Complement C3 Expression Is Decreased in Autism Spectrum Disorder Subjects and Contributes to Behavioral Deficits in Rodents

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Abstract

Autism spectrum disorder (ASD) is a neurodevelopmental disorder with hallmark symptoms including social deficits, communication deficits and repetitive behaviors. Accumulating evidence suggests a potential role of the immune system in the pathophysiology of ASD. The complement system represents one of the major effector mechanisms of the innate immune system, and regulates inflammation, and orchestrates defense against pathogens. However, the role of CNS complement system in ASD is not well understood. In the present study, we found a significant increase in C2, C5, and MASP1, but a decrease in C1q, C3, and C4 mRNA levels in the middle frontal gyrus of ASD subjects compared to controls. Significant decreases in the mRNA levels of 2 key pro-inflammatory cytokines, IL-17 and IL-23 were observed in ASD subjects. Our study further demonstrated a strong association of complement genes with IL-17 and IL-23, suggesting a possible role of the complement system in immune dysregulation in ASD. We observed significant associations between complement components and abnormality of development scores in subjects with ASD. In rodents, C3 knockdown in the prefrontal cortex induced social

interaction deficits and repetitive behavior in mice. Together, these studies suggest a potential role of C3 in the pathophysiology of ASD.

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Introduction

Autism spectrum disorder (ASD) is a complex set of neurodevelopmental disorders including pervasive developmental disorder not otherwise specified, Rett syndrome, Asperger syndrome, and autism [1, 2]. It is estimated that the prevalence of ASD is 1% of the population worldwide, with an occurrence of about 1 in 68 children in the US [3, 4]. Symptoms of ASD include repetitive or stereotyped movements, cognitive impairments, deficiencies in communication and social interaction, speech impairments, and obsessive compulsive tendencies [5]. The exact pathophysiology of ASD is unknown; however, there are theories with regard to neurotransmitters, sex hormones, genetics, environmental factors, the stress response pathway, the immune system, and many other areas [1, 3, 5, 6].

An emerging area of research is the effect of the immune system on the central nervous system due to the

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fact that some genes regulate both brain development and immune function [5]. Relatives of people with autism as well as autistic patients have high rates of autoimmune diseases [6]. Many immune cells including lymphocytes and microglia as well as proteins that influence neuronal proliferation and survival such as cytokines, neuroinflammatory markers, and immunoglobulins have been found to be altered in autistic patients [2, 7]. Specifically, TNF- α , IL-1 β , and IL-6 have been shown to be increased in blood, brain, and CSF from children with ASD [2]. There are also increased levels of complement proteins and MHC molecules in the plasma of ASD patients [2]. These changes in immune function in autism could be due to genetic factors or to the activation of the maternal immune system during pregnancy [2].

The immune system consists of the adaptive immune system and innate immune system. The complement system represents one of the major effector mechanisms of the innate immune system and serves to signal for more inflammation and clear cell debris and pathogens. The complement system consists of a number of inactive components that are activated in a cascade-like manner to exert its biological effects in the innate immune system. The complement cascade can be initiated by 3 major pathways: classical pathway, lectin pathway, and alternate pathway [8, 9]. C1q is the recognition molecule of the classical pathway and its binding to antigens or antibodies can activate the associated serine proteases C1r and C1s, leading to C2 and C4 cleavage to generate the C3 convertase, C3b2b, which in turn cleaves C3 and activates downstream cascade components [8, 10, 11]. The lectin pathway is initiated by the binding of mannose-binding lectin (MBL) to mannose residues on the cell surface [8]. This activates the MBL-associated proteases MBL serine protease 1 (MASP1) and MASP2, which then cleave C4 to generate the C4 convertase, C4b2b [9]. The alternative pathway is activated by a “tickover” mechanism (via C3 hydrolysis), which functions primarily as an amplification loop of C3b after initiation by the classical pathway and the lectin pathway [9]. All 3 pathways converge on the major complement component, C3 leading to its activation. This activates microglia to release inflammatory signals, induces phagocytosis of the pathogens, and activates T cells which create cytokines including interleukins [8, 11].

