported on decreased expression of multiple $GABA_A$ receptor mRNA species ($\alpha 1$, $\alpha 3$, $\alpha 4$, $\beta 1$, $\beta 2$, $\gamma 1$, $\gamma 2$, δ) in Fragile X mice, a

validated model for Fragile X mental retardation syndrome (El Idrissi et al., 2005; D'Hulst et al., 2006; Gantois et al., 2006). Additionally, D'Hulst et al., (2006) also found significant reduction in three $GABA_A$ receptors in Fragile X *Drosophila melanogaster*. These authors posited that the global underexpression of $GABA_A$ receptors in these animal models could be an evolutionarily conserved hallmark of Fragile X syndrome (D'Hulst et al., 2006).

Previous reports have shown presence of autistic behavior in 15-33% of patients with FXS (Cohen, 1995; Bailey et al., 2000; Kauffman et al., 2004; Hatton et al., 2006). Indeed, Hatton et al., (2006) suggested that lower levels of FMRP expression may be the contributory cause of autistic behavior and intellectual deficits in children with Fragile X syndrome (Hatton et al., 2006). By the same token, Gothelf et al., (2008) recently reported a correlation between lower levels of FMRP, aberrant behavior with various abnormalities in brain size (increased size of caudate nucleus and decreased size of posterior cerebellar vermis, amygdala and superior temporal gyrus) in a group of FXS patients. We therefore decided to investigate for the first time whether FMRP levels could be lower in the vermis of children and adults with autism as compared to age and postmortem interval (PMI) matched control subjects. This study was also undertaken to buttress the previous theories relating abnormalities in levels of FMRP to $GABA_A$ receptor underexpression observed by us and others in brains from subjects with autism.

Materials and Methods

Tissue Preparation

All experimental procedures were approved by the Institutional Review Board of the University of Minnesota School of Medicine. Postmortem blocks of vermis were obtained from the Autism Research Foundation and various brain banks (NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD; TARF; the Harvard Brain Tissue Resource Center, which is supported in part by PHS grant number R24 MH068855; the Brain Endowment Bank, which is funded in part by the National Parkinson Foundation, Inc., Miami, Florida; and the Autism Tissue Program). The tissue samples (Table 1) were prepared as described previously (Fatemi et al., 2009a,b, 2010).

SDS-PAGE and Western Blotting

Tissue samples from vermis (N=11 control, N=16 autistic), were prepared and subjected to SDS PAGE and western blotting as described previously (Fatemi et al., 2009a,b, 2010). The primary antibodies used were: anti-fragile X mental retardation protein (FMRP) (ab17722, Abcam (Cambridge, MA), 1:500), anti-metabotropic glutamate receptor 5 (mGluR5) (ab53090-100, Abcam Inc. (Cambridge, MA), 1:300), anti-$GABA_A$ receptor beta 3 (GABRβ3) (NB300-199, Novus Biologicals (Littleton, CO), 1:1,000), anti-glial fibrillary acidic protein (GFAP) (G3893, Sigma-Aldrich (St. Louis, MO), 1:2,000), anti-neuronal specific enolase (NSE) (ab16808, Cambridge, MA), 1:2,000), and anti-β actin (A5441, Sigma Aldrich (St. Louis, MO), 1:5,000). Secondary antibodies used were A9169 (Sigma Aldrich, (St. Louis, MO) goat anti-rabbit IgG 1:80,000) and A9044 (Sigma Aldrich, (St. Louis, MO) rabbit anti-mouse IgG 1:80,000). The molecular weights of approximately 224 kDa (dimer) and 112 kDa (monomer) for mGluR5; 73 kDa (FMRP); 52 kDa (GABRβ3). We have observed a range of molecular weights for GABRB3 including 52 kDa and 56 kDa which agrees with the expected molecular weight of 51-56 kDa according to the antibody data sheet from Novus Biologicals, Inc. and from previously published results (Bureau and Olsen, 1990; Sarto et al., 2002; Samaco et al., 2005); 50, 46, 42 and 38 kDa (GFAP; all bands measured together), 46 kDa (NSE), and 42 kDa (β-actin) immunoreactive bands were quantified with background subtraction. As many as 2–5 bands can be seen in some preparations of FMRP (private communication with Dr. J. Darnell of Rockefeller

University; Darnell et al., 2009) however, in our preparations we can distinctly see up to two clear bands generally, which were measured together. Results obtained were based on at least two independent experiments.

Statistical Analysis

Statistical analysis of protein data was performed as previously described (Fatemi et al., 2009a,b). We investigated the potential confounding effects of age, postmortem interval, and gender by examining group differences with postmortem interval as covariates. We further analyzed data based on stratification by age groups, i.e. adults (above age 18) and children (ages 18 and under). Analysis of medication and presence of seizures as confounds could not be tested when data were further stratified by age. While the impact of these factors could not be accurately verified, previous work by our group showed lack of such effects on GABAA receptor abnormalities in cerebellar tissues from a different group of subjects with autism (Fatemi et al., 2009a,b).

