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Original Article

Decreased number and increased activation state of astrocytes in gray and white matter of the prefrontal cortex in autism

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The cerebral cortex presents with alterations in the number of specific cell types in autism spectrum disorder (ASD). Astrocytes have many functions in the brain including a role in higher cognitive functions and in inflammatory brain processes. Therefore, an alteration in number, function, and/or activation state of astrocytes, could be present in ASD. We quantified astrocyte number in the gray and white matter of the prefrontal cortex—BA9, BA46, and BA47—in 15 ASD and 15 age- and sex-matched control cases. We labeled astrocytes with antibodies against the protein GFAP and S100β, markers of astrocytes. We found a significant decrease in the number of astrocytes in the gray and white matter of all prefrontal areas of interest with both markers. We also found an increased state of activation of GFAP+ astrocytes in all areas. A reduced number of astrocytes in the cerebral cortex in ASD could lead to impaired synaptic function and disrupted connectivity. An increased astrocyte activation may indicate a chronic mild inflammatory state of the cerebral cortex in ASD. Overall, we found that astrocytes are disrupted in ASD.

Key words: astrocytes; autism; GFAP; human; postmortem.

Introduction

Autism spectrum disorder (ASD) is characterized by abnormalities in social communication, and reciprocal interaction and repetitive behaviors. One in every 54 children in the United State suffers from ASD being the prevalence 4.3 times more frequent in males than females (Maenner et al. 2020). Although the etiology of ASD is not well understood, it is thought that genetic, environmental, and/or immune factors are the cause of ASD (Mandy and Lai 2016). Specific brain areas are disturbed in ASD including cerebellum, amygdala, hippocampus, and cerebral cortex (Bauman and Kemper 2005; Garbett et al. 2008; Teffer and Semendeferi 2012). Among these areas, the prefrontal cortex (PFC) holds some of the cognitive functions that are affected in ASD (Alexander and Stuss 2000; Ardila et al. 2017). Specifically, cortical Brodmann areas (BAs) BA9 and BA46 in the dorsolateral PFC are responsible for attention and working memory, and BA47 in the ventral PFC is specialized in language processing (Zhang et al. 2003; Friederici 2011). ASD does not have a characteristic pathology, but changes in the number of specific cell types have been reported. Among them an increase in the number of pyramidal cells (Courchesne et al. 2011; Falcone et al. 2021) and a decrease in the number of a specific interneuron population, the parvalbumin+ chandelier (PV+ Ch) cells (Hashemi et al. 2017; Ariza et al. 2018; Amina et al. 2021) in the PFC. The number or function of astrocytes in ASD has hardly been examined. Astrocytes have a crucial role in regulating neuroinflammation, dendrite development, synaptogenesis, synaptic stability, neurotransmitter release, and they are a major regulator of neurodevelopment (Nedergaard et al. 2003; Perea et al. 2009; Jacobs et al. 2010). Neuroinflammation plays a significant role in pathophysiology of ASD (Zantomio et al. 2015; Matta et al. 2019). Given the role of astrocytes in higher cognitive functions and in inflammatory brain processes (Chung et al. 2015; Lee et al. 2017; Dossi et al. 2018), we hypothesize that an alteration in the number, morphology, and activation state of astrocytes, could be present in ASD.

The number of astrocytes has only been quantified in the white matter of the cerebral cortex in ASD and was reported no evidence for alteration in astrocyte density (Lee et al. 2017). There are not data reported on the number of astrocytes in the cerebral gray matter. On the

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other hand, the amount of glial fibrillary acidic protein (GFAP), a protein contained in astrocytes and widely used as an astrocytic marker, has been evaluated by several groups. GFAP, a type III intermediate filament, is present in mature astrocytes in the adult brain. GFAP plays a role in astrocyte–neuron interaction, and is involved in myelin maintenance (Liedtke et al. 1996). Elevated level of GFAP was reported in anterior cingulate cortex (ACC) white matter, cerebellum, and superior frontal and parietal cortex using the western blot technique in homogenized ASD brain tissue (Laurence and Fatemi 2005; Vargas et al. 2005; Crawford et al. 2015). Moreover, some genetic data showed GFAP gene expression was upregulated in PFC and cerebellum of ASD brains (Purcell et al. 2001; Edmonson et al. 2014). Increasing GFAP levels indicate astrogliosis and reactive damage and can lead to increasing cytokine level that trigger intrinsic immune responses (Laurence and Fatemi 2005; Vargas et al. 2005). Genetic studies carrying out clustering nuclear profiles showed an increase in protoplasmic astrocytes in the PFC and anterior ACC in ASD brains (Velmeshev et al. 2019). In addition, there is an upregulation of genes expressed by astrocytes in frontal and temporal cortex of patients with ASD (Parikshak et al. 2016).

Despite high specificity for astrocytes, GFAP is also expressed by neural stem cells (Obernier and Alvarez-Buylla 2019). Therefore, proper detection of astrocytes should combine the use of an alternative astrocyte marker. S100β protein is a Ca^{2+}-binding protein implicated in the regulation of intracellular processes and that mediates interactions among glial cells and between glial cells and neurons (Donato 2001). S100β is primarily found in astrocytes but it is also localized to other neural cell types (Steiner et al. 2007). No data are available about S100β+ cells in ASD. To better understand the pathology of ASD, we quantified the number of astrocytes in PFC, both in the gray and white matter of the PFC. We used GFAP and S100β as astrocyte markers in postmortem brain samples from ASD and aged-matched control cases. We also examined the state of activation of astrocytes based on the morphology of GFAP+ astrocytes.

