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ORIGINAL PAPER

Brain Region-Specific Glutathione Redox Imbalance in Autism

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Abstract Autism is a heterogeneous, behaviorally defined neurodevelopmental disorder. Recently, we reported a brain region–specific increase in lipid peroxidation, and deficits in mitochondrial electron transport chain complexes in autism, suggesting the role of oxidative stress and mitochondrial dysfunction in the pathophysiology of autism. However, the antioxidant status of the brain is not known in autism. Glutathione is a major endogenous antioxidant that plays a crucial role in protecting cells from exogenous and endogenous toxins, particularly in the central nervous system. The present study examines the concentrations of glutathione (GSH, reduced form; and GSSG, oxidized form) and the redox ratio of GSH to GSSG (marker of oxidative stress) in different regions of brains from autistic subjects and age-matched control subjects. In the cerebellum and temporal cortex from subjects with autism, GSH levels were significantly decreased by 34.2 and 44.6 %, with a concomitant increase in the levels of GSSG by 38.2 and 45.5 %, respectively, as compared to the control group. There was also a significant decrease in the levels of total GSH (tGSH) by 32.9 % in the cerebellum, and by 43.1 % in the temporal cortex of subjects with autism. In contrast, there was no significant change in GSH, GSSG and tGSH levels in the frontal, parietal and occipital cortices in autism versus control group. The redox ratio of GSH to GSSG was also significantly decreased by 52.8 % in the cerebellum and by 60.8 % in the temporal cortex of subjects with autism, suggesting glutathione redox imbalance in the brain of individuals with autism. These findings indicate that autism is associated with deficits in glutathione antioxidant defense in selective regions of the brain. We suggest that disturbances in brain glutathione homeostasis may contribute to oxidative stress, immune dysfunction and apoptosis, particularly in the cerebellum and temporal lobe, and may lead to neurodevelopmental abnormalities in autism.

Keywords Autism · Brain · Glutathione · Neurodevelopment · Oxidative stress · Redox

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Introduction

Autism is a severe neurodevelopmental disorder characterized by deficits in social interaction; impairments in verbal and nonverbal communication; and restricted, repetitive and stereotyped patterns of behavior [1]. Autism belongs to a group of neurodevelopmental disorders known as autism spectrum disorders (ASDs), which include pervasive developmental disorder—not otherwise specified (PPD-NOS) and Asperger disorder. According to the Centers for Disease Control and Prevention, 1 in 110 children in the United States is diagnosed with ASDs [2].

Autism is a heterogeneous disorder, both etiologically and phenotypically. While the cause of autism remains elusive, autism is considered a multi-factorial disorder that is influenced by genetic, epigenetic, environmental and immunological factors [3, 4]. Accumulating evidence suggests that oxidative stress may be a common feature in

autism through which environmental factors exert their deleterious effects, which may be further exacerbated by the interaction of genetically susceptible alleles [3–6]. Several studies suggest that inflammatory phenomena, immune dysregulation and certain autoimmune risk factors may also contribute to the development and pathogenesis of autism [3, 7–9].

The brain is highly vulnerable to oxidative stress as a result of its limited antioxidant capacity, high energy requirement and high amounts of unsaturated lipids and iron [10]. Antioxidants, particularly glutathione, are essential for neuronal survival during the early critical period [11, 12]. Glutathione exists in the thiol-reduced form (GSH) and disulfide-oxidized form (GSSG). GSH is the most important endogenous antioxidant for detoxification and elimination of environmental toxins and free radicals, i.e., reactive oxygen species (ROS) that cause damage to cellular functions by oxidizing lipids, proteins and DNA. In addition to serving as an antioxidant, GSH plays an important role in cell differentiation, proliferation and apoptosis [11, 13–15]. There is also ample evidence on the role of glutathione in both innate and adaptive immune functions and on its anti-inflammatory role [13, 16–18].

Some studies provide evidence of the prenatal and perinatal onset for developmental abnormalities that lead to autism [19–21]. Children are more vulnerable than adults to oxidative stress, because of their low GSH levels [22, 23]. The risk from deficits in detoxification capacity in infants is higher because some environmental factors that induce oxidative stress accumulate in the placenta, and are found at higher concentrations in developing infants than in their mothers.

