Brain reactive IgG correlates with autoimmunity in mothers of a child with an Autism Spectrum Disorder

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Abstract

It is believed that in utero environmental factors contribute to Autism Spectrum Disorder (ASD). The goal of this study was to demonstrate, using the largest cohort reported so far, that mothers of an ASD child have an elevated frequency of anti-brain antibodies and to assess whether brain reactivity is associated with an autoimmune diathesis of the mother. **We screened plasma of 2431 mothers of an ASD child from Simon Simplex Collection and plasma of 653 unselected women of child-bearing age for anti-brain antibodies using immunohistology on mouse brain**. Positive and negative plasma from mothers with an ASD child were analyzed for anti-nuclear antibodies and for autoimmune disorders. Mothers of an ASD child were 4 times more likely to harbor anti-brain antibodies than unselected women of child bearing age (10.5% vs. 2.6%). **A second cohort from The Autism Genetic Resource Exchange with multiplex families displayed an 8.8% prevalence of anti-brain antibodies in the mothers of these families. .** Fifty-three percent of these mothers with anti-brain antibodies also exhibited anti-nuclear autoantibodies compared to 13.4% of mothers of an ASD child without anti-brain antibodies and 15% of control women of child-bearing age. The analysis of ASD mothers with brain-reactive antibodies also revealed an increased prevalence of autoimmune diseases, especially rheumatoid arthritis and systemic lupus erythematosus. This study provides robust evidence that brain-reactive antibodies are increased in mothers of an ASD child and **may be** associated with autoimmunity. The current study serves as a benchmark and justification for studying the potential pathogenicity of these antibodies on the developing brain. The detailed characterization of the specificity of these antibodies will provide practical benefits for the management and prevention of this disorder.

Key Words: ASD, Autoimmunity, Maternal, anti-brain antibodies

Introduction

Autism Spectrum Disorder (ASD) is a complex neurodevelopmental disorder with poorly defined and complex etiology, characterized by a broad range of symptoms in the domains of socialization and communication and by restricted behavioral patterns [1].Over the past decades, a substantial increase in the prevalence of ASD has been reported with a current estimation of over 1% incidence in the general population [2]. The need for early diagnosis and prevention is pressing. While the rapid increase in incidence may be in part attributable to better diagnosis and public awareness, environmental causes may also contribute.

It has become apparent that brain-reactive antibodies can contribute to human pathobiology. Some well characterized examples of antibody- mediated neurological disease are neuromyelitis optica, paraneoplastic syndromes and neuropsychiatric lupus for which pathogenic antibodies that target antigens in the brain have been identified (for a review see [3]). Although much less well characterized, anti-brain antibodies have also been implicated in the pathogenesis of a variety of childhood neuropsychiatric syndromes such as obsessive compulsive disorder [4], Tourette's syndrome [5] and ASD [6]. Several studies have linked maternal infections **or inflammation** during pregnancy to the development of ASD in the offspring (reviewed in [7]), suggesting that activation of the maternal immune system might lead to an increased risk of a child with ASD. Other studies have addressed the potential risk of exposure to maternal antibody in utero. Specifically, several investigators have identified the presence of antibodies that bind to human fetal brain tissue in mothers with an ASD offspring [8-9]. When these antibodies were administered to gestating mice **or** monkeys, the offspring exhibited abnormal behavior (reviewed in [6]). In an early study, serum containing antibodies reactive with neuronal antigens from a mother of an ASD child was passively administered to pregnant mice. The offspring were observed to have deficits in social behavior and motor skills and cerebellar abnormalities on histopathology [10]. In a subsequent study, IgG isolated from pooled sera of mothers of a child with ASD were administrated to pregnant mice. The in utero exposed

offspring had increased activity **at a young age** as well as anxiety-like behavior, alterations in sociability and increased startle following acoustic stimuli in adulthood [11]. In addition to these studies in rodents, IgG pooled from sera of mothers of a child with ASD were administrated to pregnant rhesus monkeys. The offspring were observed to have social deficits, increased motor activity and increased stereotypic behaviors compared to monkeys born of mothers given control IgG pooled from mothers of a normally developing child [12]. The results from these studies suggest that maternal antibodies targeting brain antigens can alter neurodevelopment in the fetus.

