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TITLE: Maternal Brain-Reactive Antibodies and Autism Spectrum Disorder

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14. ABSTRACT

We have determined that one monoclonal antibody cloned from the memory B cell population of the mother of a child with ASD binds Caspr2. This antibody disrupts fetal brain development and leads to an ASD-like phenotype in the offspring. Forty percent of women with brain-reactive serology and a child with ASD exhibit antibodies to Caspr2; thus this antibody may contribute to approximately 5% of cases of ASD.

15. SUBJECT TERMS
Fetal brain; Autism spectrum disorder; antibody; B cells; Caspr2

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Progress Report DOD – Dr. Diamond

1. Introduction:

The goal of this project is to test a series of monoclonal anti-brain antibodies, each cloned from a woman with brain-reactive serology and a child with ASD, for pathogenic potential during fetal brain development.

The etiology of ASD is complex, and includes both genetic and environmental factors. In recent years, the maternal immune contribution has been the focus of studies demonstrating that autoimmune disorders, infections, and maternal brain-reactive antibodies may play a significant role in ASD. In ASD, the concept that maternal autoantibodies potentially affect fetal neurodevelopment and contribute to ASD has been entertained for some time. Several studies [1-3] including our own [4] have demonstrated that mothers of an ASD child are more likely to harbor anti-brain IgG. When serum or purified IgG with brain-reactive antibody derived from the mother of a child with ASD was administered to gestating mice or monkeys, offspring displayed behaviors that are reminiscent some of the symptoms seen in ASD [2,5,6].Yet, the targets of the IgG in these preparations have not been fully identified, and different preparations led to different impairments. Most recently, two papers were published identifying potential antigens recognized by serum of mothers of a child with ASD [3,5]. These studies provide strong evidence that IgG present in some mothers of a child with ASD can affect fetal brain development and lead to behaviors analogous to ASD phenotypes. These studies indeed move the field forward, yet they have some important limitations including the use of polyclonal serum which limits the ability to pin point which fine specificities are those that impair brain development. For this reason and for therapeutic purposes, the generation of monoclonal antibodies is critical. We devised a novel strategy [7] and generated monoclonal IgGs from blood samples of mothers with an ASD child previously characterized to have brain-reactive IgG [4]. B cells were incubated with human fetal brain lysate labeled with biotin. B cells that bound to biotinylated fetal brain antigens were isolated with magnetic anti-biotin beads. Because we obtained blood years after the pregnancy that resulted in a child with ASD, we focused exclusively on B cells in the memory compartment. Memory B cells persist in humans for years to decades, and thus can provide a window on autoreactivity that may have been present during a pregnancy occurring years before. We used fluorescent markers to distinguish the memory B cells (CD19+, CD27+) among the brain-reactive B cell population. These were isolated by fluorescence activated single-cell sorting. Using an established technique [8] previously employed in our lab [9], we expressed IgG variable region genes from these B cells in human $\gamma 1$ and κ chain constructs. These were then co-transfected into HEK 293T cells and intact human IgG1 was harvested from the culture supernatant and purified on a protein G column. Using this approach, we have obtained ~40 monoclonal antibodies from 3 mothers of a child with ASD. So far, 20 of them have been shown to bind mouse brain. As described below we have now extensively characterized one of those monoclonal antibodies, namely C6 and also assessed the frequency of such antibodies in plasma of mothers of an ASD child. We have also identified two other monoclonal antibody to Caspr2, A7 and C9, which we will test for pathogenic potential.

2. Keywords:

Fetal brain; Autism spectrum disorder; antibody; B cells; Caspr2

3. Accomplishments:

What were the major goals of the project?

The major goal of this project is to identify antibodies that can perturb fetal brain development and lead to an ASD-like phenotype. The second goal is to determine how frequently such antibodies are present in plasma of a mother of a child with ASD and whether each antibody contributes to a distinct phenotype.

What was accomplished under these goals?

Using our novel strategy to generate a monoclonal IgG from B cells of women with brain-reactive serology and a child with ASD we have identified three monoclonal antibodies, that target the membrane protein Contactin associated protein-like 2 (Caspr2) (**Figure 1**). Caspr2, encoded by the gene CNTNAP2, is expressed mainly in neurons, and rare variants of CNTNAP2 are associated with neurodevelopmental phenotypes. We characterized extensively one of the antibodies that bind Caspr2, C6 [7]. To demonstrate the contribution of C6 to ASD, mice were exposed in utero to C6 transported from the maternal blood or to B1 control antibody (an antibody that does not bind brain). We found amoralities in the developing brain that translated into behavioral phenotype in males but not females, exposed in utero to C6 , Our results are now published in *Molecular Psychiatry* [7], Importantly, we found similar changes to the developing cortex in mice exposed in utero to the plasma of the mother from whom which C6 was isolated **(Figure 2)**. There were no changes to the developing cortex when mice were exposed in utero to the same plasma after Caspr2-binding antibodies were depleted from it [7].

We examined how frequently antibodies with this specificity are present in women with an ASD child. We analyzed plasma of mothers of a child with ASD that had brain-reactive serology using indirect immunofluorescence on non-permeabilized Caspr2-transfected HEK 293T cells. We found that 37% displayed robust Caspr2 membrane staining, compared to 12% of plasma from mothers of an ASD child lacking brain-reactive antibodies, 12% of plasma from unselected women of child-bearing age, and 7.6% of plasma from mothers of a typically developing child bound Caspr2 [7].

We have started analyzing the specific epitope in Caspr2 to which C6 binds.

The following task have been accomplished:

Major Task 1: To characterize monoclonal brain reactive antibodies

Subtask 1: To obtain regulatory approval from the DOD – completed

Subtask 2: To produce large amounts of each monoclonal antibody

We have optimized our methods and have generated the amounts for 16 of the monoclonal antibodies needed to assess must of pathogenicity.

Subtask3: To inject all brain-reactive antibodies and isotope control antibodies into 14 pregnant mice each

We have begun to administer A7 which is also an anti-Caspr 2 antibody to pregnant mice **(Figure 1)**. We are currently collecting fetuses for cortical development analysis.

Subtask4: To examine behavior in antibody-exposed offspring (7 litters for each antibody)

We have done an extensive analysis of behavior in offspring exposed in utero to C6 antibody (**Figure 3** and also [7]). We have identified a second monoclonal antibody, A7, that binds Caspr2. We are in the process of generating enough adult mice exposed in utero to A7 for behavioral testing .