Complement proteins in the blood are unable to cross the blood brain barrier (BBB) unless it is damaged [12, 13]. The brain produces complement proteins locally, so peripheral and central complement production remains isolated. In the CNS, complement proteins are expressed in neurons and glial cells [14]. Recent studies have shown

that the classical complement cascade plays an important role in synaptic plasticity [10, 11, 15, 16].

In response to complement system activation, interleukins are synthesized and released [17]. Interleukin-17 (IL-17)-secreting CD4⁺ T cells (Th17 cells) are the key immune cells responsible for immune responses against infections. Th17 cells, cytokines, and interleukins play important roles in ASD [18]. Increased levels of IL-17 have been found in blood from subjects with ASD [19]. IL-23 has been shown to promote the terminal differentiation and expansion of Th17 effector cells, and is low in the peripheral blood of autistic patients [20]. However, it is not known whether IL-17 and IL-23 levels are altered in the brain of ASD subjects.

Despite the fact that inflammation is known to be a part of ASD pathophysiology, the role of the complement system in the brain of ASD subjects has never been explored. In the present study, we examined the expression of C1q, C2, C3, C4, C5, and MASP1, the main components of classical, lectin, and alternate pathways in the postmortem middle frontal gyrus of ASD and age and gender-matched control subjects. We also measured IL-17, IL-23, and IL-10 mRNA in these subjects. In addition, we examined whether altered C3 expression in PFC induces behavioral deficits in mice.

Methods

Ethics Statement

The Augusta University Institutional Review Board has deemed this study exempt from full review due to the use of de-identified human postmortem brain samples, with no possibility to track back the identity of the donors. Human postmortem samples are from the NICHD Brain and Tissue Bank for Developmental Disorders at the University of Maryland with ethical permission granted by the institutional review boards of the University of Maryland. Animal studies were carried out in compliance with the US National Institute of Health guidelines and approved by Augusta University animal welfare guidelines.

Animals

Adult (8- to 10-week-old) C57BL/6J male mice were purchased from Charles River Laboratories (Wilmington, MA, USA). Mice were housed in groups of 4 mice in standard polypropylene cages in a 12-h light-dark cycle.

Stereotaxic Injection of Lentivirus

C3 shRNA (m) lentiviral (LV) particles and its control shRNA LV particles were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). LV-C3-shRNA is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19–25 nt (plus hairpin) shRNA designed to knock down gene expression. shRNA LV particles frozen stock contains a concentration of 1.0×10^6 infectious units of virus in

Table 1. Comparison of ASD and control samples

Covariate	Control	ASD	<i>F</i> (1, 23)	<i>p</i>
Age, years	12.22 (5.63)	11.80 (5.80)	0.03	0.856
PMI, h	14.25 (8.14)	19.00 (10.01)	1.68	0.208
Storage time, days	3,997.08 (2,143.47)	2,828.77 (1,434.07)	2.60	0.120
pH	5.98 (0.22)	6.11 (0.26)	2.47	0.129
RNA integrity	5.78 (2.52)	6.84 (1.92)	1.40	0.248

Data are presented as mean (SD). PMI, postmortem interval; RNA integrity range: 2.4–9.2.

Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Mice (6–8 per group) were injected bilaterally with 5 μ L (each side) of LV particles into PFC by stereotaxic microinjection (coordinates: *x* 0.5 mm [lateral], *y* 1.0 mm [anterior-posterior], with respect to bregma at 0), *z* 1.0 mm (dorsoventral with brain surface at 0) at a rate of 0.2 μ L/min at each site (Stoelting Co).

Behavior Experiments

Behavioral testing was performed in a room with constant background sound and ambient lighting approximately 25–30 lux (lumen/m²) unless noted. Temperature and pressure in behavioral rooms are monitored and kept constant. Animals are transferred in their home cages to behavioral rooms at least 1 h before testing and allowed to habituate to the testing room. All behavioral experiments were scored blind to treatment.