Results

All proteins of interest were normalized against both neuronal specific enolase (NSE) and β-actin and are shown as ratios of the various proteins to NSE and β-actin. We chose NSE as a housekeeping gene to account for any changes in neuronal number between subjects with autism and controls. In adults and children there were no significant differences in levels of NSE between subjects with autism and matched controls (Figure 1; Table 2). β-actin was also used as a housekeeping gene as to account for any differences in neurons and glia. Similar to NSE there were no significant differences in adults and children when comparing subjects with autism and matched controls (Figure 1; Table 3). These results indicate that any changes in proteins of interest are not due to overall differences in neuronal cell number.

FMRP was visualized as an approximately 73 kDa doublet. There was a significant 75% reduction of FMRP/NSE ($p<0.028$; Table 2) and a 75% significant reduction of FMRP/ β-actin ($p<0.038$; Table 3) in adults with autism when compared with controls (Figure 2). In contrast, there were no significant differences in FMRP expression in children despite a trend for a reduction in FMRP levels (Figure 2).

Metabotropic glutamate receptor 5 (mGluR5) appeared as a dimer at approximately 224 kDa and a monomer at 112 kDa (Figure 3). There was a significant 204% increase in the dimerized mGluR5/NSE ($p<0.0023$) and a 209% increase in total mGluR5/NSE ($p<0.0042$) in children with autism when compared with controls (Table 2, Figure 3). mGluR5/ β-actin dimer also displayed a significant 181% increase ($p<0.0084$) and total mGluR5/ β-actin was similarly significantly elevated by 232% ($p<0.0071$) in children with autism vs. controls (Table 3, Figure 3). There were no significant differences in mGluR5 expression in adults with autism vs. controls. We also measured the ratio of dimerized protein to total mGluR5 protein and found a significant increase in children with autism ($p<0.049$; Table 2).

Since FMRP expression has been shown to affect expression of $GABA_A$ receptors in animal models (El Idrissi et al., 2005; D'Hulst et al., 2006; Gantois et al., 2006), we measured $GABA_A$ receptor beta 3 (GABRβ3) levels in children and adults with autism vs. controls. We found a significant 37% reduction in GABRβ3 levels in adults with autism when compared with healthy controls ($p<0.031$ for GABRβ3/NSE; $p<0.05$ for GABRβ3/ β-actin; Tables 2 and 3; Figure 4). In contrast we did not find any differences in GABRβ3 levels between autistic and control children (Tables 2 and 3; Figure 4).

Finally, we also measured levels of glial fibrillary acidic protein (GFAP) as GFAP has previously been shown to be increased in multiple brain regions of subjects with autism

(Laurence and Fatemi, 2005; Vargas et al., 2005). We found a 967% increase in GFAP levels in vermis of adults with autism when compared with controls ($p<0.029$; Table 3; Figure 5). Likewise, we found a 128% increase in GFAP protein expression in vermis of children with autism when compared with controls ($p<0.031$; Table 3; Figure 5).

We examined the effects of confounds of age, sex, gender and PMI on our results and found no impact of these factors on levels of any proteins (data not shown).

Discussion

In the current study we have demonstrated for the first time significant reduction in levels of FMRP in the vermis of adults with autism as well as a nonsignificant reduction of FMRP in children with autism when compared with age and PMI matched controls. Additionally, we have shown for the first time that levels of mGluR5 protein is significantly increased in vermis of children with autism vs. age and PMI matched controls. Our results also show that levels of the same receptor are nonsignificantly reduced in adults with autism vs. controls. We have also demonstrated that the expression of mGluR5 in autistic children is significantly more homo-dimerized when compared with normal children. There was also a significant decrease in GABRβ3 in vermis of adult subjects with autism. Finally we found significant increases in GFAP in vermis of both children and adults with autism when compared with controls.