The biological activity of GSH resides in the sulfhydryl (thiol) group (SH) of cysteine. It acts as a reducing agent, and protects the cells from the deleterious effects of ROS by neutralizing them. In this process, GSH is oxidized to GSSG by glutathione peroxidase (GPx). GSSG can be recycled back to GSH by NADPH-dependent glutathione reductase (GR). In healthy cells and tissues, most of the total glutathione (tGSH) pool is in the GSH form, and less than 1 % [24] or 1.2 % [25] exists in the GSSG form. GSH and GSSG are the primary determinants of redox status in all human cells. A decrease in GSH-to-GSSG redox ratio is a marker of oxidative stress.

Extensive evidence from our and other groups suggests a role of oxidative stress in the development and clinical manifestation of autism. The levels of oxidative stress markers for lipid peroxidation, protein oxidation and/or DNA oxidation are increased in the blood [3, 5, 26–28], urine [29] and brains [3, 30–34] of autistic subjects as compared with control subjects. In addition, the activities of antioxidant enzymes and the levels of antioxidant proteins, namely transferrin (iron-binding protein) and ceruloplasmin (copper-binding protein) are decreased in the blood samples from autistic subjects [26–28, 35]. Several clinical studies have reported lower GSH levels and GSH/GSSG ratio in the plasma of individuals with autism [36–40]. However, the status of antioxidant capacity in the brains of individuals with autism has not been studied previously.

Brain tissue is highly heterogeneous, with specific functions localized in specific areas of the brain. The majority of free radicals, i.e., ROS, are produced in the mitochondria during oxidative metabolism and energy production, and the electron transport chain (ETC) in mitochondria is a prime source of ROS generation [41, 42]. We recently reported brain region–specific deficits in expression levels of mitochondrial ETC complexes in the cerebellum and the frontal and temporal cortices of children with autism [30]. Interestingly, the levels of ETC complexes were unaffected in the parietal and occipital cortices in autistic subjects compared to control subjects. In addition, increased lipid peroxidation was observed in the cerebellum and temporal cortex of autistic subjects, but not in other brain regions [30]. In view of the brain region–specific oxidative damage and mitochondrial ETC defects in autism, it was of interest to examine glutathione redox status in different brain regions (cerebellum and frontal, temporal, occipital and parietal cortices) from autism and age-matched control subjects.

Materials and Methods

Autism and Control Subjects

Samples of postmortem frozen brain regions, i.e., the cerebellum, and the cortices from the frontal, temporal, parietal and occipital lobes from autistic (N = 7–10 for different brain regions) and age-matched, typically developed, control subjects (N = 9–10) were obtained from the National Institute of Child Health and Human Development (NICHD) Brain and Tissue Bank for Developmental Disorders at the University of Maryland, Baltimore, MD. The age (mean ± SE) for autistic subjects was 12.6 ± 3.2 years, and for control subjects, 12.4 ± 3.3 years. All brain samples were stored at −70 °C. This study was approved by the Institutional Review Board of the New York State Institute for Basic Research in Developmental Disabilities.

The case histories for the autistic and control subjects are summarized in Table 1. Donors with autism had met the diagnostic criteria of the Diagnostic and Statistical Manual-IV (DSM-IV) for autism. The Autism Diagnostic Interview-Revised (ADI-R) test was performed for donor UMB #s 4671, 4849, 1174, 797, 1182, 4899 and 1638. Each donor's impairments in social interaction, qualitative