Subtask5: To analyze histology of antibody exposed fetal brain for each antibody (7 litters for each antibody)

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We have developed computer software for Windows to systematically quantify changes in the cortical plate. Up until now there has been no standardized method to measure the cortical plate. Since the cortical plate thickness is one of the measures that we use to evaluate the effect of in utero exposure to brain-reactive antibodies, this new program will allow us to make such assessments with increased uniformity and reliability. Specifically, with this application we are able to measure cortical plate area, cortical area, and the cortical plate to cortical width ratio (CP/CW). In order to obtain these measurements, we first manually mark the superficial and deep boundaries of the

cortical plate, and the edge of the ventricle on a fetal section stained with DAPI (**Figure 4A**). We then give the application a command to trace the curve that best fits each of these boundaries (**Figure 4B**). Once the curves are drawn, we identify the segment to be measured while designating a predetermined length along the ventricle to establish a perimeter. We then command the program to calculate the shortest distance from the ventricle to the superficial boundary of the cortex and to compute the area of the cortical plate and of the cortex to obtain the CP/CW (**Figure 4C**).

Milestone(s) Achieved

We have published a manuscript Molecular Psychiatry, 21(12):1663-1671 on the effects of the C6 anti-Caspr2 antibody on the developing mouse brain. We have demonstrated that it leads to an "autism-like" phenotype in male, but not female, mice.

IACUC Approval – yes

Major Task 2: To characterize maternal serum for reactivity to brain antigens

Subtask1: To develop ELISAs for each of the antigens bound by the monoclonal antibodies

We have developed live cell based assays for reactivity to Caspr2. We have learned that Caspr2 must be expressed in its native confirmation for C6 to bind. Therefore, solid based assay like ELISA will not be possible to develop for Caspr2 and C6 like antibodies. While cell based assays are cumbersome, one is clinically used for antibodies to Aquaporin 4, which are used to diagnose Neuromyelitis Optica [10].

We have used truncated forms of Caspr2 to localize an epitope seen by C6, but have failed to identify one. We believe that this is due to the structure of the protein which includes repeated regions and therefore truncating one region might not affect binding in a second region [11]. It is also possible that the structure of Caspr2 is affected when use truncated proteins, and therefore binding to C6 is lost.

We have now partnered with HealthTell, to use a peptide array which enables the simultaneous screening of 140,000 peptide antigens. We have sent for analysis 24 maternal monoclonal antibodies (including C6 and our B1-Control antibody) that we have expressed and screen for brain reactivity on histology and currently awaiting for results. Once peptides will be identified we will established an ELISA.

Subtask2: To test brain-reactive sera on the ELISAs~300 sera

We have analyzed 60 brain-reactive sera from mothers of a child with ASD, 60 non-brain-reactive sera from mothers of an ASD child, and 60 control sera from mothers of a typically developing child for reactivity to Caspr2 using the cell based assay, and are currently analyzing more of the sera available to us [4]

Milestone(s) Achieved

We have an assay to analyze anti-Caspr2 reactivity. Approximately 40% of women with anti-brain reactivity have antibodies to Caspr2 compared to a significantly smaller percentage in individuals without anti-brain reactivity. We have also identified 2 more antibodies that bind Caspr2 (**Figure 1**), and are assessing their pathogenicity in mice.

Research subjects

Plasma from mothers with an ASD child was obtained from the Simons Simplex Collection (SSC, http://sfari.org/resources/simons-simplex-collection) . Control plasma from women of childbearing age were obtained from the Northwell Health (previously North Shore-LIJ Health System) clinical laboratory and participants in a registry at the Feinstein Institute for Medical Research (http://www.gapregistry.org). Both cohorts were described previously [4]. Plasma of mothers of a typically developing child (determined by the mother report) were obtained from the Genotype and Phenotype registry (http://www.gapregistry.org) at the Feinstein Institute for Medical Research. The age of the

mothers at the time the plasma was drawn matched the previous cohorts [4] All individuals provided informed consent through the appropriate institutional review boards.

Sample Collection

Blood was collected into heparinized tubes from consenting mothers enrolled in the SSC, previously identified as having brain-reactive antibodies [4]. The protocol was approved by the SSC as well as by the Feinstein Institute for Medical Research Institute Review Board.

Single cell sorting

Isolation of single human memory brain-reactive B cells was performed as previously described [9] with several modifications. B cells were purified from fresh mononuclear cells by negative selection using a B cell kit (StemCell Technology). They were then incubated for 30 min at room temperature (RT) with human fetal brain lysate (3 µg per ml, Nouvus) labeled with biotin using the EZ-Link Sulfo-NHS-Biotin labeling kit (Life Technologies). Cells bound by biotinylated brain antigens were isolated with a biotin selection kit (StemCell Technologies) and stained with FITC conjugated anti-human CD19, phycoerythrin (PE) conjugated anti-human CD27 and allophycocyanin (APC) streptavidin to allow the separation of $CD19^+$, $CD27^+$, brain $lysate^+$ memory B cells. As a control, the fraction that initially was identified as non brain-reactive was incubated with biotinylated brain antigen and stained as described above. No APC positive cells were detected in this fraction. Finally, $CD19^+$, $CD27^{+,}$ APC^+ single cells were isolated on a BD FACSAria as described in [8].

cDNA Synthesis and RT-PCR

cDNA synthesis of individual IgH (γ only) and IgL chain (κ or ג) was performed as previously described [8,9]. Heavy and light chain variable region genes were ligated into IgG1 or κ constant region containing plasmids (a gift from M. Nussenzweig, Rockefeller University, NY).

Antibody Production

Antibodies were expressed in vitro as described previously [8], with few modifications. In brief, 24 h before transfection with both heavy and light chain plasmids, human embryonic kidney fibroblast 293T (HEK-293T) cells were split into a 100 x 20 mm culture dish in high glucose DMEM (HyClone, GE Healthcare), supplemented with heat inactivated fetal bovine serum (FBS, 10%), glutamine (1%) and penicillin-streptomycin (1%, HyClone, GE Healthcare). Subsequently, 8 h before transfection, the medium was changed to SFM4Transfx-293 (HyClone, GE Healthcare) supplemented with glutamine (1%) and penicillin-streptomycin (1%). Cells were then cotransfected with plasmid DNA encoding IgH and IgL chains (5 µg), using Lipofectamine 2000 (Life Technologies). Supernatants were collected after 7 days of culture. Antibodies were purified on protein G–sepharose (GE Healthcare, Life Technologies), eluted with glycine buffer (0.1 M, pH 3.5) and neutralized in Tris-HCl (1 M, pH 8). Antibody concentrations in supernatants were determined by anti–human IgG ELISA [8,9]. Purified antibodies was dialyzed extensively against PBS; their integrity was determined by nonreducing SDS gels stained with Coomassie blue and their concentration was measured by both anti–human IgG ELISA [8,9] and Nanodrop.