Significant reduction in FMRP in vermis of adults with autism is both novel and intriguing since none of the subjects used in this study had a diagnosis of FXS. This decrease in FMRP in vermis was also evident in children with autism, however, it did not reach statistical significance indicating that low power of our sample size may have contributed to this effect. The underexpression of FMRP in autistic subjects may be a universal event and could easily explain the presence of several potential endophenotypes that are shared between autism and FXS: 1) several autistic abnormalities including mental retardation, seizures, abnormal dendritic spine morphology, social anxiety, and reduced size of the cerebellar vermis (Hatton et al., 2006; Gothelf et al., 2008) are shared between both disorders; 2) valid animal models of FXS in mice and *Drosophila melanogaster* also show reduction in FMRP levels, $GABA_A$ receptor underexpression, behavioral, and glutamatergic receptor abnormalities (El Idrissi et al., 2005; D'Hulst et al., 2006; Gantois et al., 2006; Dölen et al., 2007); 3) Pak1 and Pak 3 (p21 associated tyrosine kinases) have been known to antagonize FMRP function (Hayashi et al., 2007) leading to changes in synaptic plasticity and abnormal spine morphology in animal models of FXS. These molecules may indeed be abnormal in autistic brain and their levels and functions are yet to be determined; 4) decreased FMRP levels have been associated with presence of increased colocalizable molecules such as calcium/calmodulin protein kinase II (CAMK2), activity regulated cytoskeleton-associated protein (ARC), and microtubule associated protein 1B (MAP1B) as well as homer (Antar et al., 2004; Lu et al., 2004; Irwin et al., 2005); 5) decreased FMRP is known to increase long term depression (LTD) (Bear et al., 2004) and increased epileptic discharges (Musumeci et al., 1999) as seen in autism; 6) decreased FMRP may also be associated with hypoplasia of cerebellar vermis especially since the same phenomenon has also been observed in subjects with FXS (Gothelf et al., 2008) who show a decrease in size of posterior cerebellar vermis; 7) multiple recent reports have also shown a decrease in size of cerebellar vermis in autism (Steinlin, 2008; Scott et al., 2009; Webb et al., 2009). All of these morphologic changes co-occurred with problems with language ability and cognitive abnormalities. Indeed, DeLorey et al., (2008) described GABRβ3 deficient mice who displayed hypoplasia of vermal lobules, and exhibited impaired exploratory and interactive behaviors, similar to what is observed in autism.

A second important and novel finding of the current study is the observation of significantly increased expression of mGluR5 in autistic children which has been unknown hitherto. It is quite interesting that the same receptor was non-significantly reduced in vermis of autistic adults when compared with controls. mGluR5 is a member of a group I metabotropic glutamate receptor system which modulates excitatory synaptic transmission and is involved in a number of important functions both during brain development and in adult life (Catania et al., 2007): 1) mGluR5 receptors are numerous at birth and show reductions in density later in life (Raol et al., 2001); 2) in rodents, mGluR5 receptors drop in number beyond postnatal day 18 and later in rat cerebellum (Romano et al., 1996); 3) mGlur5 receptors are present on stem cells that can give rise to neurons and glia and participate in basic developmental events that occur prior to synaptic formation such as during neuronal proliferation, differentiation and survival (Catania et al., 2007); 4) mGluR5 is also present on Cajal-Retzius cells, thus affecting the release of Reelin (Mienville, 1999; López-Bendito et al., 2002); 5) increase in mGluR5 in childhood may be responsible for early onset of seizures as seen in autism (Catania et al., 2007); 6) mGluR5 can protect against apoptosis action leading to increased cell number when activated (Copani et al., 1998); 7) Increased mGluR5 can lead to abnormal spine formation and abnormal synthesis of synaptic proteins most likely due to antagonism of FMRP (Grossman et al., 2006; Catania et al., 2007).

Abnormalities in expression of mGluRs have been observed in multiple neurological disorders including increased protein expression in Down's syndrome (Oka and Takashima, 1999) and increased mRNA without change in protein levels in adult schizophrenia (Breese et al., 1995). mGluR5 has been reported by some investigators to appear on western blots as both a dimer of approximately 224-250 kDa as well as a monomer of approximately 112-130 kDa (Copani et al., 2000; Hermans and Challiss, 2001; Goudet et al., 2005). The dimerized form may represent a desensitized version of the receptor (Naur et al., 2005); however it has been assumed that the dimer form is the natural form of the receptor (Romano et al., 2001; Goudet et al., 2005; Schwendt and McGinty, 2007) and may be induced into dimerization by oxidative stress or via auto-induction (Copani et al., 2000). This is quite interesting since our data indicate that a significant proportion of mGluR5 expression seen in children with autism is in the dimer form, further supporting the hypothesis that activation of the receptor early in life (as it may occur in autism either because of oxidative stress or ischemia), could further initiate a vicious cycle of further dimerization and activation of additional mGluR5 receptors leading to a number of consequences that are related to furtherance of pathology observed in autism: 1) abnormal regulation of apoptosis in brain as reported by our laboratory and confirmed by others (Fatemi and Halt, 2001; Araghi-Niknam and Fatemi, 2003; Sheikh et al., 2010); 2) increase in frequency of seizures; 3) increased occurrence of LTD as well as abnormal conditioned eye blink response observed in animal models of autism (Bear et al., 2004); 4) decrease in number of GABA receptors; 5) potential increased expression of amyloid precursor protein as seen in Down's Syndrome (Oka and Takashima,1999); 6) ultimate drop in mGluR5 expression in adults with autism as seen in this report may represent the final ending of a pathologic pathway that is observed in autism and is associated with decrease in long term potentiation (LTP), learning deficits, and LTD (Lu et al., 1997).