Materials and Methods

Samples

We collected prefrontal postmortem samples from the NIH NeuroBioBank, the Autism Tissue Program (ATP) (current Autism BrainNet), and the Hispano-American Brain Bank on Neurodevelopmental Disorders (CENE). We examined the cerebral cortex of 15 controls (CTs) and 15 cases with ASD. ASD diagnoses were confirmed through standard postmortem use of the ASD Diagnostic Interview-Revised (ADI-R) in all cases. Control (CT) cases were determined to be free of neurological disorders, including ASD, based on medical records and information gathered at the time of death from next of kin. Areas of interest were BA9, BA46, and BA47 (Table 1). All cases presented in this study were male except one female in the ASD group and one female in CT group. This study was designed as pair matched. There was no difference in postmortem interval (PMI) between CTs and ASD cases. The average age of the CT group was 24.18 years with a range of 7–56 years of age, and the average age for ASD group was 25.11 with a range of 7–56 years of age. More details on age, PMI, and cause of death are shown in Table 1.

Tissue Processing

We obtained human brain blocks containing BA9, BA46, and BA47 from each case based on the Brodmann cortical neuroanatomy as previously described (Hashemi et al. 2017). We immersed blocks in 10% buffered formalin for at least 8 weeks with post-fixation in 4% paraformaldehyde, cryoprotected tissue in a 30% sucrose solution in 0.1 M phosphate buffered saline with 0.1% sodium azide, embedded in optimum cutting temperature compound, and froze at −80 °C. We cut the blocks in a Leica CM 1950 cryostat to obtain 14-μm-thick slide-mounted sections. Slides were stored at −80 °C until use. We Nissl-stained one section of each block for further confirmation of cortical area based on von Economo histology as previously described (Fig. 1) (Hashemi et al. 2017). We next used one section of each block to label astrocytes with antibody against GFAP and one section to label the astrocytes with antibody against S100β.

Immunohistochemistry

We performed enzymatic immunostaining on the tissue sections with antibodies against GFAP or S100β. Briefly, we treated the tissue with 1:1 chloroform/ethanol followed by a sequential immersion in 100%, 96%, 90%, 70%, and 50% EtOH for 5 min each. Then, we immersed the tissue in DIVA at 110 °C for 8 min. After three washes with TBS (TBS twice followed by TBS + 0.05% tween once), performed endogenous peroxidase blocking with 3% H_2O_2 in a dark humidified box for 10 min, washed the slides with TBS three times and blocked with TBS + 10% NDS + 0.3% Triton-X for 1 h at room temperature. We then treated the tissue with avidin–biotin blocking kit (Vector Labs) for 15 min each followed by primary antibody application. We used monoclonal Rabbit-anti GFAP protein primary antibody (Rbt anti-GFAP, Dako, 1:400) and monoclonal Rabbit-anti S100β protein primary antibody (Rbt anti-S100β, abcam, 1:300). Primary antibody incubation was for 24 h, at 4 °C in a dark humidified box. After washing, we incubated slides with secondary antibody (biotinylated donkey anti-rabbit IgG; Jackson, 1:150) for 1 h, washed, and incubated with ABC solution (Vector Labs) for 2 h. After washing, we developed with DAB substrate (brown, Vector Labs) and washed again, dehydrated through sequential immersion in 50%, 70%, 90%, 96%, and 100% EtOH for 3 min each, cleared in xylene for 6 min, and coverslipped with Permount mounting medium.

Table 1. Clinical characteristics of control (CT) and ASD cases, including sex, age, postmortem interval (PMI), cause of death, and time in formalin; NK: not known

Case ID	Diagnosis	Sex	Age (years)	PMI (h)	Cause of death	Time in formalin (months)	BAs analyzed
13AP86	CT	M	6	44.3	NK	64	46, 47
4203	CT	M	7	24	Respiratory insufficiency	164	9, 46, 47
4337	CT	M	8	16	Blunt force	97	9, 46, 47
210	CT	M	10	18	Myocarditis	278	9
5834	CT	M	14	38	Cardiac arrhythmia	26	9, 46, 47
AN07444	CT	M	17	30.8	Asphyxia	74	9, 46, 47
AN00544	CT	M	17	28	NK	NK	9, 46, 47
5893	CT	M	19	19	Dilated cardiomegaly	21	9, 46, 47
5958	CT	M	22	24	Dilated cardiomegaly	13	9, 46, 47
AN01891	CT	M	24	35	NK	86	9, 46, 47
AN19760	CT	M	28	23	NK	NK	9, 46, 47
AN12137	CT	M	31	32.9	Asphyxia	NK	9, 46, 47
AN15566	CT	F	32	28	NK	NK	9, 46, 47
AN17868	CT	M	46	18.8	Cardiac arrest	NK	9, 46, 47
AN19442	CT	M	50	20.4	NK	NK	9, 46, 47
AN13295	CT	M	56	22.1	NK	NK	9, 46, 47
AN03221	AU	M	7	11.4	Drowning	123	9, 46, 47
AN01293	AU	M	9	3.8	Cardiac arrest	120	9, 46, 47
4305	AU	M	12	13	Serotonin syndrome	119	9, 46, 47
AN00394	AU	M	14	10.3	Cardiac arrest	197	46, 47
4899	AU	M	14	9	Drowning	128	9
5403	AU	M	16	35	Cardiac arrhythmia	82	9, 46, 47
4269	AU	M	19	45	Meningitis	135	9, 46, 47
AN00764	AU	M	20	23.7	Accident	167	46, 47
4999	AU	M	20	14	Cardiac arrhythmia	111	9
5176	AU	M	22	18	Subdural hemorrhage	106	9, 46, 47
5574	AU	M	23	14	Pneumonia	56	9, 46, 47
AN09412	AU	M	29	38	NK	42	9, 46, 47
AN18892	AU	M	31	>72	Gun shot	177	9, 46, 47
1575	AU	F	40	24	Complications of diabetes	136	9, 46, 47
AN06746	AU	M	44	30.8	Cardiac arrest	216	9, 46, 47
5137	AU	M	51	72	Pneumonia	107	9, 46, 47
AN01093	AU	M	56	NK	NK	190	9, 46, 47