A number of epidemiologic studies have found an association between a maternal history of autoimmune disease and increased risk of having a child with ASD. Increased risk of ASD in the child was found if the mother was diagnosed with psoriasis [13], rheumatoid arthritis or celiac disease [14]. With a substantial increase in the prevalence of ASD, as has also been observed for most autoimmune diseases over the past four decades [15], we hypothesize that production of brain-reactive antibodies may be associated with features of autoimmunity.

The goal of this study was to provide evidence, using the largest cohort reported so far, that mothers of an ASD child have an elevated frequency of anti-brain antibodies and to assess whether brain reactivity is associated with an autoimmune diathesis of the mother.

The implication of this work could lead, in the future, to prevention of some proportion of ASD.

Methods

Research subjects

Plasma from mothers with an ASD child was obtained from the Simons Simplex Collection (SSC, http://sfari.org/resources/simons-simplex-collection) [16] and The Autism Genetic Resource Exchange (AGRE, http://agre.autismspeaks.org). **Details on recruitment of families, consent and**

protection of the privacy of participants, evaluation and basic description of the cohort can be found for the SSC in [16] and for AGRE in [17].

Control plasma from women of child bearing age were obtained from the North Shore-LIJ health system clinical laboratory, participants in a registry at the Feinstein Institute for Medical Research (http://www.gapregistry.org) and a previously characterized adult cohort enrolled in the metropolitan New York area, as described in [18]. No other selection criteria were applied to these control subjects, and no other information was obtained.

Plasma of women with rheumatoid arthritis were derived from subjects enrolled in the The National Data Bank for Rheumatic Diseases (NDBRD), a longitudinal cohort of rheumatoid arthritis patients recruited across the U.S. [19] that has been included in previous genetic studies of rheumatoid arthritis [20]. **A full description of this cohort including demographic and clinical variables can be found in [19].**

Individuals provided informed consent through the appropriate institutional review board.

Immunohistology

Preparation of adult mouse tissue sections. 12 week old unmanipulated C57BL/6 mice (Jackson Laboratories, USA) were anesthetized with isoflurane prior to perfusion. Brains were perfused with 4% paraformaldehyde, following replacement of circulation with heparanized preperfusion buffer. Brains were removed, postfixed over-night, and infiltrated with 30% sucrose 48 h at 4°C. Brains were next frozen in freezing medium (O.C.T. compound, Sakura, CA, USA) on dry ice and stored at -80˚C until sectioning. 12 µm transverse sections were mounted on gelatin-coated slides and stored at -80˚C until use. Procedures were approved by the Institutional Animal Care and Use Committee.

Preparation of embryonic brain tissue sections: The procedure was done as described in [21] with slight modifications. Brains were sectioned as described for the adult mouse brain preparation.

Immunostaining for anti-brain antibodies. To assess IgG reactivity to the brain, 12µm sections were air dried, rinsed twice for 5 min with PBS containing 0.1% Tween-20. Following blocking for 1

hour with PBS containing 10% heat inactivated normal goat serum in 0.2% Triton X100 in at room temperature, sections were incubated for 1 hour with 1:50 human plasma diluted in **PBS with** 3% heat inactivated normal goat serum in 0.2% Triton X100. After washing in PBS/Tween, binding of plasma to the brain was detected using Alexa 488 goat anti-human IgG (Invitrogen, USA) and visualized with OpenLab software on an Axio-Plan2 microscope (Zeiss).
Intensity and localization of reactivity was determined by two independent observers. **One observer was blind to the status of the subjects (i.e. mother of an ASD child; control). A third reviewer, also blind to the status of the subjects, assessed random samples (~10%).**

Anti-nuclear antibody staining: Anti-nuclear antibodies were assayed in human plasma utilizing the Anti-Nuclear Antibody test system (MBL-Bion, USA). Plasma was diluted 1:100 in PBS. Staining was performed according to the manufacturer's instructions and visualized with OpenLab software on an Axio-Plan2 microscope (Zeiss).