Binding assays using transfected HEK-293T cells

Plasma and the human monoclonal antibodies, C6, A7, C9 and B1, [12] were analyzed for binding to Caspr2 using a live cell-based immunofluorescence assay as previously described [10]. HEK-293T cells were transfected using Tgfp-Caspr2 or Tgfp vector (Origene) and cultured for 72 h. Cells were stained with C6 or B1 (10 µg per ml in PBS/10% FBS). Antibody binding to Caspr2 transfected cells was detected by Alexa 594-conjugated goat anti-human IgG (Life Technologies). To test for the presence of anti-Caspr2 antibodies in plasma, cells were blocked with goat IgG (Sigma-Aldrich) in PBS and FCS (10%), incubated with pre-absorbed (rabbit liver powder, Sigma-Aldrich) plasma samples (dilution 1:100 and 1:200). IgG binding was detected with Alexa 594-conjugated goat anti-human IgG (Life

Technologies). Dead cells were visualized with DAPI staining (Sigma-Aldrich) and live cells were analyzed for Caspr2-IgG binding. Commercially available Anti-Caspr2 antibody directed to an extracellular epitope (NeroMab! UC Davis, CA) served as positive control. Anti-Caspr2 antibody directed to a cytoplasmic epitope (Abcam) served as negative control. Cells transfected with tGFP vector and non-transfected cell also served as controls.

Antibody administration to pregnant dams

C57BL/6 mice (6–8 weeks old) were obtained from the Jackson Laboratory. Animal use was in accordance with institutional guidelines of the Feinstein Institute for Medical Research. For timed pregnancy, 2 females and 1 male were housed together for 14 h. The time when the male mouse was removed from the cage was designated embryonic (E) day 0.5. At E13.5, either C6 antibody (200 µg) or B1 control antibody (200 µg) were administrated by retro-orbital injection to time-pregnant mice under light anesthesia[12]. Embryos were harvested at E15.5 and processed for sex identification (described in [13]) and fetal brain pathology. Additional pregnancies were allowed to reach full term.

Immunohistology of fetal brains

E15.5 brains were fixed in paraformaldehyde (4%) overnight at 4°C followed by sucrose solution (30%) for 48 h at 4°C and then frozen in OCT. compound (Sakura) on dry ice and stored (-80˚C). Sagittal sections were cut (12 µm thick) on a Cryostat (Leica) and mounted on gelatin-coated slides and stored (-80˚C). Prior to staining, sections were thawed to RT, rinsed twice with PBS and blocked for 1 h with PBS (5%) with bovine serum albumin (BSA) in Triton X100 (0.1%) at RT. Anti-$PH3^+$ antibody (1:100, Millipore) or anti-nestin antibody (1:200, Millipore) and DAPI (1 µg per ml, Life Technologies) were added overnight at 4°C. After washing in PBS/0.1%Tween, antibody binding was detected using Alexa 488 goat anti-rabbit or anti-mouse IgG (Life Technologies) and visualized with an Axio-Imager (Z-1, Axio-Vision 4.7, Zeiss). $PH3^+$ cell quantification was performed as described in [12]. Cortical plate and cortical width measurements were obtained from multiple sections of each animal, described in [12]

Behavioral assessments

Mice that were exposed in utero to C6 or B1 were assessed at 10-14 weeks of age. They were maintained on a reverse schedule of darkness (09:00 to 21:00) and light (21:00 to 9:00), with ad libitum access to food and water. One week before testing, mice were handled for 5 days in sessions of 5–10 min during the dark period of their circadian cycle. A behavioral screen [14] was conducted to ascertain that autonomic responses and neurological reflexes were normal in C6-exposed mice (data not shown). An open field test was used to examine that locomotion was normal in C6-exposed mice; each mouse was placed in the center of a square arena (40 cm on the side) with black walls (30 cm high) and was allowed to move freely for 10 min. Animal behavior was recorded with a centrally-placed video camera using video tracking software (EthoVision v8.5, Noldus, Attleboro, MA, USA). We also analyzed the occupancy of the center of the arena (10 x 10 cm square) as a measure of anxiety and found that C6-exposed mice were comparable to B1-exposed mice (data not shown). The behavioral tests resembling the core symptomps of ASD were the marble burying assay, the social preference test, and the clockmaze task. They were performed sequentially, and a resting period of at least 48 h occurred between tests. The marble burying assay [15] was conducted in a cage (38×26 cm^2, 18 cm high walls) with soft bedding (4.5 cm deep), in which 20 black glass marbles (1.2 cm diameter) were placed in a 4×5 arrangement. The day before the experiment each mouse was familiarized to the cage (without the marbles) for 20 min and the next day, it was placed in the cage (with marbles) for 30 min. The number of marbles buried (> 50% marble covered by bedding material) was recorded. The social preference test was performed in a Y-shaped maze (each arm was 27 x 14 cm^2, 20 cm high walls made of Plexiglas) with a thin layer of bedding placed on the floor. One arm of the Y maze contained a novel object (made of plastic, ~5 cm diameter and 5 cm in height, located at the end of the arm), a second arm had a mouse (placed inside an inverted strainer cup) that had never been in contact with the test mouse, while the third arm was empty. The use of the strainer cup containing the novel mouse ensured that social approach was initiated by the subject mouse only. A day prior to the test, each mouse was placed in the empty Y maze for 10 min, and the next day it was

placed for 10 min and its behavior was recorded (Ethovision v8.5). The object was cleaned with ethanol and water between each run. The times spent exploring the object and the novel mouse were used to analyze social preference. The clockmaze task [14] was performed in a circular maze with 12 exits that were located in the wall of the arena like the numbers on the face of a clock. All exits were blocked (black plugs) except for one that led to a tunnel. Mice learned to escape from the arena, which was filled with water (20°C) to 2 cm depth, sufficient to wet the underside of the belly of mice and provide motivation to find the exit. In the first phase, each animal underwent 6 trials per day, on 2 consecutive days, with an inter-trial interval of at least 20 min. In the second phase, the location of the exit was changed and mice underwent 6 trials on 1 day. The trials were recorded with software (Ethovision 8.5) and the latencies to escape were used to measure learning. A flexibility ratio was computed by the following equation: $(L2 – L1) / (L2 + L1)$, in which L1 was the average inverse of the latency for the last 3 trials in the first phase and L2 was the average inverse of the latency for the first 3 trials on the second phase of the task.