Table 1 Case history of autism and control donors of brain tissue samples

Brain tissue (UMB #)	Diagnosis	Autism Diagnostic tests	Age (year)	Sex	PMI (h)	Medications	Cause of death
4671	Autism	ADIR, VABS, BSID-II	4.5	F	13		Multiple injuries from fall
1349	Autism	ADOS, VABS, BSID-II	5.6	M	39		Drowning
4849	Autism	ADIR, BSID-II, CARS	7.5	M	20		Drowning
1174	Autism	ADIR, VABS	7.8	F	14	Depakote, Tegretol	Multiple-system organ failure
4231	Autism		8.8	M	12	Zyprexia, Reminyl	Drowning
797	Autism	ADIR	9.3	M	13	Desipramine	Drowning
1182	Autism	ADIR	10.0	F	24		Smoke inhalation
4899	Autism	ADIR	14.3	M	9	Trileptal, Zoloft, Clonidine, Melatonin	Drowning
1638	Autism	ADIR	20.8	F	50	Zoloft, Zyprexa, Mellaril, Depoprovera	Seizure-related
5027	Autism	WISC-R, Bender-Gestalt	38.0	M	26	Respirdal, Luvox	Obstruction of bowel
4670	Control		4.6	M	17		Commotio Cordis from an accident
1185	Control		4.7	M	17		Drowning
1500	Control		6.9	M	18		Motor vehicle accident
4898	Control		7.7	M	12	Concerta, Clonidone	Drowning
1708	Control		8.1	F	20		Motor vehicle accident
1706	Control		8.6	F	20		Rejection of cardiac allograft transplantation
1407	Control		9.1	F	20	Albuterol, Zirtec, Alegra, Rodact, Flovent, Flonase	Asthma
4722	Control		14.5	M	16		Motor vehicle accident
1846	Control		20.6	F	9		Motor vehicle accident
4645	Control		39.2	M	12		Arteriosclerotic heart disease

ADI-R Autism Diagnostic Interview Revised, *ADOS* Autism Diagnostic Observation Scale, *VABS* Vineland Adaptive Behavioral Scale, *BSID-II* Bayley Scales of Infant Development-Second Edition, *CARS* Childhood Autism Rating Scale, *WISC-R* Wechsler Intelligence Scale for Children-Revised

abnormalities in communication, and restricted, repetitive and stereotyped patterns of behavior were consistent with the diagnosis of autism, according to the results of the ADI-R diagnostic algorithm. All donors with autism exceeded the cut-off score in these parameters. The diagnosis of autism was assigned to donor UMB # 1349 after extensive evaluation of behavioral tests, including the Autism Diagnostic Observation Schedule (ADOS), Vineland Adaptive Behavioral Scale (VABS), and Bayley Scales for Infant Development-II (BSID-II). In addition to the ADI-R, UMB # 4849 was also evaluated by the BSID-II and the Childhood Autism Rating Scale (CARS), which indicated moderate to severe autism, and autism in UMB # 4671 was also verified by the VABS and BSID-II. Regressive autism, in which early development is normal but it is followed by loss of previously acquired language and/or social skills, was suggested in five autism cases (UMBs # 1349, 4849, 1182, 4899, 1638).

Preparation of Homogenates

The coded brain tissue samples (50–60 mg each) from autistic and control subjects were homogenized using a Polytron Tissue Trearor homogenizer with a 7.0-mm diameter stainless steel probe. The extraction solution consisted of formic acid (0.1 % v/v), potassium chloride (1.2 % w/v), EDTA (1 mM), bathophenanthroline disulfonic acid (2.4 mM) in serine-borate buffer (50 mM Tris–HCl, 25 mM borate, 25 mM serine and 100 μM diethylene-triamine pentaacetic acid; pH 7.0). The volume of the extraction solution was 750 μl (pH 2.8). The homogenization was performed twice for 30 s per sample at 4 °C,

followed by centrifugation at 18,000×*g* for 10 min at 4 °C. The supernatants were processed for assaying GSH and GSSG as described below.

Assay of GSH and GSSG

GSH and GSSG in brain tissues were measured using a modification of a method described by Santori et al. [43]. 100 µl of 10 mM iodoacetic acid in 10 mM aqueous ammonium bicarbonate and 0.5 % ammonia (V/V), pH 9.5 was added to 100 µl of above brain extracts or standards (GSH, GSSG). An aliquot of 50 ng of the internal standard (glutathione ethyl ester, i.e. GSHee) was added to each solution. The mixture was incubated in the dark for 1 h at 20 °C. Acetonitrile (400 µl) was added to stop the reaction and to precipitate the proteins. The samples were centrifuged, and the GSH and GSSG in the supernatants were separated by high performance liquid chromatography (HPLC) and measured by mass spectrometry (MS), following the method of Loughlin et al. [44]. The GSH and GSSG were detected in SRM (selected reaction monitoring) mode with a triple quadruple MS (Sciex API 3000; Ontario, Canada). The range of quantification for GSH was 150–150,000 nM and that of GSSG was 50.5–50,500 nM. In each sample, total glutathione (tGSH) level was calculated as [GSH + 2GSSG], and % GSSG was calculated as [(GSSG/tGSSG) × 100]. After the study was completed, the samples were decoded, and the contents of GSH, GSSG and tGSH, the redox ratio of GSH to GSSG, and % GSSG of tGSH were compared in the autism and control groups by unpaired student's *t* test.