Western Blot

Whole and membrane human fetal brain lysates, 10 ug per lane (Invitrogen), were separated by SDS–PAGE electrophoresis using NuPAGE (Invitrogen) and transferred to PDVF membranes. Membranes were incubated with plasma (1:100 in PBST) followed by incubation with anti-human IR Dye 800CW and visualized using an Odyssey Infrared Imaging System (LI-COR Biosciences).

Statistical analysis

Two-sample Student's t-tests were used to compare means of two independent groups. To analyze categorical data, a Chi-squared test for independence was used. When expected sample size was smaller than 5, the Fisher's exact test was used. Values were considered significant for $p<0.05$. For multiple comparisons, the Keppel multiple comparisons correction was used.

Results

To test whether mothers of an ASD child are more likely to harbor anti-brain antibodies than unselected women of childbearing age, we assessed plasma reactivity to mouse brain tissue. Intensity was determined to be an absolute negative when no staining was visualized, positive when strong staining was visualized and indeterminate for all other cases (Supplementary Figure 1). There was no disagreement between the observers on positive and negative plasma. **The study was performed on 2,431 mothers of an ASD child obtained from the SSC and 653 controls obtained from local New York cohorts [18], the Feinstein Institute (www.gapregistry.com) and the North Shore University Hospital laboratories. Table 1 describes, by each source of subjects, race, ethnicity and the age of the subject when blood was drawn.**

Mothers of an ASD child are nearly 4 times more likely to harbor anti-brain antibodies than unselected women of child bearing age ($p<0.00001$). In total, 10.7% of the plasma of mothers of an ASD child (260/2431) displayed strong reactivity to mouse brain antigens compared to 2.6% plasma of unselected women of child bearing age (17/653). Only 28% plasma of mothers of an ASD child (682/2431) showed no binding compared to 64.7% plasma of unselected women of child bearing age (423/653). The remaining women showed indeterminate binding.

Chi-Square test yielded no difference in the presence of anti-brain antibodies between the different sources of controls (see Supplementary Table 1, for detailed analysis), thus, they were analyzed as a single cohort in subsequent analyses.

The presence of antibodies to brain tissue within a subgroup of the mothers of an ASD child could not be attributed to confounding factors such as the time elapsed from giving birth to the time blood was collected (Table 2), the age when she gave birth (Table 2) or to any reported complication or illnesses during pregnancy including fever, infections, allergies and diabetes (Supplementary Table 4). **No differences in demographic, socioeconomic (Supplementary**

Table 2) and psychiatry evaluation (Supplementary Table 3) were found between mothers of an ASD child with or without anti-brain antibodies.

In order to confirm that the current observation was not restricted to subjects enrolled in the SSC, we analyzed additional 318 plasma of mothers of an ASD child from AGRE (Demographic data is described in supplementary Table 5). 8.8% of the plasma of mothers of an ASD child (28/318) displayed strong reactivity to mouse brain antigens. Only 22.6% plasma of mothers of an ASD child (72/318) showed no binding. The remaining women showed indeterminate binding. Chi-square analysis yielded no difference between sources (AGRE and SSC, p>0.5).

Staining patterns from antibody-positive woman localized to distinct regions of the adult mouse brain (Figure 1). **Generally,** binding to neurons in the frontal cortex, hippocampus and the cerebellum **was evident**. In only 5 **of 288** cases was there clear binding to glial cells in addition to neurons.

Immunohistology of adult mouse brain was corroborated **by immunohistology of** mouse fetal brain (data not shown) and Western blots of human fetal brain lysates. Distinct bands were commonly bound by IgG from positive plasma, although there was also binding on Western blot by some plasma that were negative by immunohistology (Supplementary Figure 2).