Imaging and Quantification

We selected a region of tissue that was used for cell quantification. For the gray matter, we selected a 3-mm-wide bin parallel to the pial surface that spanned from the pia through the thickness of the cortical gray matter to include all cortical layers. For the white matter, we selected 3-mm^2 area (1-mm height × 3-mm width) bin parallel to the white matter–layer VI junction. We took brightfield images on an Olympus microscope equipped with high-resolution camera, using a 40X oil objective. We quantified the number of immune-positive cells for each astrocyte marker. Image J. from Fiji software (RRID:SCR_002285) was used for total cell quantification in the selected area. Two blinded researchers analyzed the data.

Activation Analysis

GFAP+ astrocytes activation state was evaluated according to the semiquantitative score by Gouw et al. (2008). Resting: normal cell bodies with visible ramifications and low staining of glial processes; mild reactive astrogliosis: slight enlargement of cell bodies and slightly increased staining of glial processes; moderate reactive astrogliosis: significant enlargement of cell bodies, ramifications of glial processes not visible due to increased staining; and severe reactive astrogliosis: gemistocytic appearance of cell bodies and dense staining of glial processes (Gouw et al. 2008).

Statistics

We compared the total number of astrocytes labeled with GFAP and S100β in CT and ASD groups. We performed paired *t*-test as a statistical analysis and statistical significance was granted when $P < 0.05$. The influence of other variables (age, sex, and PMI) on the total amount of cells in any of the areas of interest was analyzed by analysis of covariance ($P > 0.05$). SPSS 26 (IBM) was used for statistical analyses and graphs were generated with Prism 6 (GraphPad).

Results

We identified prefrontal BA9, BA46, and BA47 in cortical tissue obtained from 15 cases with ASD and 15 age- and sex-matched CTs. We isolated blocks of PFC BA9, BA46, and BA47, cut cryosections, performed Nissl staining, and based on von Economo layer histology determined the

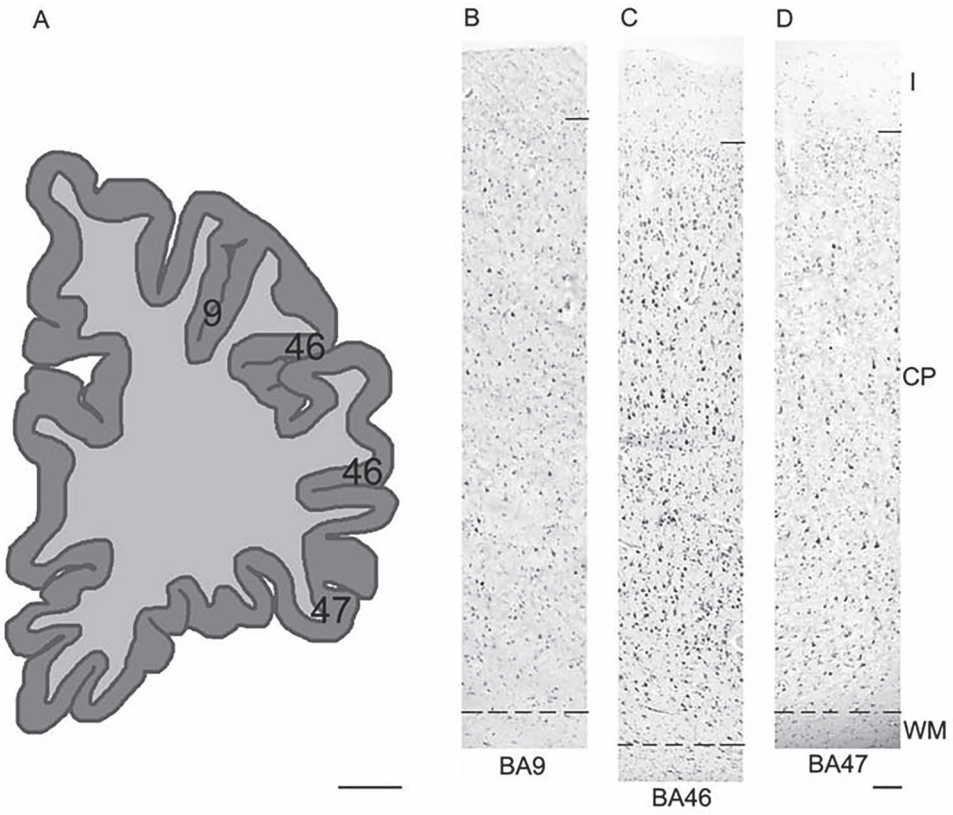

Figure 1. Prefrontal Brodmann areas BA9, BA46, and BA47. Tissue blocks were isolated based on Brodmann anatomy and areas chosen based on von Economo histology. (*A*) Coronal section from left hemisphere. (*B–D*) Nissl staining of BA9 (*B*), BA46 (*C*), and BA47 (*D*). Scale bar in *A*: 0.5 cm; *D* (*B–D*): 200 μm.

exact area of interest (Fig. 1). In adjacent sections, we labeled astrocytes with an antibody against the GFAP (Fig. 2) or S100β (Fig. 3) proteins. We quantified the number of GFAP+ and S100β+ in both gray matter and white matter in the three PFC areas of interest. We also determined the state of activation of GFAP+ astrocytes in the gray and white matter based on their morphology. We found a generalized decreased in the number of GFAP+ and S100β+ cells (Fig. 4), and an increased number of reactive astrocytes in ASD cases when compared with control cases (Fig. 5).