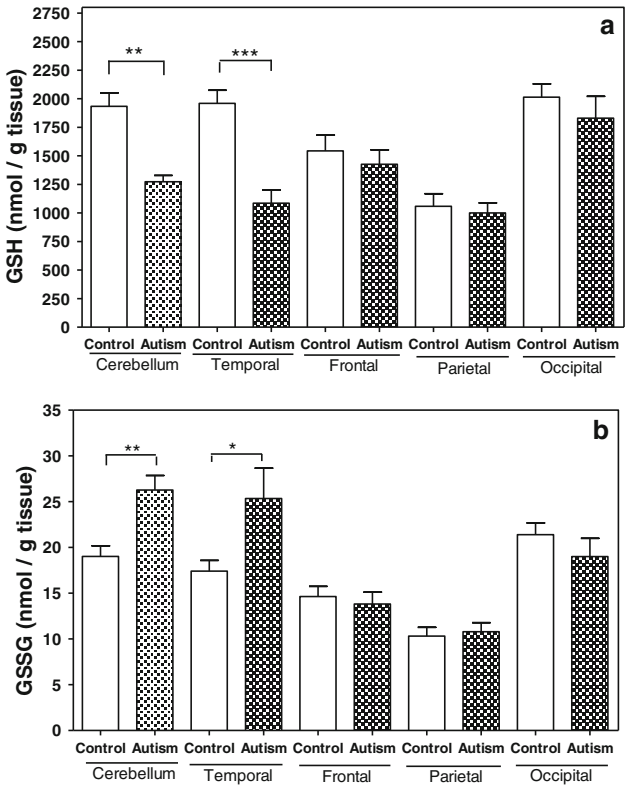

Fig. 1 Levels of reduced form of glutathione (GSH) and oxidized form of glutathione (GSSG) in the cerebellum and different regions of the cerebral cortex in subjects with autism and age-matched control subjects. There was a significant decrease in GSH levels (**a**) and increase in GSSG levels (**b**) in the cerebellum and temporal cortex in autism compared with the control group (*$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$). No significant change in the levels of GSH and GSSG was observed in the frontal, parietal and occipital cortices between the autism and control groups

Results

The levels of GSH and GSSG in the brain tissue samples from the cerebellum and frontal, temporal, parietal and occipital cortices from individuals with autism and age-matched normal subjects are represented in Fig. 1a, b, respectively. The levels of GSH (Fig. 1a) were significantly decreased by 34.2 % in the cerebellum ($p = 0.001$), and by 44.6 % in the temporal cortex ($p = 0.0008$) in autistic subjects compared to control subjects. There was also a significant increase in the levels of GSSG (Fig. 1b) by 38.2 % in the cerebellum ($p = 0.0021$) and by 45.5 % in the temporal cortex ($p = 0.0214$) in autistic subjects compared with the control group. On the other hand, the levels of GSH and GSSG were similar in other brain regions, i.e., frontal, parietal and occipital cortices between the autism and control groups (Fig. 1a, b).

Table 2 represents the data for tGSH levels, GSH/GSSG redox ratio, and % GSSG of tGSH in the cerebellum and different regions of cerebral cortex from autism and control subjects.