We hypothesized that brain reactivity would be associated with an autoimmune predisposition; we, therefore, tested for the presence of anti-nuclear antibodies (ANA) which are **commonly** present in individuals with many autoimmune diseases.[22] Reactivity was assessed as positive, negative or indeterminate (Supplementary Figure 3).

As can be seen in Table 3, mothers of an ASD child **who** were positive for anti-brain antibodies were significantly more likely to harbor anti-nuclear autoantibodies than mothers of an ASD child or unselected women of child bearing age **who** lacked **anti-brain** antibodies (chi-square, $p<0.0001$). There was no significant difference between mothers of an ASD child with no anti-brain antibodies and controls. In total, 53% (152/284) of mothers of an ASD child with anti-brain

antibodies also exhibited anti-nuclear autoantibodies compared to 13.4% (99/738) of mothers of an ASD child without anti-brain antibodies and 15% (52/345) of unselected women of child-bearing age. Only 23.2% (66/284) of mothers of an ASD child with anti-brain antibodies showed no binding compared to 54.6% (403/738) of mothers of an ASD child without anti-brain antibodies and 62.3% (215/345) of unselected women of child- bearing age. The remaining subjects showed indeterminate binding. These data are consistent with a predisposition to more generalized autoimmunity in **some** mothers with **anti-brain** antibodies who have a child with ASD.

Next, we used the self-reported data available from the SSC to analyze whether autoimmune diseases were more common in mothers of an ASD child with anti-brain antibodies compared to mothers of an ASD child who lack anti-brain antibodies. Data was available only for subjects from the SSC and included the following diseases: juvenile inflammatory arthritis, rheumatoid arthritis, systemic lupus erythematosus (SLE), Hashimoto's thyroiditis, celiac disease, type 1 diabetes, psoriasis, multiple sclerosis, adrenal deficiency. The number and percentage of cases for each disease are presented in Supplementary Table 6. The analysis of ASD mothers with **anti-brain** antibodies revealed an increased incidence of autoimmune diseases especially rheumatoid arthritis, and systemic lupus erythematosus (Table 4).

Finally, to ask whether autoimmunity predisposes to production of anti-brain antibodies, we determined whether women with rheumatoid arthritis (n=363) were more likely to have anti-brain antibodies compared to the control women. Interestingly, we found that women with rheumatoid arthritis are as likely to have anti-brain antibodies as mothers of an ASD child [rheumatoid arthritis positive, 13.5% (49/362); negative 32.3% (117/362); indeterminate 54.1% (196/362)]. **Supplementary Table 7 describes race and ethnicity, age of disease onset and the age when blood was drawn of women with rheumatoid arthritis. None of these variables could account for the difference in the presence of anti-brain antibodies.**

Discussion

The passage of maternal antibodies across the placenta is a well-known mechanism for fetal immune protection. In the fetus, the blood-brain barrier is not fully formed, making the developing brain vulnerable to blood-borne substances [23]. In utero exposure to maternal anti-brain antibodies has been posited as an important potential trigger for abnormal brain development [3]. Growing evidence suggests that maternal antibodies can target the fetal brain, as maternal antibody can penetrate fetal brain tissue which is not protected by a fully functional blood-brain barrier. For example, anti-DNA, anti-N-methyl-D aspartate receptor (NMDAR), cross-reactive antibodies specifically present in women with SLE, have been shown in mouse models to be neurotoxic to the developing brain [24]. More recently, it has been shown that these antibodies have differential effects on male and female fetuses, causing cognitive impairments in the former and fetal death in the latter [25]. These antibodies affect the adult mouse if, and only if, there is a compromise to blood-brain barrier integrity [24].

Limited reports have consistently shown that when anti-brain antibodies from mothers of an ASD child were administrated to pregnant mice or pregnant monkeys, the offspring **exhibit behavioral alterations** analogous to those seen in ASD **children** [11-12].