The Number of Astrocytes Is Decreased in the Prefrontal Cortex in ASD

The number of GFAP+ astrocytes in the gray matter in CTs was similar in BA9 and BA47 (BA9 = 1991, BA47 = 1912), and higher in BA46 (2364). We found that in gray matter, there was a 37% decrease in the number of GFAP+ astrocytes in BA9, and 38% decrease in BA47 in ASD when compared with CTs. There was also a decrease of 17% in BA46, but this was not statistically significant (BA9 = 1239, BA47 = 1180; BA46 = 1973; BA9 ($P = 0.006$), BA47 ($P = 0.001$), and BA46 ($P = 0.1$)) (Fig. 4A).

The number of GFAP+ astrocytes in the white matter in CTs was also similar in BA9 and BA47 (BA9 = 717, BA47 = 785), and was also higher in BA46 (1333). We found that in the white matter there was a 39% decrease in the number of GFAP+ astrocytes in BA9 and 37% in BA47 in ASD when compared with CTs, while this decrease was only of 29% in BA46 (BA9 = 441, BA47 = 493; BA46 = 953; BA9 ($P = 0.004$), BA47 ($P = 0.004$), and BA46 ($P = 0.01$)) (Fig. 4A).

The number of S100β+ astrocytes in the gray matter of the selected regions was 2501 (BA), 2251 (BA46), and 2250 (BA47). We found that in gray matter there was a 38% decrease in the number of S100β+ astrocytes in BA9, of 29% in BA46, and 30% in BA47 in ASD when compared with CTs (BA9 = 1550, BA46 = 1606, BA47 = 1588; BA9: $P = 0.001$, BA46: $P = 0.0004$, BA47: $P = 0.0001$) (Fig. 4B).

The number of S100β+ astrocytes in the white matter of the selected regions was 877 (BA9), 972 (BA46), and 1239 (BA47). We found that in the white matter there was a 23% decrease in the number of S100β+ astrocytes in BA9, of 46% in BA46, and 32% in BA47 in ASD when compared with CTs (BA9 = 678, BA46 = 529, BA47 = 845; BA9: $P = 0.0138$, BA 46: $P = 0.0026$, BA47: $P = 0.0322$) (Fig. 4B).

Overall, we found a significant and comparable decrease in both cell types, GFAP+ cells and S100β+ cells, in ASD when compared with CTs. Based on that these are markers for astrocytes, our data support the hypothesis that astrocyte number is consistently decreased in the PFC in ASD.

Astrocyte Activation Is Increased in ASD

We evaluated the state of activation of astrocytes in ASD and CTs (Fig. 5A). We determined the state of activation

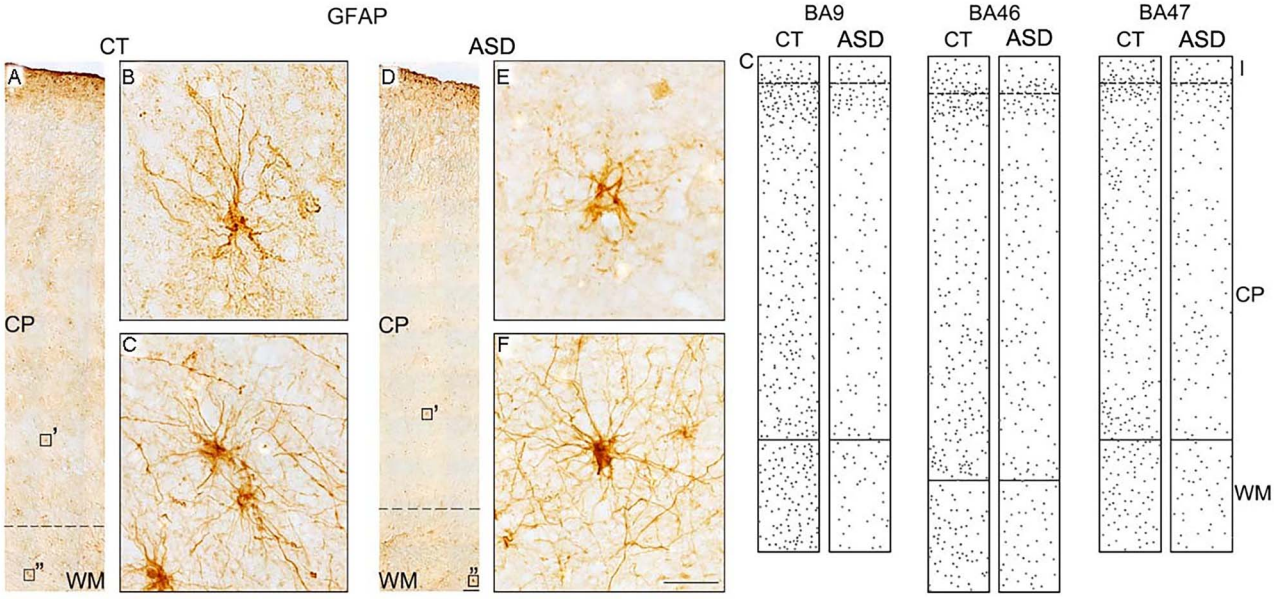

Figure 2. Prefrontal cortex immunostained with an antibody against GFAP that labels astrocytes in the cortical plate (CP) and the white matter (WM) in (*A–C*). controls (CTs) and (*D–F*). ASD cases. (*B*, *C*, *E*, *F*) High magnification of GFAP+ cells squared in (*A* and *D*). (*C*) Representative diagram demonstrating GFAP+ astrocytes location in CP and WM of BA9), BA46, and BA47 (*C*) of the CTs and ASD cases. Scale bar in *A* and *D*: 500 μm; (*B*, *C*, *E*, *F*): 20 μm; *C*: 500 μm.