Three Chamber Test

This test was performed to measure sociability and social deficits. The test mouse was placed in a box with 3 chambers. Each chamber is 19 $\times$ 45 $\times$ 22 cm, and the dividing walls are made from clear Plexiglas[®], with openings on each wall for free access to the other 2 chambers. Two identical wire containers that were large enough to house a single mouse were placed vertically inside the apparatus with one in each side chamber and weighted down. The test mouse was habituated to the apparatus for 5 min while freely exploring. After the habituation period, the stranger mouse was placed in one of the wire containers while the test mouse was still allowed to freely move outside of the container. The wire containers allow air exchange between the interior and exterior, but the holes are small enough to prevent direct physical contact between the stranger mouse and test mouse. The free test mouse was allowed to interact through the wire container with the stranger mouse for 5 min. During this time, time spent in chambers (stranger mouse, empty cage, and center) was recorded by an examiner with a stopwatch. The stranger mouse chamber is defined as the chamber containing the wire container with the stranger mouse inside. The empty cage chamber is the chamber containing an empty wire container. The stranger mouse was a mouse of similar age, same sex, and similar weight as the test mouse.

Reciprocal Social Interaction Test

The test mouse was placed in a neutral box (57 $\times$ 45 $\times$ 22 cm) made from clear Plexiglas and allowed to habituate for 5 min. After habituation, a stranger mouse was placed in the box and the test mouse was allowed to freely interact with the stranger mouse. In-

teraction is defined as close physical contact, nose to nose sniffing, anogenital sniffing, and grooming. Time spent interacting (initiated by the test mouse) was recorded by an examiner with a stopwatch. The stranger mouse was a mouse of similar age, same sex, and similar weight as the test mouse.

Marble Burying Test

Each mouse was placed alone in a lidded standard polypropylene cage with approximately 2 inches of woodchip bedding and 10 equidistantly placed marbles, all of the same size and texture. The test mouse was left undisturbed in the cage for 30 min and allowed to freely move about the cage and dig in the bedding. The number of buried marbles out of the total of 10 was counted by the examiner. This test serves as another measure of repetitive behavior.

Open Field Test

Mouse activity in an open field chamber was measured over a 15-min period. The open field chamber was 40 $\times$ 40 $\times$ 40 cm and made of white opaque Plexiglas. A video camera was fixed over the chamber by an adjacent rod. Ethovision XT 10 (Noldus Information Technologies Inc., USA) software was used for analysis. Trial totals for total distance travelled were taken.

Ultrasonic Vocalizations

Mice were habituated to the testing chamber for 5 min, then introduced to an intruder mouse (stranger mouse of the similar age and weight as well as the same gender as the test mouse) for 5 min. The intruder mouse was then removed from the testing chamber and the test mouse was recorded for 5 min. The calls were recorded using Avisoft Recorder USGH (Avisoft Bioacoustics, Glienicke, Germany). The files were analyzed using SASLab Pro (Avisoft Bioacoustics), and Fast Fourier Transform (FFT) was performed using the following settings: sampling rate: 250 Hz, FFT-length of 512 points, time window overlap of 75% (100% frame hamming window). Frequency resolution was 488 Hz, time resolution was 1 ms, and the lower cutoff frequency was 20 kHz. Mean duration of calls, total duration of calls, mean peak amplitude, and vocalizations per minute were analyzed for each mouse, and group averages were plotted.

Histology

Mice were perfused transcardially with 4% (wt/vol) paraformaldehyde in 0.1 M phosphate buffer (PB). The brains were dissected and postfixed for 4 h. Thereafter, tissue was incubated in 30% (wt/vol) sucrose/0.1 M PB. The brain tissue was cut into 20 μ m

cryostat sections and stained using a TUNEL kit (ApopTag Fluorescein in situ Apoptosis Detection Kit; EMD Millipore, Temecula, CA, USA) as per the manufacturer's protocol.

Human Postmortem Samples

Postmortem middle frontal gyrus tissues were from ASD ($n = 13$) and control ($n = 12$) subjects [1, 3, 5]. Demographic information is included in Table 1. Autism Diagnostic Interview-Revised (ADI-R) scores were available for 9 out of the 13 ASD subjects. There were no significant differences between tissues of ASD and control subjects in the areas of postmortem interval, refrigeration interval, age, RNA integrity, and brain pH (Table 1).