Using the largest cohort reported so far, we have provided evidence that mothers of an ASD child are significantly more likely to harbor anti-brain antibodies than age-matched unselected women. **This observation was true with respect to women in the SSC and with respect to women in the AGRE collection.**

Our results are **also consistent** with previous relatively small studies [8-9, 26].

The majority of previous reports have used Western blotting of brain antigens as a primary screening tool. The specific brain antigens detected in these studies have not yet been identified, although some studies have identified 39 and 73 kDa bands that are often seen on

Western blots of brain lysates [8-9, 26]. In this study, we have screened for anti-brain antibodies by **histology** of adult mouse brain sections. We, as others, have not identified a specific antigenic target; yet, the use of an **immunohistologic** assay allowed us to identify the brain regions exhibiting highest reactivity with maternal IgG, including the frontal cortex, hippocampus and cerebellum. These regions have been implicated in ASD through imaging and post mortem studies [27]; Cytoarchitectural organizational abnormalities of the cerebral cortex, limbic area, cerebellum, as well as other subcortical structures have been documented. In particular, studies have demonstrated neuronal abnormalities such as loss and atrophy of Purkinje cells, abnormal development of neurons in the hippocampus and changes in neuronal size and number in the cerebellum and midbrain [27-28]. The immunostaining patterns we observed were almost exclusively neuronal and confined to regions that have consistently been shown to be involved in ASD, thus strongly supporting the physiologic relevance of the antibodies. Their specificity and functional role remain to be determined.

The utilization of immunohistology of adult mouse brain allowed us to screen relatively quickly the large number of samples reported in this study as well as to broadly identify the brain regions targeted by antibody. In order to confirm the relevance of the findings to mouse fetal brain and human, we performed immunohistology on fetal mouse brain sections as Western blotting using commercially available human fetal brain lysates. **Without expectation, plasma binding to adult brain bound Fetal brain as well, but it was not possible to localize regions of immunoreactivity in fetal brain.** While we were also able to demonstrate bands on Western blot, the bands identified were variable **using** a limited number of seropositive and seronegative plasma making it difficult to characterize plasma as positive or negative. **These** data, **did however,** show that binding to tissue antigens was not species-specific. Reactivity across species is also consistent with the studies mentioned above showing that **anti-brain** serum or IgG from mothers of an ASD child given to pregnant mice or monkeys cause cognitive and behavioral abnormalities in the offspring [11-12]. It has also been shown that many human

autoantibodies target highly conserved region of the autoantigen and therefor reacts with antigen present in multiple species [29-30].

We demonstrated that ANA antibodies and autoimmune disease are increased in mothers with anti-brain antibodies and with an ASD child. The possibility of autoimmune mechanisms being a contributing factor in ASD has been entertained since early studies suggested that individuals with ASD have a family history of autoimmune disease [14, 31]. A recent large study examined autoimmune disorders in women with over 600,000 births and showed that women with either rheumatoid arthritis or celiac disease have an increased risk of having a child with ASD [14]. Interestingly, there was no increased incidence of rheumatoid arthritis in fathers of a child with ASD, consistent with the hypothesis that the in utero experience **may contribute to ASD in** the offspring.

Our findings of increased autoimmune disease and specifically rheumatoid arthritis and systemic lupus erythematosus are consistent with **the hypothesis** that autoimmune diseases **may confer** risk for ASD in the offspring of mothers with these disorders.

We have shown that an autoimmune diathesis such as is present in women with rheumatoid arthritis predisposes to the production of anti-brain antibodies in a similar proportion to what we reported for mothers with an ASD child. **Plasma of women with rheumatoid arthritis showed similar staining patterns as plasma of mothers with an ASD child. Future studies will need to determine whether common antigens are involved**. **It is interesting to postulate** that production of anti-brain antibodies **may be** the mechanism for the reported associations between autoimmune disease and ASD. We have shown that mothers of an ASD child who were positive for anti-brain antibodies had more self-reported autoimmune conditions than mothers of an ASD child with no anti-brain antibodies. We also found that anti-nuclear

antibodies, a common marker of both subclinical autoimmunity as well as clinical autoimmune disease, are more frequent in mothers of an ASD child who harbor anti-brain antibodies.