The comparison of the tGSH contents showed a significant decrease of tGSH levels by 32.9 % ($p = 0.0013$) in the cerebellum, and by 43.1 % ($p = 0.0011$) in the temporal cortex of subjects with autism as compared to control subjects (Table 2). In the control group, percent GSSG of tGSH was 0.97 and 0.91 in the cerebellum and temporal cortex respectively (Table 2), which is in agreement with the literature values of GSSG to be less than 1–1.2 % in the human tissues under normal conditions [24, 25]. In comparison to the control group, GSSG % in the autism group increased by twofold to 1.98 in the cerebellum ($p < 0.0001$), and by 2.4-fold to 2.19-fold in the temporal cortex ($p < 0.0001$) (Table 2), suggesting oxidative stress condition in autism. The redox ratio of GSH/GSSG, an indicator of oxidative stress was significantly reduced by 52.8 % in the cerebellum ($p < 0.0001$) and by 60.8 % in

Table 2 Redox ratio of GSH/GSSG, levels of total glutathione, and percentage of oxidized glutathione in the cerebellum and different regions of cerebral cortex in the autism and control groups

Basin tissue	GSH/GSSG redox ratio	Total glutathione (tGSH)	% GSSG of tGSH
Cerebellum			
Autism (A)	48.7 ± 1.7	1,326 ± 59	1.98 ± 0.07
Control (C)	103.4 ± 5.9	1,976 ± 119	0.97 ± 0.05
Change (A vs. C)	↓ 52.8 %	↓ 32.9 %	↑ 2.0-fold
p value	<0.0001	0.0013	<0.0001
Temporal cortex			
Autism (A)	44.7 ± 3.1	1,136 ± 120	2.19 ± 0.12
Control (C)	113.9 ± 8.2	1,996 ± 157	0.91 ± 0.07
Change (A vs. C)	↓ 60.8 %	↓ 43.1 %	↑ 2.4-fold
p value	<0.0001	0.0011	<0.0001
Frontal cortex			
Autism (A)	103.8 ± 4.3	1,453 ± 128	0.96 ± 0.04
Control (C)	105.7 ± 4.6	1,574 ± 140	0.94 ± 0.04
Change (A vs. C)	↓ 8 %	↓ 7.7 %	None
p value	ns	ns	ns
Parietal cortex			
Autism (A)	93.0 ± 3.0	1,022 ± 90	1.06 ± 0.03
Control (C)	103.0 ± 5.4	1,080 ± 116	0.98 ± 0.05
Change (A vs. C)	↓ 9.7 %	↓ 5.4 %	None
p value	ns	ns	ns
Occipital cortex			
Autism (A)	96.5 ± 2.9	1,868 ± 195	1.02 ± 0.03
Control (C)	94.4 ± 2.5	2,057 ± 118	1.04 ± 0.03
Change (A vs. C)	↑ 2.2 %	↓ 9.2 %	None
p value	ns	ns	ns

GSH reduced glutathione, *GSSG* oxidized glutathione. Total glutathione (tGSH) was calculated as [GSH + 2GSSG], and % GSSG of tGSH was calculated as [(GSSG/tGSH) × 100]. A significant decrease in GSH/GSSG redox ratio and tGSH levels, and increase in % GSSG of tGSH was observed in the cerebellum and temporal cortex in the autism group as compared with the control group. There was no significant change in these parameters in other brain regions, i.e. frontal, parietal, and occipital cortices between the autism and control groups

the temporal cortex ($p < 0.0001$) in autistic subjects compared with control subjects (Table 2). However, there was no significant change in the tGSH levels, GSH/GSSG redox ratio, and % GSSG of tGSH in other brain regions, i.e., frontal, parietal and occipital cortices between the autism and control groups (Table 2). Taken together, a decrease in GSH levels, increase in GSSG levels and % GSSG of tGSH, and a decrease in the redox ratio of GSH/GSSG in the cerebellum and temporal cortex from autism subjects, but not in other brain regions, suggest brain region-specific glutathione redox imbalance in autism.

There was no significant difference in postmortem interval (PMI) between the autistic and control groups. The mean ± SE of PMI was: 22.0 ± 4.2 h in the autism group (n = 10), and 16.1 ± 1.22 h in the control group (n = 10). Because GSH and GSSG levels were affected in the cerebellum and temporal cortex but not in the frontal, parietal and occipital cortices of individuals with autism, these findings also suggest that PMI was not a contributing factor to the alterations in GSH and GSSG levels observed in the cerebellum and temporal cortex of individuals with autism.