Quantitative Reverse Transcriptase PCR

The SV Total RNA Isolation System (Promega, Madison, WI, USA) was used to isolate mRNA. A SuperScript III Platinum SYBR Green One-Step qRT-PCR kit (Invitrogen, Carlsbad, CA, USA) was then used to perform qRT-PCR on a MasterCycler (Eppendorf, Hauppauge, NY, USA). Incubation was initiated at 55°C for 1,200 s, then at 95°C for 120 s and preceded by 35 cycles at 95°C for 15 s, 50°C for 30 s, 72°C for 30 s for PCR amplification. Then, a melting curve was created from 55 to 95°C with 0.2°C/s. Each gene was normalized to a housekeeping gene (18S) for human samples. RPS3 was used as a housekeeping gene for mouse samples. Primer sequences are located in online supplementary Table 1 (see www.karger.com/doi/10.1159/000465523 for all online suppl. material).

Statistical Analysis

For mouse studies, data were analyzed using 2-tailed Student t tests (for 2-group comparisons) or analysis of variance (ANOVA; for multiple-group comparisons). $p < 0.05$ was considered significant. We used a multivariate analysis of covariance (MANCOVA) model to compare the postmortem samples of people with ASD with control samples on the expression of complement genes – C1q, C2, C3, C4, C5, and MASP1 and cytokines IL-10, IL-17, and IL-23. The MANCOVA model served to evaluate and control for the effects of age, storage time, postmortem interval, pH, and RNA integrity of the postmortem sample on measurements of gene expression. All covariates were included in the MANCOVA, and partial η^2 (η^2p) was used to index effect size differences between ASD and control subjects. All analyses were completed using SPSS Statistics 20 software (IBM).

Results

The postmortem samples included those of 13 ASD subjects and 12 control subjects. There were no significant differences between the ASD group and control samples on any of the evaluated covariates. In the MANCOVA model, storage time (Wilk's $\lambda = 0.422$, $F(6, 13) = 2.965$, $p < 0.05$, $\eta^2p = 0.578$) and sample pH (Wilk's $\lambda = 0.426$, $F(6, 13) = 2.922$, $p = 0.05$, $\eta^2p = 0.574$) were significant predictors of complement gene expression. In contrast, none of the covariates significantly predicted the expression of interleukins.

ASD Status and Complement Gene Expression

In the overall multivariate model, there was a significant difference between ASD subjects and controls in complement gene expression (Wilk's $\lambda = 0.009$, $F(6, 13) = 238.11$, $p < 0.001$, $\eta^2p = 0.991$, observed power = 1.00). An examination of the univariate between-subject effects showed that ASD status was associated with an increased expression of C2 ($F(1, 18) = 19.41$, $p < 0.0001$, $\eta^2p = 0.52$), C5 ($F(1, 18) = 184.60$, $p < 0.0001$, $\eta^2p = 0.91$), and MASP1 ($F(1, 18) = 198.39$, $p < 0.0001$, $\eta^2p = 0.92$) (Fig. 1). However, ASD was also associated with a decreased expression of C1q ($F(1, 18) = 520.82$, $p < 0.0001$, $\eta^2p = 0.97$), C3 ($F(1, 18) = 306.49$, $p < 0.0001$, $\eta^2p = 0.95$), and C4 ($F(1, 18) = 8.93$, $p < 0.01$, $\eta^2p = 0.332$) genes (Fig. 1).

ASD Status and Inflammatory Cytokines

The overall MANCOVA revealed significant differences between the postmortem samples of people with ASD and controls in the expression of interleukins (Wilk's $\lambda = 0.011$, $F(3, 16) = 474.76$, $p < 0.001$, $\eta^2p = 0.989$, observed power = 1.00). The univariate between-subject analysis revealed that ASD status was especially associated with a decreased expression of IL-17 ($F(1, 18) = 1,182.79$, $p < 0.0001$, $\eta^2p = 0.985$) and IL-23 ($F(1, 18) = 138.16$, $p < 0.0001$, $\eta^2p = 0.885$) relative to control samples (Fig. 2). In contrast, there were no significant differences between ASD and control samples in the expression of IL-10 ($F(1, 18) = 1.81$, $p > 0.05$, $\eta^2p = 0.092$) (Fig. 2).