A large number of genetic risk variants have been identified with autoimmune disorders [32], and we have considered the possibility that these variants may also predispose to the development of anti-brain antibodies in mothers of an ASD child. Our preliminary studies of mothers in the SSC for whom genetic data was available have not revealed strong evidence of associations with established autoimmune loci. However, our sample size is small and large cohorts will be required to perform a definitive analysis of autoimmune risk alleles in mothers of a child with ASD.

In a preliminary analysis on a subset of ASD subjects, we did not find an association between the presence of maternal anti-brain antibodies and the age of diagnosis **of the child**, **severity of disease,** IQ or distinct behavioral patterns in the child (data not shown). We believe that this may reflect the heterogeneity of the **antigenic** specificity of anti-brain antibodies. One previous report, however, did demonstrate a correlation between maternal antibody reactivity with specific bands on a Western blot of brain lysates and irritability and communication impairments in the affected offspring [33].Once distinct antibody specificities are identified, we will repeat the analysis if phenotypes of children **with ASD relate to** target antigens.

We evaluated antibodies from blood that was obtained from the mother years after pregnancy. Our assumption is that these antibodies reflect a chronic immune state of the mother, in which the abnormal serology persists for years, and thus was present at the time of pregnancy. **Autoantibodies have been shown to be present years before the clinical diagnosis of disease [34]. We, however, cannot rule out that at least in some cases the anti-brain antibodies appeared after pregnancy**. Only one study has assessed mid-pregnancy antibodies to fetal brain as an early marker for ASD [8]. This study suggested that reactivity to 39 and 73kDa proteins tended to be more common in mothers of an ASD child compared to

mothers of a normally developing child. Of note, the same antigenic reactivities have been identified in blood collected after pregnancy from mothers of an ASD child [33].

Control samples were obtained from various different sources, enhancing the reliability and validity of the data. No differences were observed among the sources despite some variability in ethnicity and race.

We should further note that child bearing age was the only criteria applied for selecting the control group of women. We have, therefore, no data whether those women have had a child or have an autoimmune disease. Yet, if indeed our control group is representative of overall female population, 80% have children (according to the Census Bureau) and around 6% have an autoimmune disease [35]. This latter frequency of autoimmune disorders is similar to what we report in the current paper for the mothers of an ASD child without **anti-brain** antibodies.

We did not control for sex of the mice used to assess anti-brain reactivity. Since the male to female ratio is skewed toward males in ASD, there might be a specific immune reaction to male brain antigens. We are, however, not aware of sex specific brain antigens.

Several ASD susceptibility genes have been identified in the past decade; collectively may account for 10-20% of ASD cases [36]. Once pathogenicity of anti-brain antibodies **is** determined, our study has the potential to explain up to 10% of additional cases of ASD.

Our data suggest that **anti-brain** antibodies are associated with autoimmunity and are increased in mothers of an ASD child. Work is currently under way to assess the etiologic role of these antibodies in autism. The detailed characterization of the **antigenic** specificity of these antibodies is likely to shed light on the neurobiology of autism as well as provide practical benefits to the management and prevention of this disorder.

Conflict of Interest

The authors declare no conflict of interest to report.

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Supplementary information is available at Molecular Psychiatry's website

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Figure legend:

Figure 1: Representative plasma showing reactivity of plasma of an unselected woman of child bearing age (first panel) and mother of an ASD child to mouse adult brain (second and third panel). (a-c) Transverse tissue sections were incubated with plasma of mother of an ASD child or control. Anti-brain IgG was detected using Alexa 488 anti-human IgG. As can be seen, plasma of mother of an ASD child primarily labeled the (a) Hippocampus, (b) Cerebellum and (c) Frontal cortex. x5, x40 state the magnification.

Table 1: Demographic of subjects.