Our mGluR5 results in children with autism have to be analyzed with respect to sources for these receptors in cerebellar vermis. Group I mGluRs (consisting of mGluR1 and mGluR5), upon activation, couple to phospholipase C and regulate the IP_3/Ca^{2+} signaling system (Knöpfel and Grandes, 2002). Quite interestingly, mGluR5 is localized to about 10% of the Golgi cells mainly in lobules I, II, VII-X of the cerebellar vermis (Neki et al., 1996; Négyessy et al., 1997) and to Lugaro cell, a type of inhibitory interneuron (Neki et al., 1996; Négyessy et al., 1997; Melik-Musyan and Fanardzhyan, 2004).

In rat cerebellar cortex, Négyessy et al., (1997) showed the presence of two splice variants of mGluR5 gene, namely mGluR5a and mGluR5b. These authors showed the presence of a range of molecular weight species, i.e., 128 kDa (mGluR5a), 132 kDa (mGluR5b) and a band of greater than 250 kDa as a dimeric form in the rat cerebella (Négyessy et al., 1997). These authors posit that activation of mGluR5 on Golgi cells might synchronize groups of granule cells to secrete Reelin. Supposedly, this activity of Golgi cells may lead to inhibition of Golgi cells releasing GABA as well as granule cells releasing Reelin. Both phenomena occur in autistic subjects (i.e. underproduction of Reelin and GABA in autism). Thus, both GABA and Reelin will be produced less in autistic cerebellum.

Neki et al., (1996) also showed that ≈ 8.8% of all Golgi cells carried mGluR5 receptors with 71% of these cells localized to vermis and 28% localized to hemispheric region of cerebellum. Thus, it appears clear that mGluR5 results obtained in this study reflect activation of mGluR5 localized to vermal Golgi and Lugaro cells.

There are, however, additional reports mostly supportive of the presence of mGluR5 on GFAP-bearing glial cells resident in vermis. Aronica et al., (2000) showed that status epilepticus (SE) resulted in hypertrophy of astrocytes and microglia along with an increase in mGluR5 protein expression in glial cells, which persisted up to three months after SE. Finally, Ferraguti et al., (2001) showed that administration of a subconvulsive dose of kainic acid to CA3 pyramidal cells led to proliferation of mGluR5 in glial cells which was positive by GFAP labeling. Thus, it appears that while the largest mGluR5 expression in subjects with autism is due to activation of these receptors resident on Golgi and Lugaro cells, presence of significant GFAP upregulation in the same tissue may also contribute to activation of these receptors. This scenario seems less likely since a further increase in GFAP in adults with autism did not match the activation or lack thereof in mGluR5 receptor levels in adults with autism.

The one dissenting report is by Tilleux et al., (2007) who showed downregulation of mGluR5 expression in cultured astrocytes treated with LPS, probably secondary to effects of tumor necrosis factor, since application of PGE_2 and NO resulted in upregulation of mGluR5. The upregulation of mGluR5 in surviving neuronal cells is probably a consequence rather than a cause of the epileptic seizures as seen in TLE-resistant patients (Notenboom et al., 2006).

Our results with GABRβ3 reached statistical significance in adults with autism, while in children with autism we showed a trend for abnormality [i.e. elevation in children, possibly in response to a negative feedback loop; and significant reduction in adults as seen to be true in our previous published data with a different collection of brain samples with autism (Fatemi et al., 2009a)]. These results provide us with four potential avenues of treatment yet to be tested: 1) hyperactivation of mGluR5 in children with autism, at a time when receptors should not be as active, can be treated with negative allosteric modulators of the mGluR5 receptor tested in both animal models and recently in a group of patients with FXS (Berry-Kravis et al., 2009). This indicates that early treatment in children with autism may arrest the vicious cycle of mGluR5 activation, decreased FMRP and subsequent reduction in some or all $GABA_A$ receptors which can lead to permanent brain abnormalities including spine abnormalities and reduction in size of vermis; 2) in adult subjects with autism, when mGluR5 is reduced or at normal levels, it may be treated with positive allosteric modulators of the mGluR5 receptor which can improve cognition without provoking any seizures (Lecourtier et al., 2007) and are candidate treatments in schizophrenia; 3) in subjects with autism, use of drugs that mitigate the loss of FMRP on mGluR5-mediated translation (Bear et al., 2004; Berry-Kravis et al., 2008). An open label treatment trial of lithium in patients with FXS found improvement in total Aberrant Behavior Checklist score, Vineland

Adaptive Behavior Scale, and Clinical Global Improvement Scale (Berry-Kravis et al., 2008). These results are consistent with results from animal models of FXS. Lithium reduces mGluR-mediated translation and it is hypothesized that repletion of FMRP has the same effect (Bear et al., 2004; Berry-Kravis et al., 2008); and finally; 4) Norbin, a neuronal protein, acts as a positive mediator of mGluR5 signaling and genetic deletion of norbin leads to altered LTP, LTD, and synaptic transmission (Wang et al., 2009). Treatment with norbin may reverse increased mGluR5 signaling in subjects with autism.