Statistical analysis
We used analysis of variance (ANOVA) as well as Student's t-test for datasets that were normally distributed (and with samples larger than 10). For smaller datasets, we used the Mann-Whitney test. To analyze categorical data, a Chi-squared test for independence was used. The nonparametric Kolmogorov Smirnov test was used for large datasets that were not normally distributed. All tests were performed with the statistical toolbox of Origin (versions 9 and 11), and are indicated in the text. Values were considered significant for $p < 0.05$.

What opportunities for training and professional development has the project provided?
This project has allowed post doctoral fellows to learn single cell cloning, histology quantification techniques and behavioral phenotypes in mice. Lior Brimberg has been invited to present her work at a podium session at the Society for Neuroscience annual meeting in 2015, and put together a panel session focuses on maternal immune system and autism for 2017 International Meeting for Autism Research (IMFAR) (pending approval).

How were the results disseminated to communities of interest?
A manuscript on these data has been published.

What do you plan to do during the next reporting period to accomplish the goals?
During the next year, we will identify the epitope of Caspr2 recognized by the monoclonal antibody. We will then determine which women have antibodies to this epitope and explore the phenotype of their affected children

4. Impact:
These data demonstrate unequivocally that anti-brain antibody can cause an ASD-like phenotype.

What was the impact on the development of the principal discipline(s) of the project?
Nothing to Report

What was the impact on other disciplines?
Nothing to Report

What was the impact on technology transfer?
Nothing to Report

What was the impact on society beyond science and technology?
Nothing to Report

5. Changes/Problems:

Changes in approach and reasons for change.
Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them.
Nothing to Report

Changes that had a significant impact on expenditures.
Nothing to Report

Significant changes in use or care of human subjects - Nothing to Report
Significant changes in use or care of vertebrate animals - Nothing to Report
Significant changes in use of biohazards and/or select agents - Nothing to Report

6. Products:

Publications, conference papers, and presentations:
International Society of Neuroimmunology (ISNI), The International Meeting for Autism Research (IMFAR), Society for Neuroscience

Journal publications:

Brimberg, L; Mader, S; Jeganathan, V; Berlin, R; Coleman, RT, Gregersen, PK; Huerta ,P; Volpe, BT; Diamond, B (2016): Caspr2-reactive antibody cloned from a mother of an ASD child mediates an ASD-like phenotype in mice. Molecular Psychiatry, 21(12):1663-1671
Also highlighted in Nature Reviews Neurology (Malkki, October 2016)

Books or other non-periodical, one-time publications:

Other publications, conference papers, and presentations

Brimberg, L; Mader, S; Jeganathan, V; Berlin, R; Coleman, RT, Gregersen, PK; Huerta ,P; Volpe, BT; Diamond, B (2016): "Caspr2-reactive antibody cloned from a mother of an ASD child mediates an ASD-like phenotype in mice" International congress of Neuroimmunology (ISNI);2016, Jerusalem, Israel.

Website(s) or other Internet site (s)
http://www.feinsteininstitute.org/2015/10/feinstein-institute-researchers-discover-that-maternal-antibodies-are-risk-factors-for-autism-spectrum-disorder/

https://spectrumnews.org/news/maternal-immune-molecule-triggers-autism-symptoms-in-male-mice/

https://www.autismspeaks.org/science/science-news/another-study-implicates-maternal-antibodies-one-cause-autism

Technologies or techniques

Inventions, patent applications, and/or licenses

Other products

7. Participants & other collaborating organizations
What individuals have worked on the project?

Dr. Lior Brimberg – No Changes
Dr. Betty Diamond: No Changes
Dr. Peter Gregersen – No Changes
Dr. Patricio Huerta – No Changes
Dr. Bruce Volpe – No Changes

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Dr. Betty Diamond

Complete Research:
1 R01 AR049126-06 (Diamond) 09/15/02 – 08/31/14
NIH/NIAMS - Neuropsychiatric SLE
Antibodies to NR2 in SLE

1P01AI073693-02 (Diamond) 08/01/08 – 07/31/14
NIH/NIAID – **Anti-NMDA receptor antibodies in adult brain dysfunction and fetal brain development: lessons from lupus**
Project 4 (Diamond)
Preventing anti-NMDAR Ab neurotoxicity: Rational Therapies for NPSLE

NIH R01AR057084 (Diamond) 07/01/10 – 06/30/15
Toward a new approach to SLE therapy

Alliance for Lupus Research (Gregersen & Diamond) 07/01/12 – 06/30/14
Functional analysis of CSK: A newly defined risk gene for lupus

R21 AR063929 (Diamond) 07/01/13-06/30/15
Nelfinavir in SLE: A pilot phase IIa clinical trial

NIH R21 AR065199 (Gregersen) 07/01/13 – 06/30/15
(Diamond co-investigator)
TNIP1 risk haplotypes and immune endophenotypes

NIH U34 AR063407 (Mackay) 09/25/13-08/31/15
Treatment of SLE with ajulemic acid, a non-psychoactive cannabinoid derivative
(Diamond co-investigator)

Empire Clinical Research Investigator Program (ECRIP) 01/15/14-02/15/15

An evolutionary approach to SLE therapy
Project Director (Diamond)

Biogen Idec (Diamond & Gregersen) 07/01/14 - -6/30/16
Investigation of Blimp1 in SLE Patients, Unaffected Sisters and Normal Controls
NIH R43 MH106195 (Burch, R) 02/05/15 – 01/31/16
(Diamond PI)

Autism Spectrum Disorder Diagnostic/Therapeutic Agent
The goal of this research project is to translate molecular insights on pathogenic mechanisms in ASD.
This proposal is the study of T_{FH} cells in SLE

Active Research:
1P01AI073693-06 (Diamond) 07/1/14-06/30/19
NIH/NIAID – **Anti-NMDA receptor antibodies in adult brain dysfunction and fetal brain development: lessons from lupus**
Project 3 (Diamond)
Antibody modulation of neural endocrine network
This is a study of prolactin modulation by anti-NMDAR antibodies.

R21 AR063929 (Diamond) 07/01/13 – 06/30/17
Nelfinavir in SLE: A pilot phase IIa clinical trial
This is a study of the ability of nelfinavir to block DNA binding.

NIH U34 AR063407 (Mackay) 09/25/13-08/31/16
Treatment of SLE with ajulemic acid, a non-psychoactive cannabinoid derivative
(Diamond co-investigator)
This Phase IIa clinical trial will test the safety and efficacy of a non-psychoactive cannabinoid derivative, ajulemic acid, on patients with lupus that have mild to moderate disease activity.