Table 2 depicts the associations among complement genes and the expression of interleukins. In the ASD group, there was a significant negative association between C3 expression and the expression of IL-10. In contrast, more associations between complement genes and IL-17 and IL-23 were apparent in the complete sample including ASD and control samples.

The association of complement genes and interleukin expression with ASD features as measured by the ADI-R is given in Table 3. Whereas C1q, C3, and C4 had significant positive association with abnormality of development, C5 and MASP1 were negatively associated with abnormality of development. Of the interleukins, IL-17 and IL-23 were both significantly correlated with abnormality of development.

C3 Inhibition in Mouse PFC Induces Social Interaction Deficits and Repetitive Behavior

Among the complement components, complement C3 is the convergence point of all activation pathways and the molecular hub for crosstalk with multiple pathogenic pathways. Therefore, we examined whether altering C3

Fig. 1. C1q, C2, C3, C4, C5, and MASP1 mRNA levels in the middle frontal gyrus of ASD subjects. Levels of mRNA of complement proteins from the middle frontal gyrus of 12 controls and 13 ASD subjects were measured. C1q (a), C2 (b), C3 (c), C4 (d), C5 (e), and MASP1 (f) mRNA was normalized to the housekeeping gene 18S. Student *t* tests were performed. *** $p < 0.0001$.

Fig. 2. IL-10, IL-17, and IL-23 mRNA levels in the middle frontal gyrus of ASD subjects. Levels of mRNA of IL-10, IL-17, and IL-23 from the middle frontal gyrus of 12 controls and 13 ASD subjects were measured. IL-10 (a), IL-17 (b), and IL-23 (c) mRNA was normalized to 18S. Student *t* tests were performed. *** $p < 0.0001$.

expression using a viral expression approach in PFC induces behavioral changes in mice. We found significant reduction in C3 mRNA levels in the PFC of mice given bilateral PFC infusion of C3 shRNA ($p < 0.05$; Fig. 3a). Next, we performed TUNEL assay to examine whether C3 depletion using shRNA in PFC induces any neuronal damage. No TUNEL-positive cells were detected in brain sections of mice injected with shRNA, which indicates

that C3 depletion in PFC does not induce any neuronal damage (Fig. 3b). In the 3-chamber test, we found that whereas control shRNA-injected mice spent more time in the chamber housing stranger mouse than the empty cage chamber, C3 shRNA-injected mice had no preference for either chamber ($p < 0.05$; Fig. 3c). In the reciprocal social interaction test, C3 shRNA-injected mice showed decreased interaction with a stranger mouse when com-

Table 2. Correlations among complement gene and proinflammatory cytokines

	C1q	C2	C3	C4	C5	MASP1	IL-10	IL-17	IL-23
C1q	–	–0.701**	0.953**	0.687**	–0.938**	–0.932**	0.341	0.972**	0.957**
C2	–0.109	–	–0.741**	–0.396	0.818**	0.810**	–0.162	–0.786**	–0.698**
C3	–0.029	–0.223	–	0.646**	–0.940**	–0.933**	0.300	0.967**	0.931**
C4	0.293	–0.232	0.172	–	–0.670**	–0.548**	0.302	0.677**	0.646**
C5	0.032	0.789**	–0.237	–0.249	–	0.917**	–0.340	–0.962**	–0.895**
MASP1	–0.210	–0.070	0.515	0.636*	–0.332	–	–0.340	–0.959**	–0.889**
IL-10	–0.072	–0.069	–0.637*	–0.039	0.206	–0.282	–	0.296	0.231
IL-17	0.279	–0.325	0.447	0.081	–0.261	0.242	–0.295	–	0.946**
IL-23	0.536	0.091	0.354	–0.028	0.075	0.079	–0.407	0.831**	–