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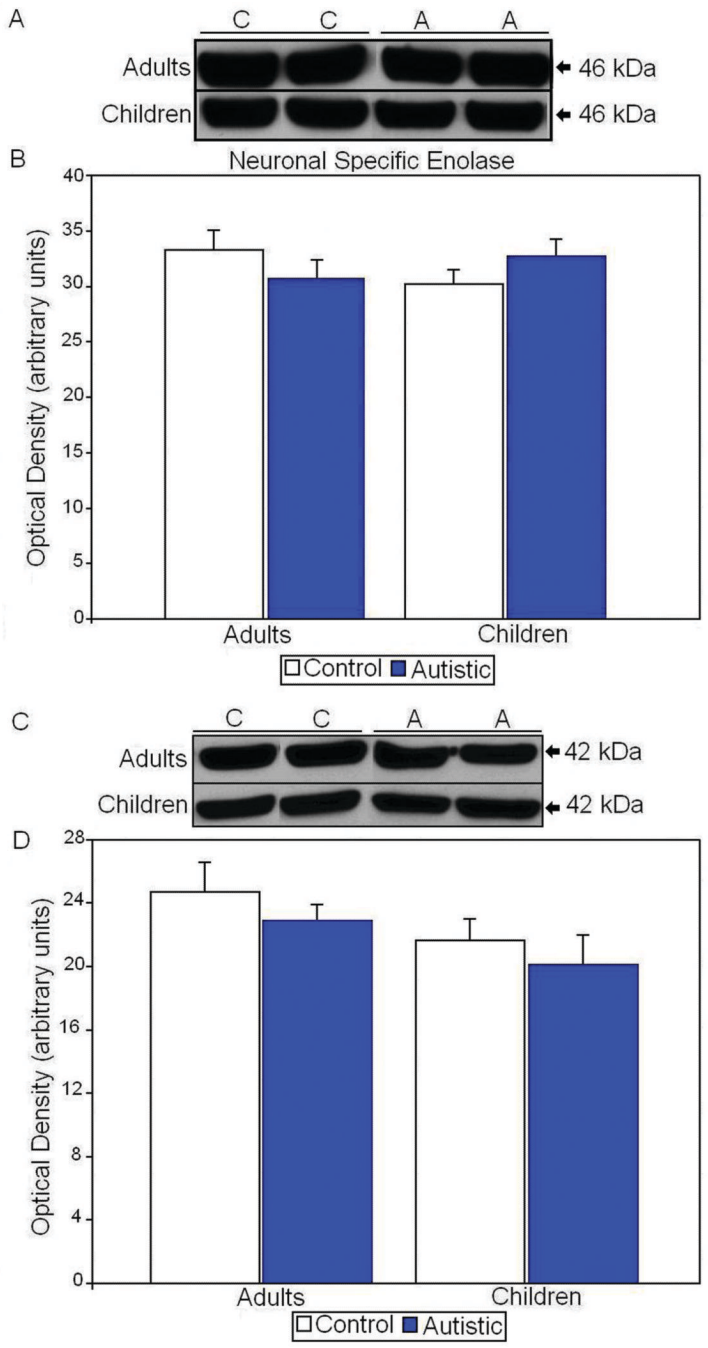

Figure 1.

A. Representative samples of neuronal specific enolase from control (C) and autistic (A) subjects. B. Mean NSE values for control and autistic (filled histogram bars) subjects are shown for vermis. C. Representative samples of β-actin from control and autistic subjects. D. Mean β-actin values for autistic (filled histogram bars) and control subjects are shown for vermis. (Error bars expressed as standard error of the mean.)

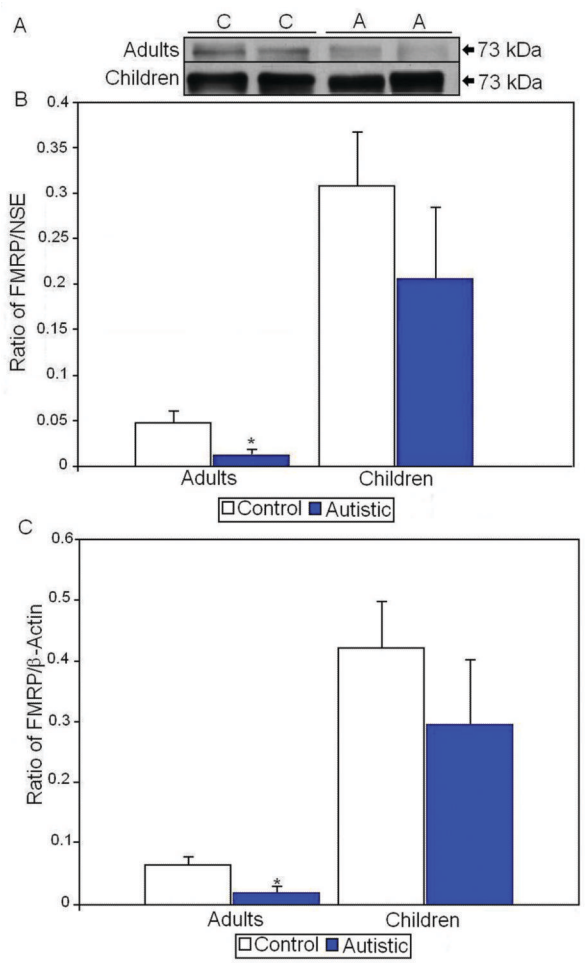

Figure 2.