Lupus Research Institute (Diamond) 02/01/14 – 01/31/17
Autoantibodies and prolactin participate in a positive feedback loop
This is a study of the role of anti-NMDAR antibodies in triggering autoantibody production. The overlap with P01AI073693 is being addressed.

NIH 1UM1AI110494 (Aranow) 05/01/14 – 04/30/19
Proposal for The Feinstein Center for Clinical Research in Autoimmune Disease
(Diamond-Mechanistic Investigator)
This is a proposal to establish an Autoimmunity Centers of Excellence Basic Science Center.

NIH P01 AI102852 (Diamond & Tracey) 08/01/14 – 07/31/19
NIH/NIAID - **Cognitive and immune impairments in sepsis survivors**
Project 2 (Diamond)
Immune function in sepsis survivors
Study of immune impairment in monocytes following sepsis.

NIH R01 AR065209 (Kim) 09/01/14-08/31/18

(Diamond co-investigator)
The Blimp-1 SLE risk variant regulates inflammatory function in dendritic cells
This grant is to study the function of the PRDM1 SNP rs548234 in dendritic cells for predisposition to SLE.

NIH UH2 AR067688 (Diamond) 09/24/14-05/31/19
PEARL: Pathway exploration and analysis in renal lupus
This a proposal to apply state of the art genomic and immunophenotyping technologies to lupus nephritis.

NIH/NIAID IUM1AI1095650 (Nepom. G) 05/01/14 – 06/30/19
Co-Chair Diamond
CALIBRATE – Rituximab Plus Cyclophosphamide followed by Belimumab
This is a clinical trial to determine if rituximab followed by belimumab educes autoreactive B cell repertoire.

The NLM Family Foundation (Diamond) 04/01/15 – 03/31/18
Maternal antibody as a contributor to Autism Spectrum Disorder
This is a proposal to develop a decoy antigen to protect the fetal brain from autism related antibodies.

NIH R21AR067012-01A1 (Diamond) 06/01/15 – 05/31/17
Function of the IRF5 risk allele for SLE in B cells
This is a study of the functionality of the IRF5 risk allele in B cells of healthy individuals.

NIH 1UG3OD023391-01 (Gregersen & Diamond) 09/21/16 -08/31/18
Prenatal autoimmune and inflammatory risk factors for Autism Spectrum Disorders
This is a study of neurodevelopmental abnormalities in offspring of women with autoimmune disease

Dr. Peter Gregersen

Completed Research:

Biogen/IDEC **Biomarkers of Anti-TNF-α Therapy Efficacy in Rheumatoid Arthritis to Define Unresponsive Patients (BATTER-UP)** 6/1/10-5/31/15

Alliance for Lupus Research- **Functional Analysis of Csk** 8/1/12-7/31/14
Gregersen/Diamond (PIs)

NIH - R21 **TNIP1 risk haplotypes and immune endophenotypes** 8/1/13-7/31/15
Gregersen (PI), Diamond (Co-PI)

NIH-UH2AR067694-01– AMP-**Molecular and Cellular Dissection of Early Rheumatoid Arthritis**
Gregersen (PI) 10/01/14-05/31/16
Janssen R&D 07/01/15 – 08/31/16
Gregersen (PI)
The goal is to achieve a deeper understanding of the response to biologic therapy, with a view to developing clinically useful predictive biomarkers that can guide current therapies, and enhance the efficiency of clinical trials.

Active Research:

NIH– R01AR065209
Blimp-1 SLE risk variant regulates inflammatory function in dendritic cells
Kim (PI) 09/01/14 – 08/31/18
This grant is to study how a Blimp-1 variant associated with systemic lupus regulates the function of dendritic cells and promotes lupus pathogenesis.

NIH ACE – 1UM1AI110494-01
The Feinstein Center for Clinical Research in Autoimmune Diseases
Aranow (PI) 05/01/14 – 04/30/19
The goal is to establish an Autoimmunity Centers of Excellence Basic Science Center

Dr. Patricio Huerta

Completed Research:

5R01AR049126-10 (Diamond) 7/1/2002 – 6/30/2014
NIH/NIAMS
Antibodies to NR2 in SLE
The goal is to determine the brain neurotoxicity of DNA-reactive autoantibodies, produced by patients with SLE, which cross-react with the NR2 subunits of the N-methyl-D-aspartate receptor.

5P01AI073693-05 (Diamond) 8/1/2008 – 7/31/2014
NIH/NIAID
Anti-NMDA receptor antibodies in brain dysfunction: Lessons from lupus
Project 1 (Huerta)
NIH/NIAID
Mechanisms of neurotoxicity of lupus anti-NMDAR antibodies: Electrophysiology to behavior
The goal is to establish the molecular mechanisms of neurotoxicity of NMDAR-reactive antibodies, using ex vivo and in vivo approaches, and their effects on cognitive behaviors.

5P01AI073693-05 (Huerta)
NIH/ NIAID
Behavioral Core
The goal is to develop a core facility to perform behavioral assessments of mice immunized with a diverse panel of NMDAR-reactive antibodies.

R01-AG042508 (Marambaud) 5/9/2013 – 6/30/2017
NIH/NIA
Mechanisms of regulation of amyloid beta metabolism by CALHM1
The goal is to determine the role of the CALHM1 protein in the onset of amyloid beta deposition with the use of murine models.

Active Research:

5P01AI073693-06 (Diamond) 8/1/2008 – 06/30/2019
NIH/NIAID
Anti-NMDA receptor antibodies in adult brain dysfunction, & fetal brain Development
Project 1 (Co-PI)
"Modeling variable outcomes of antibody exposure"
This is a study of maintaining the integrity of the blood-brain barrier which does not overlap with the current proposal.

NIH P01 AI102852 (Diamond & Tracey) 04/01/14 – 03/31/19
NIH/NIAID – "Cognitive and immune impairments in sepsis survivors"
Project 1 (Co-PI)
"Nervous system alterations in sepsis-surviving mice"
This project investigates the effects of cytokines on neuroanatomy and has no relation to the current efforts
Core C: Behavioral Function (PI)
The goal is to assess animal models of sepsis-related cytokine elevation through behavioral and neural testing.

Dr. Bruce Volpe

Completed Research:

NIH, PO1 AI 073693 (Diamond PI) Sept 08- August 2014
"Anti-NMDA receptor antibodies in brain dysfunction, lessons from lupus"
Project 2 – (Volpe PI)
"Developmental Neurotoxicity: Maternal anti-NMDAR antibodies and fetal development"
Multiple families of antibodies that recognize the NMDA receptor are produced in patients with lupus. This project will study the effects of these molecules wit in vivo and in vitro techniques and with clinical experiments. There is no overlap with the current proposal.