	Race (%)			
	White	**African-American**	**Asian**	**Other**
SSC	80	3.9	5	11.1
NYC population cohort	100	0	0	0
GAP	76	3.3	8.9	11.8
North-Shore LIJ laboratories	70	13	6	11

	Ethnicity (%)	
	Hispanic	**Non-Hispanic**
SSC	5	95
NYC population cohort	0	100
GAP	7	93
North-Shore LIJ laboratories	5	95

	Age at the time blood was drawn			
	Mean	**SD**	**Min**	**Max**
SSC	40.28	5.73	22	58
NYC population cohort	43	3.42	37	49
GAP	38.8	9.6	19	50
North-Shore LIJ laboratories	36.17	8.14	18	50

Table 2: Characterization of Mothers of an ASD child

	Mothers of an ASD child								
	Anti-Brain-Antibodies Negative (N=644)				Anti-Brain-Antibodies Positive (N=238)				P value*
Age at the time the blood was drawn	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	
	39.9	5.74	22	57	36.61	6.05	23	56	N.S.
Years elapsed between birth of the child and the time blood was drawn	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	
	8.87	3.45	4	18	9.09	3.71	3	19	N.S.
Age at the time of giving birth	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	
	31.01	5.021	16	45	31.5	5.36	18	44	N.S.
Have the subject had pregnancies that did not end in live birth? (%)	**Yes**		**No**		**Yes**		**No**		
	31		69		30		70		N.S.

* Student t-test or chi-square for categorical data, all p values >0.12. N.S, not significant.

Table 3: Mothers with anti-brain antibodies and a child with ASD are more likely harbor for anti-nuclear antibodies.

Subjects	Source	Number of subjects	Anti-nuclear antibodies	
			Negative	**Positive**
Mothers of an ASD child Anti-brain IgG POSITIVE	SSC	256	22.65% (n=56)	**53.5%** (n=137)
	AGRE	28	35% (n=10)	**53%** (n=15)
Mothers of an ASD child Anti-brain IgG NEGATIVE	SSC	667	55.5% (n=357)	**12.7%** (n=85)
	AGRE	71	64.7% (n=46)	**19.7%** (n=14)
Unselected women of child bearing age Anti-brain IgG NEGATIVE	*Combined	345	62.3% (n=215)	**15%** (n=52)

Chi-Square analysis yielded no significant difference between the two cohorts for mothers of an ASD child positive for anti-brain antibodies or mothers of an ASD child negative for anti-brain antibodies, or controls. The remaining subjects in each cohort showed indeterminate binding (see Supplementary Figure 3).
*Control includes subjects for the NYC population study, GAP, and North Shore-lij laboratories.

Table 4: Autoimmune diseases are more common in mothers with anti-brain antibodies and a child with ASD.

	Mothers of an ASD child					
Disorder	**Anti-Brain antibody Negative (n=622)**		**Anti-Brain antibody Positive (n=233)**		**Chi Value**	**P value***
Rheumatoid Arthritis	n=9	**1.45%**	n=9	**4%**	4.82	0.028
Systemic Lupus Erythematosus	n=1	**0.16%**	n=5	**2.22%**	9.58	0.0068
Total number of autoimmune diseases	n=55	**8.8%**	n=43	**18.4%**	15.4	0.0005

Table 4 presents the significant results from the data that was available for subjects from SSC. For each disease the number of positive cases and the percentage that these cases represent is shown.

The full analysis (Supplementary Table 4) includes the following diseases: Juvenile inflammatory arthritis, rheumatoid arthritis, systemic lupus erythematosus (SLE), Hashimoto's thyroiditis, celiac disease, type 1 diabetes, psoriasis, multiple sclerosis, adrenal insufficiency.