A. Representative samples of FMRP from control (C) and autistic (A) subjects. B. Mean FMRP/NSE ratios for autistic (filled histogram bars) and control subjects are shown for vermis. C. Mean FMRP/β-actin ratios for autistic (filled histogram bars) and control subjects are shown for vermis. (Error bars expressed as standard error of the mean.) *, $p<0.05$.

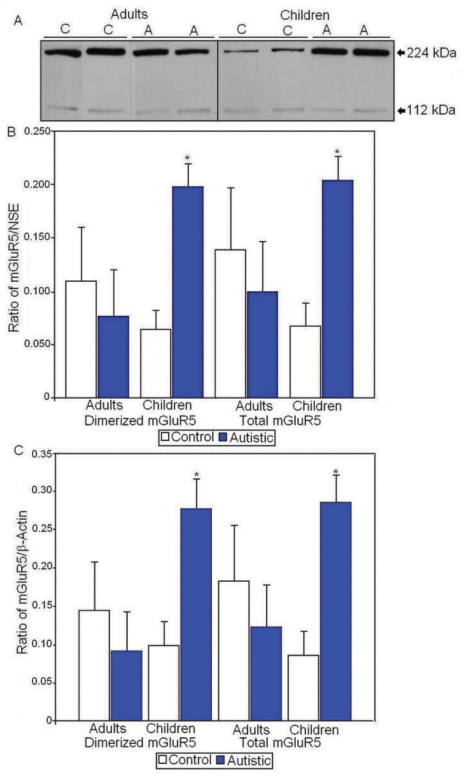

Figure 3.

A. Representative samples of mGluR5 from control (C) and autistic (A) subjects. B. Mean mGluR5/NSE ratios for autistic (filled histogram bars) and control subjects are shown for vermis. C. Mean mGluR5/ β-actin ratios for autistic (filled histogram bars) and control subjects are shown for vermis. (Error bars expressed as standard error of the mean.) *, $p<0.05$.

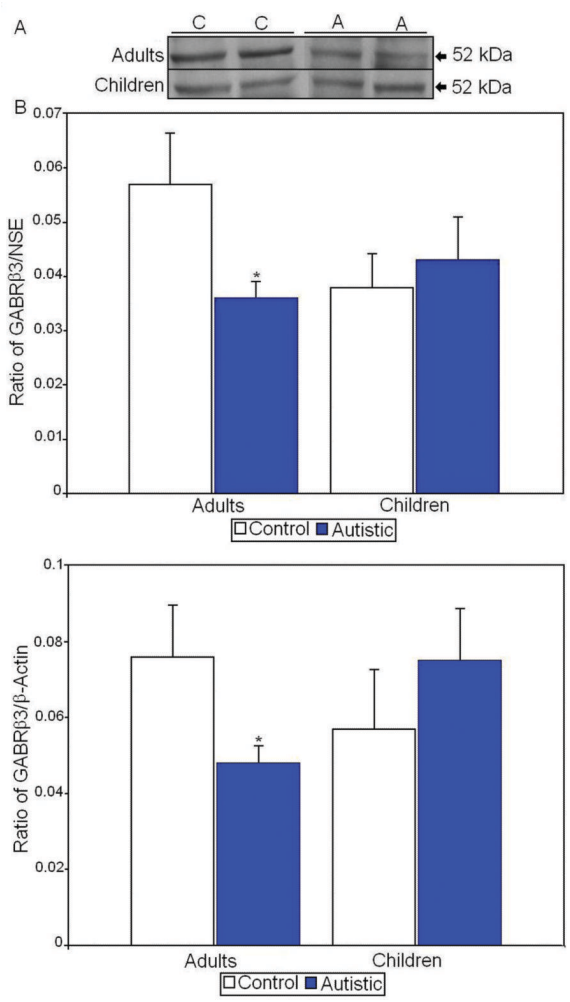

Figure 4.

A. Representative samples of GABRβ3 from control (C) and autistic (A) subjects. B. Mean GABRβ3/NSE ratios for autistic (filled histogram bars) and control subjects are shown for vermis. C. Mean GABRβ3/ β-actin ratios for autistic (filled histogram bars) and control subjects are shown for vermis. (Error bars expressed as standard error of the mean.) *, $p<0.05$.