NIH, RO1 AR49126-01 (Volpe co-I) Sept 07- August 2014
"Antibodies to NR2 in SLE"
The goal is to develop the scientific foundation for preventative therapies for cognitive decline in SLE. There are no experiments in this proposal that overlap with the current request.

Active Research:

NIH, PO1 AI 073693 (Diamond PI) Sept 2008- August 2019
"Anti-NMDA receptor antibodies in adult brain dysfunction, & fetal brain Development"
Project 1 – (Volpe PI)
"Modeling variable outcomes of antibody exposure"
This is a study of maintaining the integrity of the blood-brain barrier which does not overlap with the current proposal and a study of peptide therapy which includes partial overlap with the current proposal.
There is no overlap with the current proposal.

NIH P01 AI102852 (Diamond & Tracey) April 2014 – March 2019
NIH/NIAID – "Cognitive and immune impairments in sepsis survivors"
Project 1 (Volpe)
"Nervous system alterations in sepsis-surviving mice"
This project investigates the effects of cytokines on neuroanatomy and has no relation to the current efforts.
Core D **Histology** (Volpe)
The histology core will provide services to all the investigators in each of the projects. Quantitative pathological neuroanatomy is important to several of the experiments. Also the pathological correlations are important in animals where behavior is measured.

NIH 1 RO1 HD 069776-01 (PI D Edwards) Jan 12- Dec 2017
"Transcranial direct current stimulation and robotic training in stroke"
This project will focus on a randomized controlled test of tDCS provided before robot therapy, using outcomes measures that will provide insight into mechanisms of the effect of the tDCS via changes in brain physiology and motor control. There is no overlap of the experiments in this proposal.

Appendices:
None
What other organizations were involved as partners? None
8. **SPECIAL REPORTING REQUIREMENTS** – NA
9. **APPENDICES -** NA

References

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3. Braunschweig, D., *et al.* Autism-specific maternal autoantibodies recognize critical proteins in developing brain. *Transl Psychiatry* **3**, e277 (2013).
4. Brimberg, L., Sadiq, A., Gregersen, P.K. & Diamond, B. Brain-reactive IgG correlates with autoimmunity in mothers of a child with an autism spectrum disorder. *Mol Psychiatry* **18**, 1171-1177 (2013).
5. Bauman, M.D., *et al.* Maternal antibodies from mothers of children with autism alter brain growth and social behavior development in the rhesus monkey. *Transl Psychiatry* **3**, e278 (2013).
6. Singer, H.S., *et al.* Prenatal exposure to antibodies from mothers of children with autism produces neurobehavioral alterations: A pregnant dam mouse model. *Journal of neuroimmunology* **211**, 39-48 (2009).
7. Brimberg, L., *et al.* Caspr2-reactive antibody cloned from a mother of an ASD child mediates an ASD-like phenotype in mice. *Mol Psychiatry* **21**, 1663-1671 (2016).
8. Tiller, T., *et al.* Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. *J Immunol Methods* **329**, 112-124 (2008).
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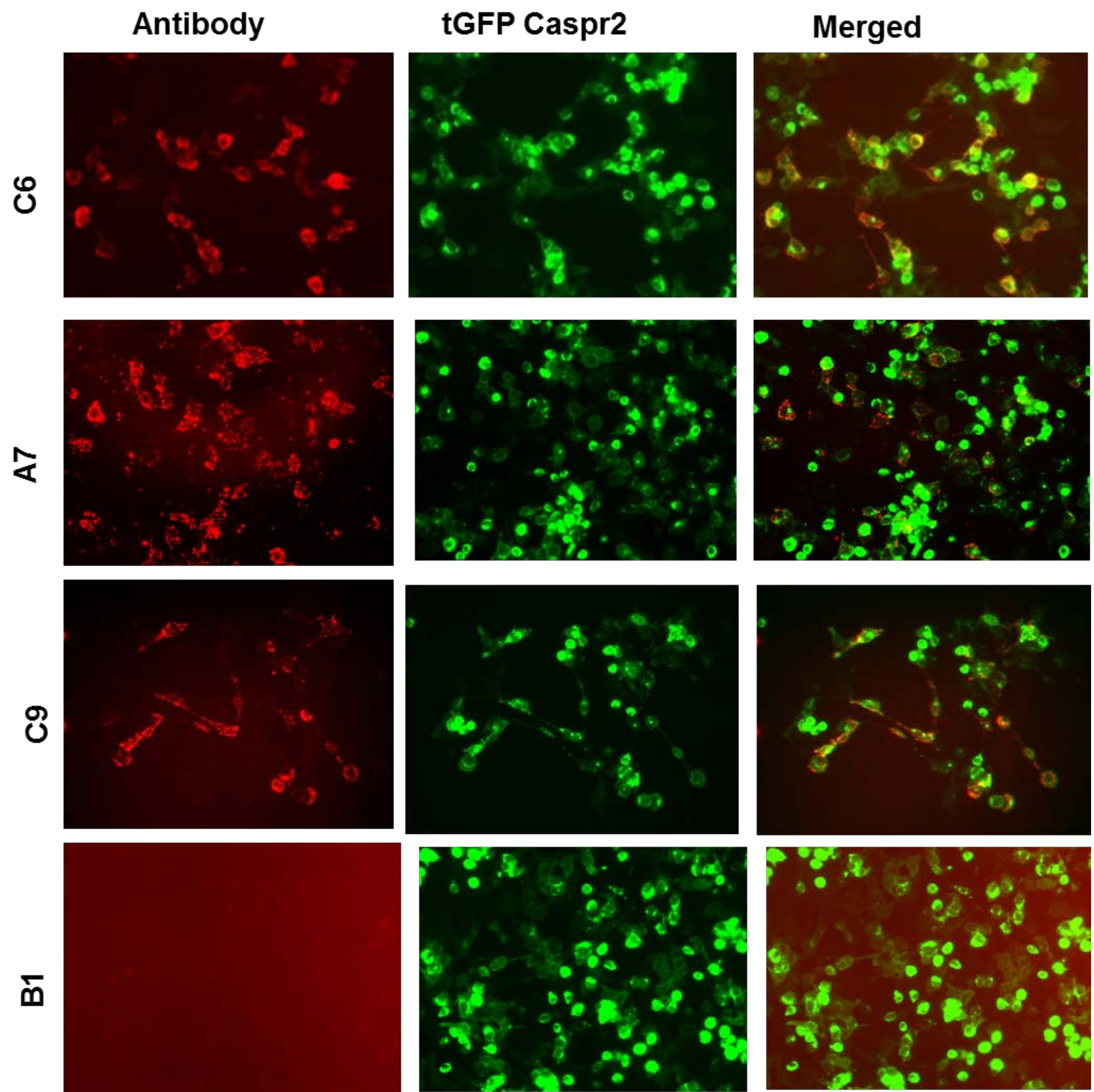
15. Thomas, A., *et al.* Marble burying reflects a repetitive and perseverative behavior more than novelty-induced anxiety. *Psychopharmacology (Berl)* **204**, 361-373 (2009).