* Keppel multiple comparison correction, $p<0.035$

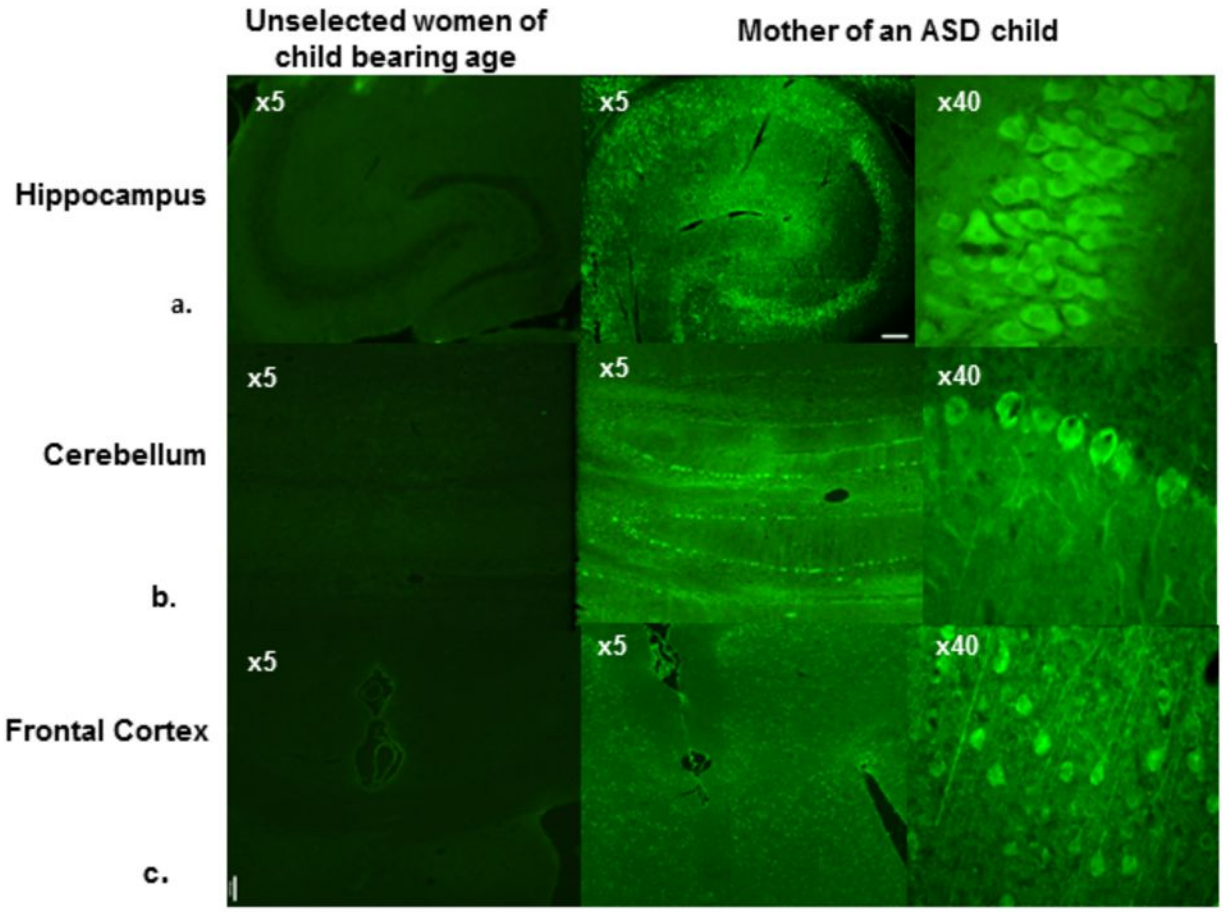

Figure 1: Representative plasma showing reactivity of plasma of an unselected women of child bearing age (first panel) and mother of an ASD child to mouse adult brain (second and third panel). **(a-c**) Transverse tissue sections were incubated with plasma of mother of an ASD child or control. Anti-brain IgG were detected using Alexa 488 anti-human IgG. As can be seen, plasma of mother of an ASD child primarily labeled the (a) Hippocampus, (b) Cerebellum and (c) Frontal cortex. x5, x40 state the magnification.