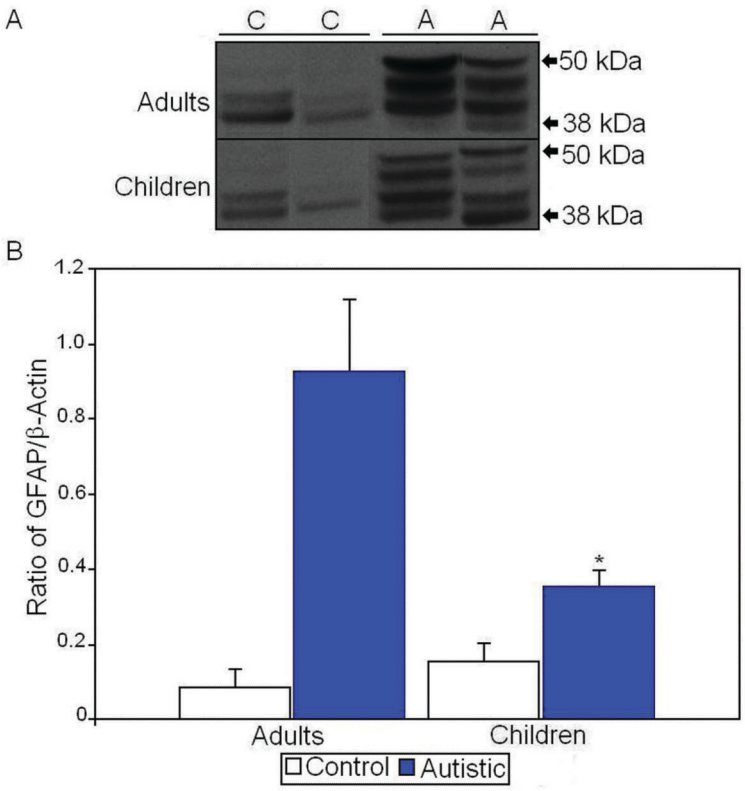

Figure 5.

A. Representative samples of GFAP from control (C) and autistic (A) subjects. B. Mean GFAP/β-actin ratios for autistic (filled histogram bars) and control subjects are shown for vermis. (Error bars expressed as standard error of the mean.) *, $p<0.05$.

Table 1

Demographic Data for Subjects with Autism and Controls

Case	Dx	Sex	Age	PMI (Hrs.)	Ethnicity	Medication History	Cause of Death	Seizure	MR
4670	Control	M	4	17	Caucasian	None	Commotio Cordis	No	No
4898	Control	M	7	12	Caucasian	Concerta, Clonidone	Drowning	No	No
1674	Control	M	8	36	Caucasian	None	Drowning	No	No
4787	Control	M	12	15	African American	Singulair, Albuteral, Prednisone, Claritin	Asthma	No	No
1823	Control	M	15	18	Caucasian	None	MVA	No	No
6396	Control	M	18	19.83	Unknown	None	Unknown	No	No
1846	Control	F	20	9	Caucasian	None	MVA	No	No
6316	Control	F	32	28.92	Unknown	None	Unknown	No	No
1169	Control	M	33	27	African American	Metoclopramide, Claritin D	Dilated Cardiomyopathy (morbid obesity)	No	No
1376	Control	M	37	12	African American	None	ASCVD	No	No
6420	Control	M	41	30.4	Unknown	None	Heart Attack	No	No
7002	Autism	F	5	32.73	Asian	None	Drowning	No	No
1349	Autism	M	5	39	Caucasian	None	Drowning	No	No
5666	Autism	M	8	22.16	Caucasian	None	Cancer	Yes	No
4231	Autism	M	8	12	African American	Zyprexa, Reminyl	Drowning	No	Yes
5342	Autism	F	11	12.88	Caucasian	Adderall, Dexedrine, Dilantin, Klonopin, Lamictal, Tegretol, Topomax	Drowning	Yes	Yes
4899	Autism	M	14	9	Caucasian	None	Drowning	No	No
6294	Autism	M	16	24	Asian	Allegra, Buspirone, Topamax	Seizure disorder	No	No
6337	Autism	M	22	25	Caucasian	Abilify, Lamictal, Zonegran	Aspiration	Yes	No
6994	Autism	M	29	43.25	Caucasian	Allegra, Geodon, Tegretol	Seizure (suspected)	Yes	No
6640	Autism	F	29	17.83	Caucasian	Luvox	Seizure disorder	Yes	No
6677	Autism	M	30	16.06	Caucasian	None	Heart Failure (congestive)	No	No
5173	Autism	M	30	20.33	Caucasian	Cisapride, Clorazepate, Depakote, Dilantin, Folic Acid, Mysoline, Phenobarbital, Prilosec, Propulsid, Reglan, Tranxene	Gastrointestinal bleeding	Yes	No
5027	Autism	M	37	26	African American	None	Obstruction of bowel due to adhesion	No	No
6401	Autism	M	39	13.95	Caucasian	None	Cardiac Tamonade	No	No
6469	Autism	F	49	16.33	Caucasian	Coumadin, Effexor, Erythromycin, Prevacid, Risperidal, Metformin, Neurontin, Propranolol, Synthroid	Pulmonary Arrest	No	Yes
4498	Autism	M	56	19.48	Caucasian	Cogentin, Haldol, Lithobid, Thorazine, Xanax	Anoxic encephalopathy	Yes	No