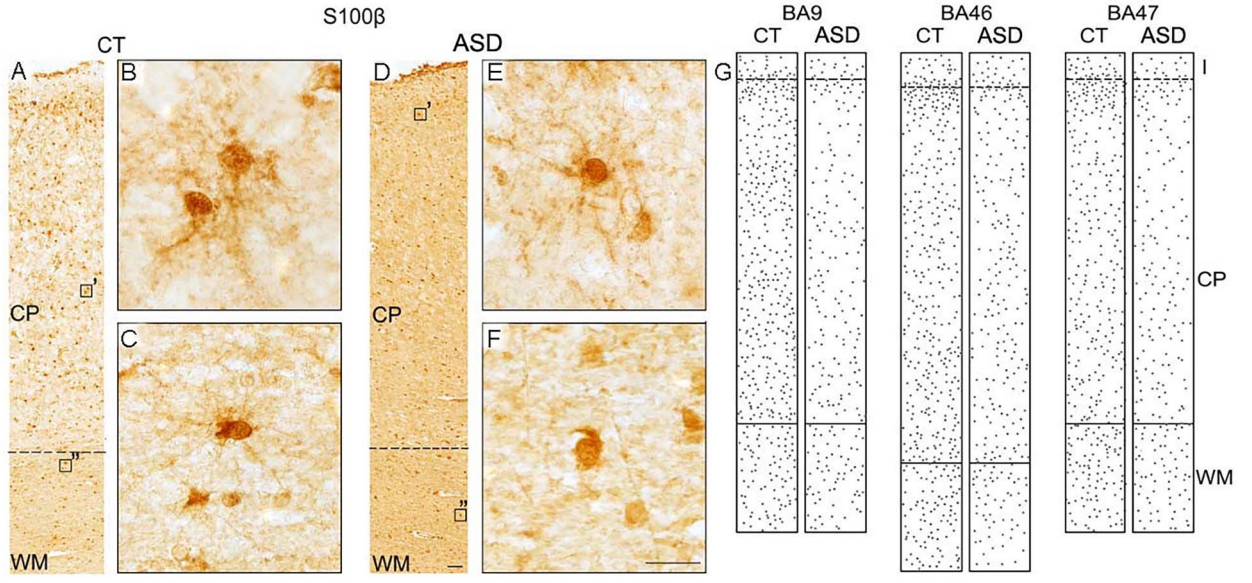

Figure 3. Prefrontal cortex immunostained with antibody against S100β to label astrocytes in cortical plate (CP) and white matter (WM) in (*A–C*). control (CT) and (*D–F*). ASD (AU) cases. (*B*, *C*, *E*, *F*) High magnification of GFAP+ cells squared in (*A* and *D*). (*C*) Representative diagram demonstrating the S100β+ astrocytes location in CP and WM of BA9, BA46, and BA47 (*C*) in CT and ASD cases. Scale bar in *A* and *D*: 500 μm; (*B*, *C*, *E*, *F*): 20 μm; C: 500 μm.

of GFAP+ astrocytes based on their morphology according to the semiquantitative score system by Gouw et al. (2008). We found a generalized increase in the number of activated astrocytes in the three prefrontal areas of interest (Fig. 5*B*). Most of the activated astrocytes were mildly reactive (stage 2), followed by moderate, and by severe activation.

In BA9 there was a 4.7-fold increase in the number of reactive astrocytes in gray matter in ASD compared with CTs (CT: mild = 68, moderate = 14, severe = 1, total number of cells = 1991; ASD: mild = 264, moderate = 95, severe = 35, total = 1239; $P = 0.003$), and a similar increase (5.1-fold) in the number of reactive astrocytes in white matter (CT: mild = 22, moderate = 2, severe = 0, total = 717; ASD: mild = 81, moderate = 31, severe = 12; total = 441; $P = 0.0006$).

In BA46 there was a 2.3-fold increase in the number of reactive astrocytes in gray matter in ASD compared with CTs (CT: mild = 66, moderate = 34, severe = 4, total = 2364; ASD: mild = 105, moderate = 72, severe = 71;

total = 1973; $P = 0.017$), and a 1.3-fold increase in the number of reactive astrocytes in white matter (CT: mild = 58, moderate = 16, severe = 4, total = 1333; ASD: mild = 60, moderate = 22, severe = 19, total = 953; $P = 0.372$).

In BA47 there was a 4.9-fold increase in the number of reactive astrocytes in gray matter in ASD compared with CTs (CT: mild = 52, moderate = 8, severe = 3, total = 1912; ASD: mild = 120, moderate = 71, severe = 119, total = 1180; $P = 0.0001$), and a 3.1-fold increase in the number of reactive astrocytes in white matter (CT: mild = 45, moderate = 2, severe = 1, total = 785; ASD: mild = 54, moderate = 42, severe = 53, total = 493; $P = 0.0001$).

Overall, we found a significant increase in the state of astrocyte activation in ASD when compared with CTs. Our data support the hypothesis that astrocytes are mildly activated in the PFC in ASD.

Discussion

The Number of Astrocytes Is Decreased in the PFC in ASD

We used two markers to label astrocytes and quantified their number. We found a decrease in the number GFAP+ (Fig. 4A) and S100β+ (Fig. 4B) astrocytes in both gray matter and white matter of the PFC in ASD. This result was replicated in the three prefrontal areas investigated.