Figure 1: Brain-reactive monoclonal antibody C6 binds to Caspr2.
(A) C6 , A7 and C9 but not control B1 (bottom panels) antibody co-localize with Caspr2 on HEK 293T cells, expressing tGFP-Caspr2. No staining was seen on cells expressing only tGFP or non-transfected cells (data not shown).


Antibody
tGFP Caspr2
Merged
C6
A7
C9
B1

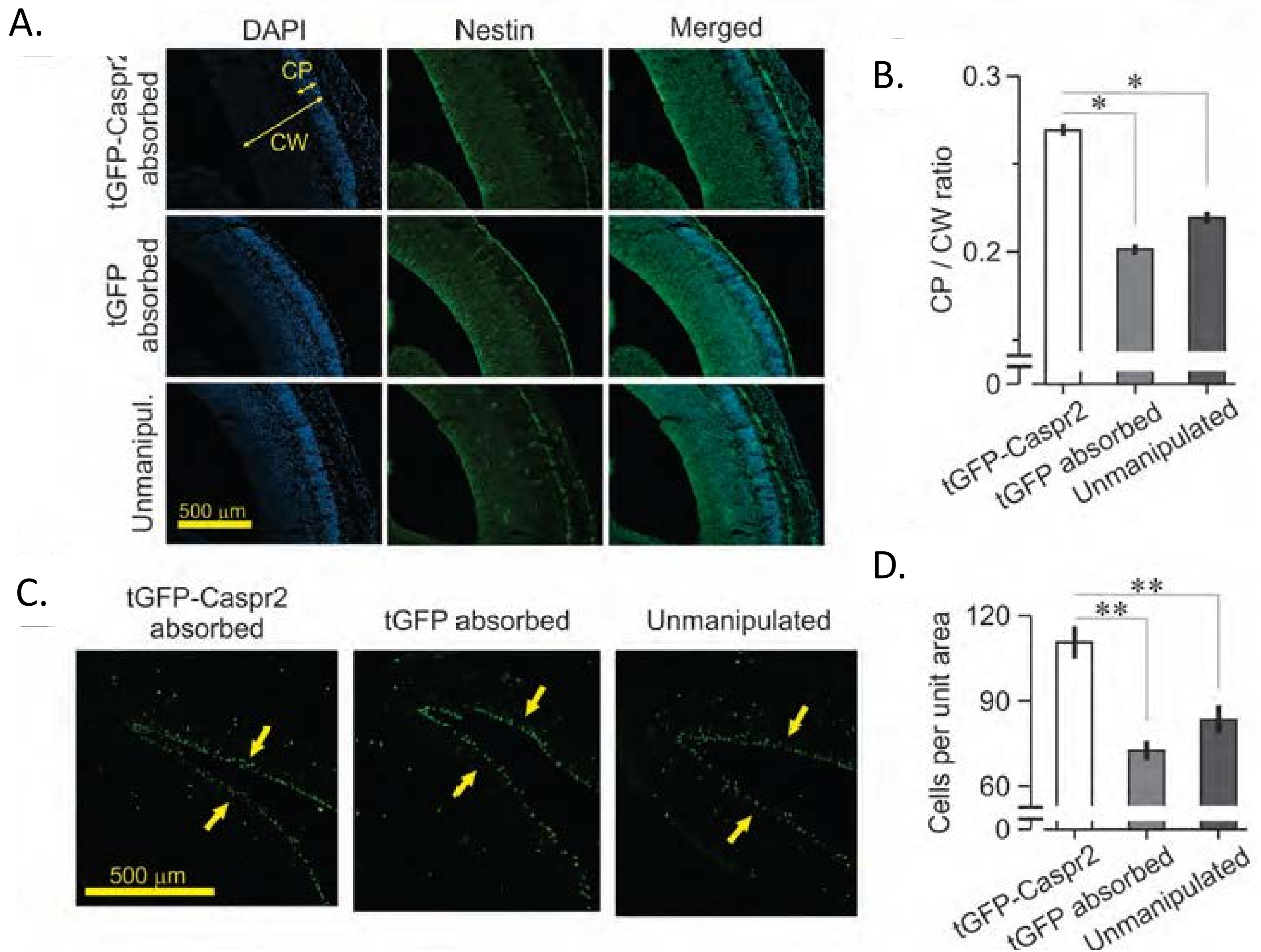


Figure 2: (A) The cortical plate is thinner in male fetal brains exposed to tGFP-absorbed plasma (middle panel) and unmanipulated plasma (bottom panel) relative to tGFP-Caspr2 absorbed plasma (top panel) as revealed by DAPI (left) and nestin (middle) staining (B) Quantification of cortical plate thickness, the ratio of cortical plate to cortical width, * $P < 0.05$, $Z = 2.5$, Mann-Whitney test. (C) Exposure *in utero* to tGFP-absorbed plasma (middle panel) and unmanipulated plasma (right panel) leads to fewer mitotic cells than exposure to tGFP-Caspr2 absorbed plasma (left panel) revealed by PH3 staining in the ventricular zone of male brains. (D) Quantification of mitotic cells (PH3+), ** $P < 0.001$, $t = 5.7$, t test. Unit area = 62.5 mm^2. Mice used: tGFP-Caspr2 absorbed plasma, n = 5; tGFP-absorbed plasma, n = 3; unmanipulated plasma, n = 5.