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Dx, diagnosis; Hrs, hours; PMI, postmortem interval; M, male; F, female; EtOH, alcohol; MVA, motor vehicle accident; MR, Mental retardation

Table 2

Western Blotting Results for FMRP, mGluR5, GABRβ3, and NSE in Vermis

Adults	Control	Autistic	Change	P
FMRP/ NSE	0.048 ± 0.025	0.012 ± 0.016	↓75%	**0.028**
mGluR5 Dimer/ NSE	0.110 ± 0.112	0.077 ± 0.108	↓30%	ns
mGluR5 Total/ NSE	0.139 ± 0.115	0.100 ± 0.115	↓28%	ns
mGluR5Dimer/mGluR5Total	0.945 ± 0.064	0.603 ± 0.327	↓36%	ns
GABRβ3 /NSE	0.057 ± 0.019	0.036 ± 0.008	↓37%	**0.031**
NSE	33.3 ± 3.8	30.7 ± 5.28	↓7.8%	ns
Age ± SD (years)	32.6 ± 7.89	35.7 ± 10.9	↑9.5%	ns
PMI ± SD (years)	21.5 ± 10.1	22.0 ± 8.91	↑2.3%	ns
Gender	3M:2F	7M:2F	--	--
Children	**Control**	**Autistic**	**Change**	**P**
FMRP/ NSE	0.308 ± 0.132	0.206 ± 0.191	↓33%	ns
mGluR5 Dimer/ NSE	0.065 ± 0.041	0.198 ± 0.044	↑204%	**0.0023**
mGluR5 Total/ NSE	0.066 ± 0.044	0.204 ± 0.046	↑209%	**0.0042**
mGluR5Dimer/mGluR5Total	0.897 ± 0.058	0.972 ± 0.017	↑8.4%	**0.049**
GABRβ3 /NSE	0.038 ± 0.015	0.043 ± 0.021	↑13%	ns
NSE	30.2 ± 3.44	32.7 ± 4.04	↑8.2%	ns
Age ± SD (years)	10.7 ± 5.28	9.57 ± 4.28	↓11%	ns
PMI ± SD (years)	19.6 ± 8.45	21.7 ± 11.3	↑11%	ns
Gender	6M:0F	5M:2F	--	--

ns, not significant

Table 3

Western Blotting Results for FMRP, mGluR5, GABRβ3, GFAP, and β-Actin in Vermis

Adults	Control	Autistic	Change	P
FMRP/ β-actin	0.064 ± 0.031	0.016 ± 0.026	↓75%	**0.038**
mGluR5 Dimer/ β-actin	0.145 ± 0.144	0.092 ± 0.126	↓36%	ns
mGluR5 Total/ β-actin	0.183 ± 0.147	0.123 ± 0.138	↓33%	ns
mGluR5Dimer/mGluR5Total	0.945 ± 0.064	0.603 ± 0.327	↓36%	ns
GABRβ3 / β-actin	0.076 ± 0.029	0.048 ± 0.012	↓37%	**0.050**
GFAP/ β-actin	0.087 ± 0.084	0.928 ± 0.491	↑967%	**0.029**
β-actin	24.7 ± 3.17	22.9 ± 3.28	↓7.3%	ns
Age ± SD (years)	32.6 ± 7.89	35.7 ± 10.9	↑9.5%	ns
PMI ± SD (years)	21.5 ± 10.1	22.0 ± 8.91	↑2.3%	ns
Gender	3M:2F	7M:2F	--	--
Children	**Control**	**Autistic**	**Change**	**P**
FMRP/ β-actin	0.422 ± 0.166	0.295 ± 0.263	↓30%	ns
mGluR5 Dimer/ β-actin	0.099 ± 0.071	0.278 ± 0.077	↑181%	**0.0084**
mGluR5 Total/ β-actin	0.086 ± 0.064	0.286 ± 0.071	↑232%	**0.0071**
mGluR5Dimer/mGluR5Total	0.897 ± 0.058	0.972 ± 0.017	↑8.4%	**0.049**
GABRβ3 / β-actin	0.057 ± 0.038	0.075 ± 0.036	↑32%	ns
GFAP/ β-actin	0.156 ± 0.088	0.355 ± 0.101	↑128%	**0.031**
β-actin	21.64 ± 3.41	20.13 ± 5.15	↓7%	ns
Age ± SD (years)	10.7 ± 5.28	9.57 ± 4.28	↓11%	ns
PMI ± SD (years)	19.6 ± 8.45	21.7 ± 11.3	↑11%	ns
Gender	6M:0F	5M:2F	--	--

ns, not significant