Discussion

ASDs are considered multi-factorial disorders in which environmental factors may act as a trigger in genetically susceptible individuals, and oxidative stress may serve as a common link between genes and environmental factors. GSH is a major intracellular antioxidant and plays a crucial role in the maintenance and regulation of the thiol-redox status of the cell. In its reduced form, GSH protects the proteins, lipids and DNA from free radicals–mediated damage by providing the reduced environment, and during this process, it gets oxidized to GSSG by GPx. Therefore, decreased levels of GSH and increased levels of GSSG are suggestive of the oxidative stress environment in cells and tissues. The redox ratio of the GSH/GSSG serves as an important indicator of redox environment in the cell and plays an important role in cell differentiation, proliferation and apoptosis [11, 13–15]. Several reports have suggested that decrease in GSH levels can also be associated with immune system dysfunction and inflammation [13, 16–18].

This is the first study to compare glutathione redox status in the brain regions of autistic subjects and

age-matched control subjects. Our results indicate that (a) the levels of GSH, tGSH and also the redox ratio of GSH to GSSG are significantly decreased, and GSSG content and % GSSG of tGSH are significantly increased in the cerebellum and temporal cortex of the brains of individuals with autism compared with age-matched control subjects, and (b) glutathione redox imbalance and oxidative stress in autism is brain region–specific because in the frontal, parietal and occipital cortices, GSH, GSSG, tGSH and GSH/GSSG were similar in the autism and control groups. Reduced glutathione-mediated redox status has also been previously reported in blood samples from individuals with autism [36–40]. In addition, several studies have provided evidence for GSH depletion and disturbances in glutathione homeostasis in other neurobehavioral and neurodegenerative disorders, including schizophrenia [45, 46], bipolar disorder [47], Parkinson's disease and Alzheimer's disease [18, 48].

Extensive evidence from our and other groups has indicated that oxidative stress and inflammatory markers are increased in autism [3, 5, 7–9]. Numerous clinical studies in autism have provided evidence for increased oxidative stress, as revealed by elevated lipid peroxidation [5, 26–28] and reduced antioxidant defense [26–28, 35]. Recent postmortem studies have also shown evidence of increased lipid, protein and DNA oxidation in the cerebellum and temporal cortex of individuals with autism compared with control subjects [3, 30–34]. However, oxidative stress condition may not be the sole mechanism responsible for the deficit in GSH content in the cerebellum and temporal cortex from subjects with autism. There are several pathways by which cells maintain intracellular GSH homeostasis, including GSH redox cycling, direct uptake, and de novo synthesis. Further studies are needed to understand whether synthesis, consumption and/or regeneration of GSH are affected in the brain of subjects with autism. GSH serves as an essential cofactor or substrate for GPx, glutathione S transferase, and glyoxalase I, which are involved in antioxidant defense or detoxification [49]. Recently, reduced levels of NADPH were reported in the plasma of children with autism compared to those of controls [39], which may affect NADPH-dependent GR activity and thus, recycling of GSSG to GSH.

The free radicals are generated endogenously during oxidative metabolism and energy production by mitochondria, and the ETC in mitochondria is a prime source for ROS generation [41, 42]. Accumulating clinical, genetic and biochemical evidence suggests that mitochondrial dysfunction in ASDs occurs more commonly than expected [50, 51]. Recently, we reported brain region–specific changes in the levels of ETC complexes in the cerebellum and the frontal and temporal cortices but not in the parietal and occipital cortices in children with autism [30]. Mitochondria contain approximately 10–15 % of GSH, which is synthesized in the cytosol and transported into the mitochondria via an energy-dependent transporter [52]. A decrease in GSH availability in the brains of individuals with autism suggests that mitochondria may also be subjected to altered redox status, which will promote mitochondrial damage via increased ROS and affect cellular energy production [53]. We have also reported that the activities of Ca^{2+}–Mg^{2+}-ATPase and Na^{+}–K^{+}-ATPase are affected in the cerebellum and the frontal cortex of autistic subjects [54].

