



# Maternal Antibody and ASD: Clinical Data and Animal Models

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Over the past several decades there has been an increasing interest in the role of environmental factors in the etiology of neuropsychiatric and neurodevelopmental disorders. Epidemiologic studies have shifted from an exclusive focus on the identification of genetic risk alleles for such disorders to recognizing and understanding the contribution of xenobiotic exposures, infections, and the maternal immune system during the prenatal and early post-natal periods. In this review we discuss the growing literature regarding the effects of maternal brain-reactive antibodies on fetal brain development and their contribution to the development of neuropsychiatric and neurodevelopmental disorders. Autoimmune diseases primarily affect women and are more prevalent in mothers of children with neurodevelopmental disorders. For example, mothers of children with Autism Spectrum Disorder (ASD) are significantly more likely to have an autoimmune disease than women of neurotypically developing children. Moreover, they are four to five times more likely to harbor brain-reactive antibodies than unselected women of childbearing age. Many of these women exhibit no apparent clinical consequence of harboring these antibodies, presumably because the antibodies never access brain tissue. Nevertheless, these maternal brain-reactive antibodies can access the fetal brain, and some may be capable of altering brain development when present during pregnancy. Several animal models have provided evidence that *in utero* exposure to maternal brain-reactive antibodies can permanently alter brain anatomy and cause persistent behavioral or cognitive phenotypes. Although this evidence supports a contribution of maternal brain-reactive antibodies to neurodevelopmental disorders, an interplay between antibodies, genetics, and other environmental factors is likely to determine the specific neurodevelopmental phenotypes and their severity. Additional modulating factors likely also include the microbiome, sex chromosomes, and gonadal hormones. These interactions may help to explain the sex-bias observed in neurodevelopmental disorders. Studies on this topic provide a unique opportunity to learn how to identify and protect at risk pregnancies while also deciphering critical pathways in neurodevelopment.

**Keywords:** brain-reactive antibodies, autism spectrum disorder, neurodevelopmental disorders, sex bias, gonadal hormones, sex chromosomes, microbiome

## INTRODUCTION

The increasing evidence of an immune mediated pathogenesis for neuropsychiatric and neurodevelopmental disorders has shifted the focus of epidemiologic studies to include the contribution of cytokines and brain-reactive antibodies. The brain was originally thought to be an immune privileged organ due to the presence of the blood brain barrier (BBB), a structure composed of endothelial cells knit together by tight junctions and supported by astrocytic endfeet (1). We now know that even though the BBB isolates the central nervous system (CNS) from factors in the blood, it is a dynamic semipermeable structure. Immune molecules including antibodies can access the CNS during both physiologic and pathologic states. Even though antibodies cannot cross the BBB and access brain tissue in healthy adults, these molecules may cross the BBB during *in utero* development when the BBB is immature and more permeable (2). Alternatively, antibodies can penetrate the adult brain when there is a BBB breach as occurs during inflammation (3–6) or at sites of limited BBB protection such as the choroid plexus. Factors affecting BBB integrity include: trauma, ischemia, stress, aging, antibodies, and specific agonists of endothelial cell receptors, such as cytokines, complement, and antibodies themselves (7–12). Once in the CNS, antibodies can lead to pathology if they recognize antigens expressed in the brain or spinal cord. In this review we discuss how maternal brain-reactive antibodies affect fetal brain development, contributing to the risk of neuropsychiatric and neurodevelopmental disorders. We focus on antibodies implicated in Autism Spectrum Disorder (ASD) and propose a role for the microbiome, sex chromosomes and gonadal hormones in determining the susceptibility to the effects of maternal antibody and the development of neurodevelopmental disorders.