Upper half represents correlations among complement genes and cytokines in the entire sample, whereas the lower half of the table represents correlations within the autism sample alone ($n = 13$). * $p < 0.05$; ** $p < 0.01$.

pared with those from control shRNA-treated group ($p < 0.05$; Fig. 3d). In the marble burying test, the C3 shRNA-injected mice showed enhanced marble burying ($p < 0.05$; Fig. 3e). We did not find any significant change in vocalizations per minute and total duration in the ultrasonic vocalization test between control and C3 shRNA-injected mice (Fig. 3f). There was no effect on distance traveled, indicating that locomotor activity was unaffected (Fig. 3g).

Discussion

Our data provide the first ever evidence of altered complement gene expression in the brain of subjects with ASD. One theory on the development of ASD is that inflammation during pregnancy of any of the maternal, neonatal, and fetal compartments leads to an increase of proinflammatory cytokines. This increase could result in abnormal brain development and the development of ASD [21]. Although a number of studies have shown increased levels of inflammatory cytokines in the offspring exposed to maternal viral infections [22, 23], the mechanism(s) leading to ASD-like phenotype is not known. Viral infection is known to activate all 3 pathways of the complement cascade [24]. Complement activation leads to a number of effector functions that contribute to virus inactivation and elimination. It is known that complement activation promotes phagocytosis, and enhancement of several arms of the immune response through the production of anaphylatoxins and chemotactic factors [24].

We found that C3 inhibition in PFC leads to social deficits and repetitive behavior in mice. In the CNS, com-

Table 3. Correlations of complement genes and interleukins with ADI-R scores

	ADI-A	ADI-BV	ADI-BNV	ADI-C	ADI-D
C1q	0.138	–0.358	0.295	0.028	0.826*
C2	–0.043	–0.458	–0.593	0.277	–0.469
C3	0.212	–0.304	0.302	0.037	0.887**
C4	0.068	–0.216	0.422	0.025	0.822*
C5	–0.074	0.118	–0.471	0.131	–0.777*
MASP1	–0.110	0.305	–0.447	0.097	–0.830*
IL-10	0.261	–0.443	0.200	–0.382	0.664
IL-17	0.244	–0.328	0.336	–0.054	0.881**
IL-23	0.163	–0.410	0.127	0.145	0.786*

ADI-A, social interaction; ADI-BV, verbal communication; ADI-BNV, non-verbal communication; ADI-C, stereotyped behavior; ADI-D, abnormality of development. * $p < 0.05$; ** $p < 0.01$.

plement proteins are widely expressed in neurons and glia [14]. C1q deficiency in mice resulted in decreased synapse loss, enhanced activity-dependent synaptic potentiation and an improvement in cognitive function [25, 26]. C1q is also important in neurodevelopment, specifically for synaptic elimination [10]. C3 tags weak or inactive synapses to signal to microglia for pruning, suggesting that C3 deficiency may limit the synaptic pruning process [14]. Interestingly, higher spine density in pyramidal neurons has been reported in the temporal cortex of ASD patients than in controls [27]. In addition, aged C3 knock out mice performed better on learning and memory tests than aged WT mice [28]. A recent study has

Fig. 3. Injection of C3 shRNA into PFC induces social interaction deficits and repetitive behavior in male mice. Adult male mice were injected with control or C3 shRNA lentiviral particles and allowed to recover for 2 weeks. **a** C3 mRNA in the PFC normalized to the housekeeping gene RPS3. **b** Representative images of TUNEL staining of the frontal cortex. **c** Three chamber test. M, stranger mouse; E, empty cage; C, center. **d** Social interaction test. **e** Marble bury test. **f** Ultrasonic vocalization recordings. **g** Open field test. $n = 6-8$. * $p < 0.05$ and ** $p < 0.01$ vs. control shRNA group; # $p < 0.05$ vs. stranger mouse chamber; ns, not significant.

found increased C4 mRNA levels in postmortem brain samples from schizophrenia, and reduced synaptic pruning in mice lacking functional C4 [29]. These studies suggest that a number of complement proteins have diverse functions in the brain.