The number of astrocytes has only been quantified in the white matter of the cerebral cortex in ASD (Lee et al. 2017). Lee et al. quantified GFAP+ cells in the white matter of the dorsolateral PFC (encompassed BA9) from eight individuals with ASD and seven age-matched CTs and found no evidence for alteration in astrocyte density (Lee et al. 2017). In our previous work by Falcone et al., using Nissl-stained sections we quantified the number of specific cell types in the PFC (BA9, BA46, and BA47) in ASD and age-matched CTs. Some of the cases used in our previous study were also included in this report, but not all. Specifically, the previous study analyzed 10 ASD and 10 CTs, while the current study analyzed 15 cases per group per area. Of these, five CTs and four ASD cases were in common between studies for BA9, four CTs and four ASD cases were in common for BA46, and six CTs and five ASD cases were in common for BA47. The rest of the cases did not overlap. Overall, one third or less of cases used in the current study were used in the previous study. We found that the number of neurons was increased, and the number of astrocytes was decreased in layer II of all three prefrontal areas. In addition, BA47 was widely affected presenting with an increased number of neurons and decreased number of astrocytes also in deeper layers of the cortex (Falcone et al. 2021).

More information is available regarding the amount of GFAP in the brain with ASD. Studies using SDS-PAGE and western blotting to measure the amount of GFAP protein found an increased level of GFAP protein in the cerebellum, superior frontal and parietal cortex, and white matter of the anterior cingulate gyrus in patients with ASD (Purcell et al. 2001; Laurence and Fatemi 2005; Crawford et al. 2015). This may seem to contradict our data; however, the amount of GFAP is not an indicator of the number of astrocytes but of the amount of GFAP per individual astrocyte. An increase in the expression of GFAP is a well-known marker for reactive astrocytes. In addition to fewer GFAP+ astrocytes, we also found a reduction in the number of S100β+ astrocytes, supporting our first finding (Fig. 4B). However, S100β is a less specific astrocyte marker than GFAP since it is also expressed by a subpopulation of oligodendrocytes (Steiner et al. 2007). Accordingly, it must be taken into account that this result may be confounded by a decreased number of oligodendrocytes since reduced axonal myelination, or immature myelination, have been reported in ASD brains (Courchesne et al. 2007; Minshew and Williams 2007; Zikopoulos and Barbas 2010). In accordance with our data, Fatemi et al. reported decreased cerebellar level of the astrocyte marker aquaporin 4 (Fatemi et al. 2008). On the other hand, there are reports of upregulation of gene sets expressed in astrocytes in the PFC, temporal cortex, and cerebellum in ASD (Edmonson et al. 2014; Parikshak et al. 2016).

Our result is on line with data gathered from other neurodevelopmental and psychiatry disorders that reported decreased glial density, for example, in the orbitofrontal (encompass part of BA47) and dorsolateral PFC (BA9 and BA47) in patients with major depression (Rajkowska et al. 1999; Steiner et al. 2007) and in the PFC in patients with schizophrenia (Benes et al. 1986; Webster et al. 2001), bipolar disorder, and mood disorders (Gittins and Harrison 2011).

Astrocytes Are Activated in ASD

To investigate whether the previously reported increased amount of GFAP resulted from an increased in astrocyte activation in the cortex in ASD, we quantified the state of activation of astrocytes and found a significant increase (Fig. 5). The first study that examined astrogliosis and microglial activation in ASD did not show evidence (Bauman and Kemper 2005); however, recent work indicates a role for glial activation and neuroinflammation in ASD (Ahlsén et al. 1993; Laurence and Fatemi 2005; Pardo et al. 2005; Vargas et al. 2005). Accordingly, microglial cells, involved in inflammatory states, have been shown to be more numerous and activated in ASD (Morgan et al. 2010, 2012). In addition, brains with ASD present with an increased expression of proinflammatory chemokines such as macrophage chemoattractant protein 1 in the anterior cingulate cortex and cerebellum, and activation regulated chemokines and proinflammatory cytokines including IL6 and IL10 also in the ACC (Pardo et al. 2005), indicating neuroinflammation in ASD.

As an increased number and activation state of microglial cells and an increased activation state of astrocytes are present in ASD, one would also expect an