Recent studies support a prenatal onset for developmental abnormalities leading to autism [19–21]. Several studies have reported the adverse effects of endogenous or xenobiotic-enhanced generation of ROS and the resultant oxidative stress on embryonic and fetal development [55]. GSH is the major endogenous antioxidant produced by the cells, which participates directly in the neutralization of ROS. Through direct conjugation, it detoxifies many xenobiotics and carcinogens. The depletion of GSH has been reported to enhance embryopathies [56]. Exposure of the developing embryo or fetus to radiation and xenobiotics, including drugs and environmental chemicals, can affect development by increasing ROS levels [56, 57]. Excess of ROS may alter development by oxidatively damaging cellular lipids, proteins and DNA, and/or by altering signal transduction via Ras, NFκB and related transducers [55].

GSH also plays a central role in cell death, including apoptotic cell death [13–15]. GSH depletion is a common feature and an early hallmark in apoptotic cell death in response to a variety of apoptotic stimuli [14, 15]. GSH levels have also been reported to affect caspase activity, transcription factor activation, Bcl-2 expression and function, thiol-redox signaling and phosphatidylserine externalization [13]. Several lines of evidence suggest the involvement of apoptosis in the cerebellum of autism subjects, including loss and atrophy of Purkinje cells [58–60], reduced levels of Bcl2 and increased levels of p53 [61]. We suggest that the alteration in brain glutathione homeostatasis observed in this study may also play a role in apoptotic cell death in the brains of individuals with autism.

Our results suggest that PMI cannot account for the observed brain region-specific glutathione redox imbalance in autism. Other factors, such as medications (reported for six autism cases, and two control cases), and regression (reported for five autism cases) do not seem to be contributing factors to the decrease in GSH levels and GSH/GSSG redox ratio in the cerebellum and temporal cortex in autism. However, further studies with a larger autistic group are needed to explore this issue.

The brain region-specific location of changes in GSH/GSSG observed in the cerebellum and temporal cortex

from autistic subjects in this study fits to the brain region specificity of other manifestations of autism. There is substantial evidence from neuroimaging and postmortem neuropathological studies that dysfunctions in the cerebellum and the temporal lobe may result in autistic symptoms. Loss of Purkinje and granule cells throughout the cerebellar hemispheres in autism has been reported [58–60]. Other studies suggested neuroimmune activation/neuroinflammation in the cerebellum [9] as well as the presence of autoantibodies against cerebellar proteins [62]. The neuropathological and immunological abnormalities have also been suggested in the temporal lobe of the brain in autism. The main autistic symptoms were seen most consistently with a neurological model involving bilateral dysfunction of the temporal lobes [63]. Positron emission tomography and voxel-based image analysis also showed localized dysfunction of the temporal lobes in children with autism [64]. Recent magnetic resonance imaging (MRI) studies have shown abnormalities in the superior temporal gyrus (STG) region of the brain in autism, which is of particular interest because of its role in language processing and social perception [65–67]. Gene expression profiles in this region provided evidence of increased transcript levels of many immune system–related genes and immune signaling pathways suggesting neuroimmune activation of the STG in autism [68]. Furthermore, fewer and smaller neurons in the fusiform gyrus (FG), located in the temporal lobe, have been reported in autism [69]. The functional MRI studies also showed hypoactivation of the FG in face perception tasks in autistic subjects [70, 71]. The changes observed in the glutathione levels in the cerebellum and temporal lobes of subjects with autism suggest that oxidative stress may be one of the contributing factors to these pathological changes in the cerebellum and temporal lobes.

In conclusion, this study implicates disturbance in glutathione homeostasis and deficit in glutathione antioxidant capacity in specific brain regions, i.e., cerebellum and temporal cortex, of individuals with autism. Our previous report on increased lipid peroxidation and deficit in mitochondrial ETC complexes in these brain regions of autistic subjects also suggests increased oxidative damage and mitochondrial dysfunction in autism. GSH deficit in many diseases has been linked to immune dysfunction, inflammation and apoptosis. Taken together, these studies indicate oxidative damage coupled with deficit in glutathione antioxidant status in the brain of autistic subjects that may be associated with mitochondrial dysfunction, inflammation and immune abnormalities in ASDs.

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