Despite an intense interest in inflammation in ASD, the role of CNS complement system in ASD has never been well explored. Warren et al. [30] found increased C4B alleles in autistic patients and their mothers. A few studies have reported altered levels of complement molecules in the periphery of subjects with ASD. For example, an increase in C1q levels has been shown in the plasma of children with ASD [31]. Higher levels of C3 and C3

fragments were also found in the plasma of children with ASD [32]. Moreover, complement factor I activity, a degradation factor of C3, was found to be higher in the plasma of ASD patients [33]. Overall, the above findings indicate increased activity of the complement system in the periphery of subjects with ASD.

In the present study, we found significant increases in mRNA levels of C2, C5, and MASP1 in the PFC of ASD subjects as compared to controls. However, the mRNA levels of C1q, C3, and C4 were significantly decreased in ASD subjects. The mechanism behind the above observed differential regulation of complement genes in the brain of ASD subjects is unknown. It is known that C3a and C5a

enhance inflammation by activating mast cells which then release histamine as well as synthesize and release cytokines [17]. However, it is not clear whether the changes in the levels of complement molecules in the CNS correlate with peripheral changes. In traumatic brain injury, it has been shown that the majority of complement proteins in the brain are from the periphery due to the dysfunction of the BBB [34]. A number of studies suggest mast cell activation as a mechanism for BBB disruption and brain inflammation in ASD [35, 36]. However, further studies are warranted to better understand the brain versus peripheral contribution of the complement system in ASD.

Complement components are known to enhance the production of cytokines [17, 37]. It is known that complement components regulate the effector function of activated T cells by regulating the development of Th1, Th2, and Th17 helper cells [37, 38]. Th17 cells and their cytokine mediators have been suggested to have a role in ASD. Increased levels of IL-17 have been found in blood from subjects with ASD [19, 39, 40]. In the maternal immune activation (MIA) mouse model of ASD, systemic blockade of IL-17 inhibited the MIA-induced ASD-like behavior in the offspring, suggesting that IL-17 plays an important role during the pathophysiological process of ASD [41]. We found a significant decrease in IL-17 mRNA levels in the brain of ASD subjects. It is known that the production of IL-17A is reciprocally regulated by the anaphylatoxins with C5a signaling limiting the frequency of Th17 cells, while C3a signaling enhanced Th17 responses [37]. Moreover, the opposing actions of C3 and C5 on IL-17A were mediated via reciprocal regulation of dendritic cell (DC) IL-23 production [37]. In agreement with the above findings, we found a significant negative association of C5 with IL-17 and IL-23, but positive association of C3 with these interleukins in our samples. IL-17 being a proinflammatory cytokine, we expect to find an increase in IL-17 mRNA in the brain of ASD subjects. The observed decrease in IL-17 levels in the brain of ASD subjects is counter to the previously published results of increased IL-17 levels in the blood of subjects with ASD. Although the exact mechanism for a decrease in IL-17 mRNA is not known, further studies should investigate whether peripheral IL-17 through interacting with its receptor on endothelial cells could down regulate IL-17 synthesis in the PFC via a negative feedback mechanism.

In summary, the findings from this study provide the initial evidence on the role of the complement system in the CNS of ASD subjects. A limitation of this study is that Autism Diagnostic Interview-Revised was available for only 9 of the 13 ASD individuals, which needs further in-

vestigation using large samples before a conclusion can be drawn. Previous studies have shown altered levels of complement proteins and interleukins in the periphery of ASD subjects, but no previous evidence was available on the status of the complement system in the brain of these subjects. It is important to note that although the complement system is typically deleterious to brain function, it is sometimes neuroprotective. For example, C5a has been shown to be neuroprotective against neurodegenerative excitotoxicity and apoptosis in neuronal cells [42]. The complement system is a potential target for drug development as it has a number of receptors that bind to different components of the innate immune system. A number of drugs targeting the complement system are currently being developed [43], suggesting the complement system may be a promising therapeutic target in ASD.

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Disclosure Statement

There are no conflicts of interest to report.

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