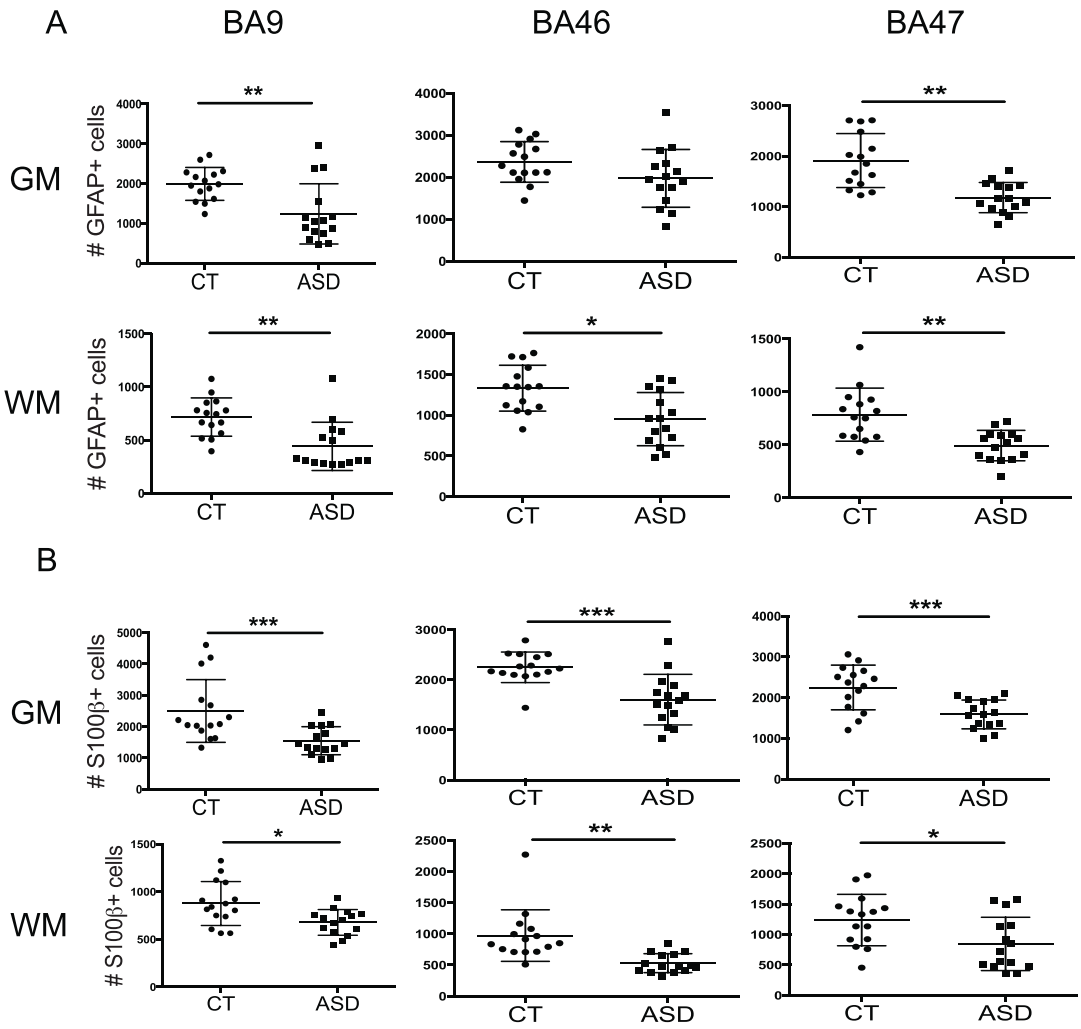

Figure 4. (A) GFAP+ cells in gray (GM) and white matter (WM) of BA9, BA46, and BA47 in control (CT) and ASD cases. There was a significant reduction in the number of GFAP+ cells in GM in ASD cases compared with CTs in BA9 and BA47. There was also a significant decrease in GFAP+ cells in the WM of ASD compared with CT cases in BA9, BA46, and BA47. (B) S100β+ cells in GM and WM in BA9, BA46, and BA47 in CT and ASD cases. There was a significant reduction in S100β+ cells in the GM and WM in ASD compared with CTs in BA9, BA46, and BA47. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

increased number of astrocytes. However, astrocytes and microglia have different origins: while astrocytes derive from neural precursor cells, microglia cells derive from the yolk sack or from monocytes. A prenatal alteration in the generation of astrocytes should not affect the generation of microglia because they are generated from unrelated types of progenitor cells. It is possible that astrocytes are first subjected to a pathological developmental event resulting in a decreased number, and to an inflammatory event later in life that would induce activation.

Our data demonstrate a mild but significant state of activation in the PFC with ASD.

Decreased Number of Astrocytes May Be Caused by Neurodevelopmental Alterations

A decreased number of astrocytes may be most likely due to a prenatal alteration of cell generation, and/or to a prenatal or postnatal increase in cell death. There is not a correlation between the number of astrocytes and age, indicating that the event that produced a decrease in the number of astrocytes takes place in prenatal development or early childhood.

An alteration in the number of cortical-specific cell types, among them astrocytes, may result from altered programs of prenatal stem cell proliferation and cell migration. During cortical development, individual radial glial cells (RGCs) produce neurons and astrocytes (Noctor et al. 2001, 2004). During early stages of cortical formation, new-born cells are generated by RGCs and migrate to their laminar destination in the cortex following an inside-out pattern (Noctor et al. 2001, 2004, 2008). Later RGCs loose contact with the pia and their cell body translocate into the subventricular zone where they are known as outer RGCs (oRGCs), (Hansen et al. 2010). During this translocation, oRGCs continue producing neurons but also give rise to glial daughter cells and eventually transform into astrocytes (Noctor et al. 2001, 2004). We and others described an increased number of neurons in ASD. The increased number of neurons in ASD