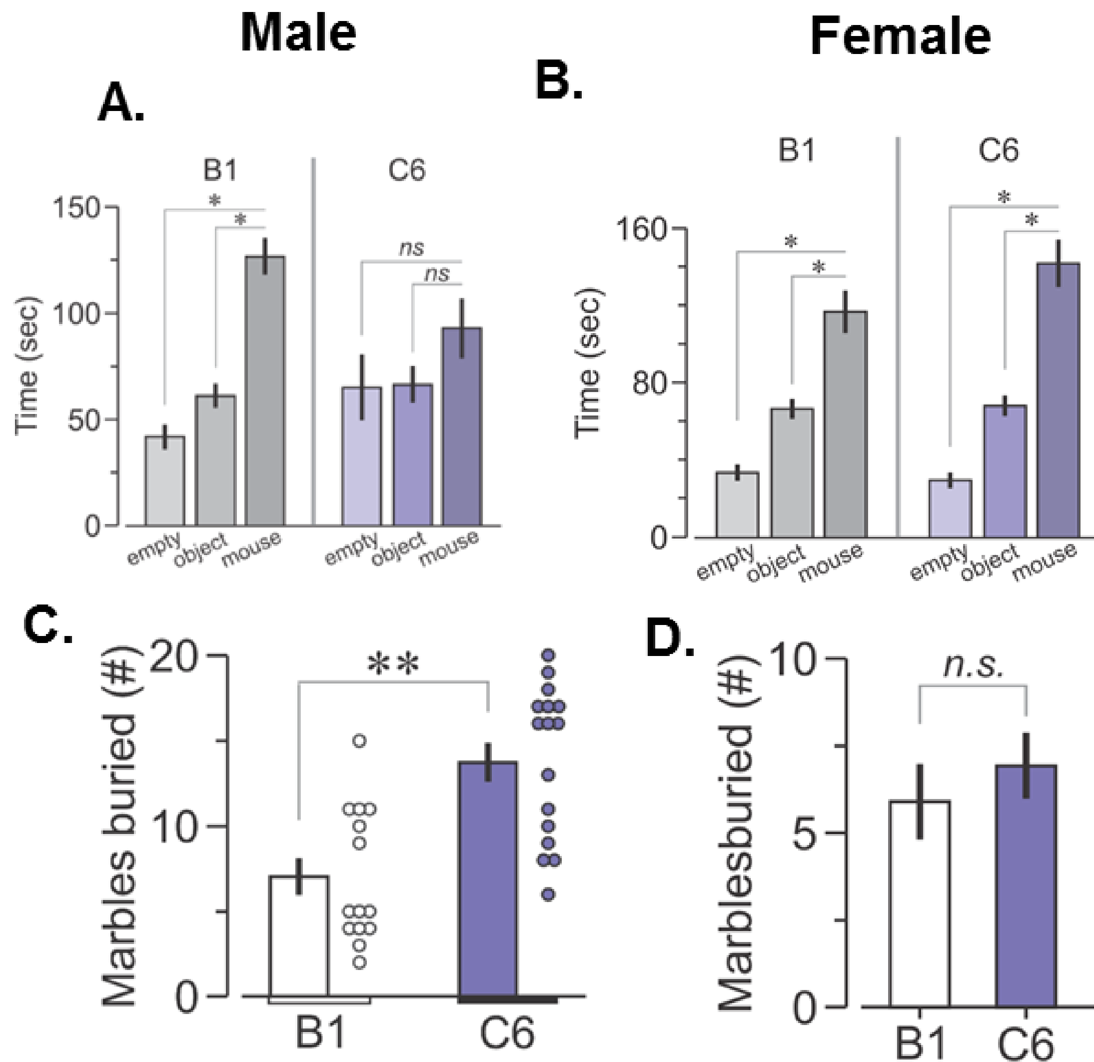


Figure 3: Examples of behavioral alterations of C6-exposed male but not female offspring in tasks that resemble core ASD symptoms. Maternal C6 antibody-exposed mice were subjected to behavioral assessment at adulthood (10–14 weeks). (A-B) Social approach task. (A) Control B1 (non brain-reactive antibody)-exposed male mice displayed normal sociability, defined as spending significantly more time with the novel mouse compared to the novel object, whereas C6-exposed male mice spent a similar amount of time near the novel object and the novel mouse. ANOVA, followed by Bonferroni test, $P < 0.05$. (B) B1- and C6-exposed female mice displayed normal sociability, ANOVA, Bonferroni , $P < 0.05$. (C-D) Marble burying task. (C) C6-exposed mice display enhanced stereotypic behavior i.e, they bury more marbles than the B1-exposed mice; ** $P < 0.0005$, *t*- test;. (D) B1- and C6-exposed female mice buried similar number of marbles.

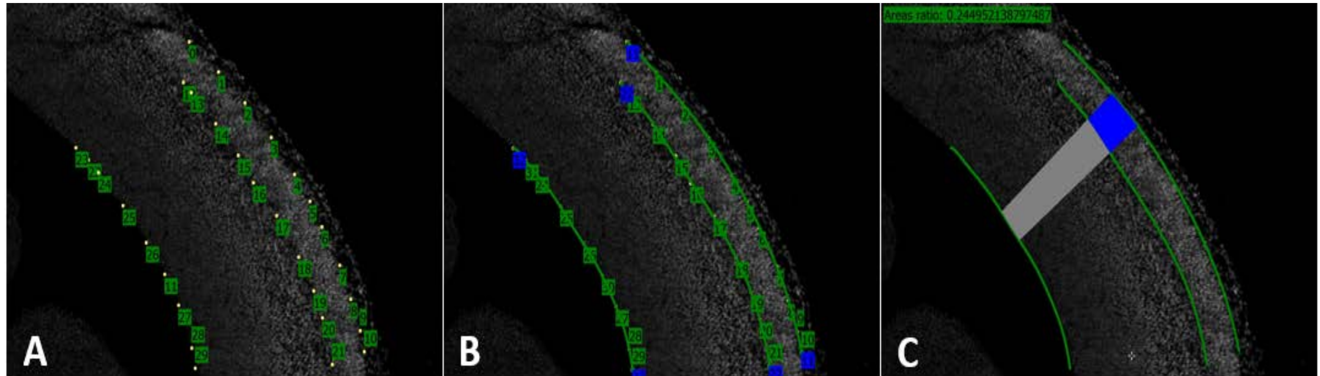


Figure 4. Cortical plate measurement software illustration.
(A) Boundary marking. Marks 0-10 and 12-21 delineate the superficial and the deep boundaries of the cortical plate respectively. Marks 11 and 22-29 trace the edge of the ventricle. (B). Best fit curves. The application traces the curve that best fits each boundary (Line 11 = superficial boundary of the cortical plate, Line 22 = deep boundary of the cortical plate, and Line 32 = edge of the ventricle). ©. Calculation of the areas and CP/CW. After the user selects the region to be measured, the program calculates the shortest distance from the ventricle to the superficial boundary of the cortex and computes the area of the cortical plate (blue) and of the cortex (blue and grey) to obtain the CP/CW.