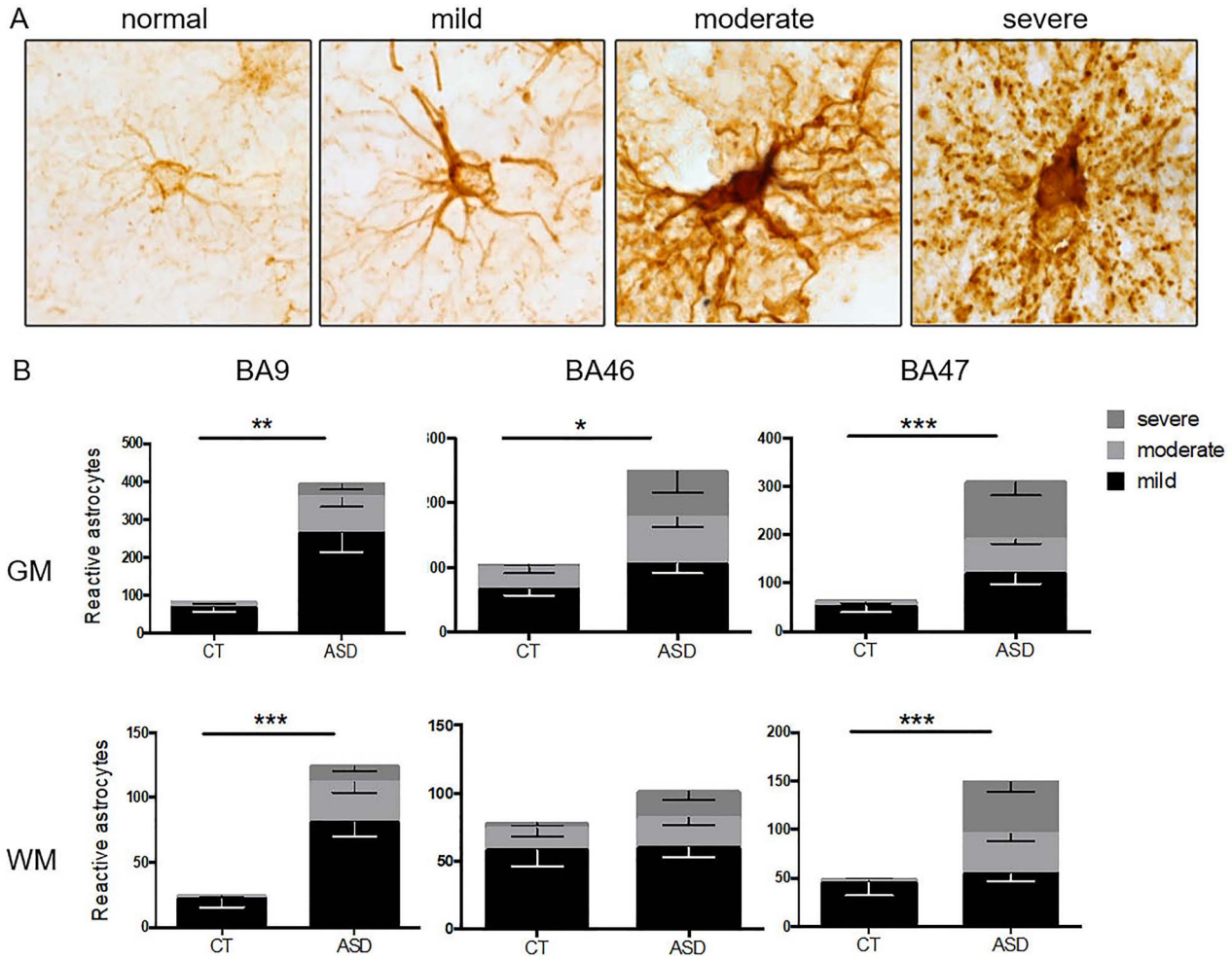

Figure 5. (*A*) Representative images of astrocytes in resting, mild, moderate, and severe states of activation. (*B*) There was a significant increase in the number of reactive astrocytes in the gray (GM) and white matter (WM) in the ASD compared with the controls (CT) in BA9, BA46, and BA47. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

could result from a delay in the switch of oRGC daughter cell production from neurons to astrocytes, which would be associated with a decreased number of astrocytes.

Most probably, both a neurodevelopmental first followed by a neurodegenerative inflammatory scenario, are behind the decreased number of astrocytes in the cerebral cortex in ASD.

Role of Astrocytes in Neurodevelopmental and Neurodegenerative Disorders

Astrocytes play a critical role in synaptogenesis and network connectivity as well as regulating excitation/inhibition balance, and their dysfunction can result in neurodevelopmental and neuropsychiatric disorders. Astrocyte loss could have detrimental impact on neuronal networks and lead to glutamate accumulation in the extracellular matrix, neuronal injury, and excitotoxicity (Purcell et al. 2001; Lin et al. 2012).

In mouse model of fragile X syndrome, that present with "autism," co-cell culture of mutant fragile X astrocytes with healthy neurons led to dendritic maturation delay and abnormal development of excitatory synapses (Jacobs et al. 2010). The reduction in astrocyte number in deep cortical layers in schizophrenia, resulted in a decreased expression of astrocyte glutamate transporter EAAT2 and a dysregulation of glutamatergic homeostasis (Bauer et al. 2010). Genetic studies have also shown astrocyte glutamate transporter EAAT1 gene polymorphism and mutation of the astrocyte potassium channel Kir4.1, responsible for potassium homeostasis, in patients with ASD (Gadow et al. 2010; Sicca et al. 2011). Moreover, elevated level of glutamine synthetase, which is expressed in astrocytes to maintain proper level of glutamate, was observed in the plasma of ASD patients (Hamed et al. 2018). Astrocytes also play a role in neurodegenerative diseases. Astrocytes regulate amyloid β protein clearance and prevent plaque formation in the brain (Wyss-Coray et al. 2003). In an animal model of Alzheimer's disease (AD), astroglia atrophy occurs in the early stages of the disease which leads to failure in proper synaptic function. Astrocytes become reactive at the later stages followed by neuroinflammation and neurodegeneration in AD (Verkhratsky et al. 2010). In addition, in animal models of amyotrophic lateral sclerosis and Huntington's disease, reduction in astrocyte glutamate transporters and glutamate uptake deficiency result in excitotoxicity

and neurodegeneration (Rossi and Volterra 2009; Faideau et al. 2010).

Conclusion

The number of astrocytes is significantly decreased, and their state of activation mildly activated in BA9, BA6, and BA47 of prefrontal gray matter and white matter in ASD. This astrocyte alterations may have detrimental impact on cortical neuronal network function.

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