**Abbreviations:** ASD, Autism Spectrum Disorder; BBB, Blood Brain Barrier; CNS, Central Nervous System; AD, Autoimmune disease; SLE, Systemic Lupus Erythematosus; NMO, Neuromyelitis Optica; LPS, lipopolysaccharide; NSPA, neuronal surface P antigen; NMDAR, N-methyl-D-aspartate receptors; DNRAb, SLE anti-DNA antibodies that cross-react with N-methyl-D-aspartate receptors; FcRn, neonatal Fc receptor; ADHD, Attention Deficit and Hyperactivity Disorders; DSM-V, Diagnostic and Statistical Manual of Mental Disorders Fifth Edition; ND, neurodevelopmental and neuropsychiatric disorders; NT, neurotypically developing; PPD, Pervasive Developmental Disorders; LD, Learning disabilities; TS, Tourette Syndrome; RA, rheumatoid arthritis; TPOAbs, Thyroid peroxidase antibodies; Abs, antibodies; P, post-natal day; IHC, Immunohistochemistry; NB, Neuroblastoma; WB, Western blot; LDH-A, lactate dehydrogenase A; LDH-B, lactate dehydrogenase B; YBX1, Y-box bonding protein 1; STIP1, stress-induced phosphoprotein 1; CRMP1, collapsing response mediator protein 1; CRMP2, collapsing response mediator protein 2; GDA, guanine deaminase; MSEL, Mullen Scales of Early Learning; VABS, Vineland Adaptive Behavioral Scales; ABC, Aberrant Behavioral Checklist; SVZ, subventricular zone; USVs, ultrasonic vocalizations; CNTNAP2, Contactin Associated Protein-Like 2; HEK, Human embryonic kidney cells; tGFP, turbo Green fluorescent protein; KO, Knockout; CDFE, cortical dysplasia-focal epilepsy; E, embryonic day; IV, intravenous; IP, intraperitoneal; SGZ, subgranular zone; VZ, ventricular zone; FCG, Four Core Genotype; Sry, testes determining gene; Y<sup>-</sup>, Y chromosome with deleted Sry; TgSry, Sry transgene; GI, gastrointestinal; MIA, Maternal Immune Activation; SFB, Segmented filamentous bacteria.

## BRAIN-REACTIVE ANTIBODIES

Antibodies that recognize CNS antigens are primarily detected in three settings: autoimmune disease (AD), paraneoplastic syndromes, and infectious diseases (13). Individuals with AD in which B cell tolerance is impaired can harbor brain-reactive antibodies with the development of neurological and neuropsychiatric disorders as seen in Systemic Lupus Erythematosus (SLE) (5, 14–20), celiac disease (21, 22), and Neuromyelitis Optica (NMO) (23–26). Due to the fact that the BBB sequesters brain antigen from the immune system, these brain-reactive antibodies may be produced against non-CNS antigens, but cross-react with structurally similar epitopes in the CNS. In paraneoplastic syndromes brain cross-reactive antibodies can result from an immune response to tumor antigens that are routinely expressed by brain cells but only by non-brain cells under pathologic states. These antibodies can trigger neurologic symptoms (27), a phenomenon that has been described in breast cancer (28, 29), testicular tumors (28), small-cell lung cancer (28, 30), ovarian teratoma (31, 32), and more (33–36). Finally, exposure to microbial antigens can stimulate the production of antibodies that cross-react with CNS antigens, a process known as molecular mimicry. Infection with HTLV-1 (37, 38), *Trypanosoma brucei* (39–41), and group A  $\beta$ -hemolytic streptococcus (42–47) has been shown to produce antibodies that cross-react with brain antigens and cause neurologic disorders.

The potential for pathology to arise from brain-reactive antibodies accessing the brain parenchyma depends on multiple factors. Vulnerability to the brain-reactive antibodies requires that the anti-brain antibody be present in the CNS at a time when the antigen is expressed. Furthermore, when a BBB breach is necessary for the antibody to penetrate the brain parenchyma, the nature of the BBB insult will restrict access to specific regions of the brain, determining whether the antibody will encounter its cognate antigen. For instance, lipopolysaccharide (LPS) causes a BBB breach in the hippocampus in mice (5) while epinephrine results in a BBB breach in the amygdala (4). Additionally, genetic variants and differences in protein expression between males and females (48–50) may also influence brain vulnerability to immune assault.

The symptoms and deficits observed in disorders caused by brain-reactive antibodies are dependent on the antigen recognized and its distribution in the brain. For example, antibodies to ribosomal P protein (anti-P antibodies) present in SLE patients cross-react with a brain antigen termed neuronal surface P antigen (NSPA) (19). While these antibodies bind to several regions of the mouse brain, when injected into the lateral cerebral ventricles they lead to smell alterations (51) and depression-like behavior (52), implicating the piriform cortex, the cingulate cortex, and the hippocampus. Furthermore, upon LPS-induced BBB breach, which enables access to the hippocampus, these antibodies cause memory impairment in mice (15). SLE anti-DNA antibodies that cross-react with N-methyl-D-aspartate receptors (NMDAR) termed DNRAb cause cognitive impairment (5) and an abnormal stress response (4) in mice, consistent with the function of NMDARs in the hippocampus and the amygdala, respectively. While NMDARs

are expressed in both the hippocampus and the amygdala, the regional selectivity of symptoms observed in the mouse models is determined by the agent used to compromise BBB integrity; cognitive impairment is seen only when LPS damages BBB integrity in the hippocampus while the abnormal stress response is seen with exposure to epinephrine and antibody penetration of the amygdala. This example highlights the importance of antigen distribution and the region of BBB breach.

## MATERNAL BRAIN-REACTIVE ANTIBODIES AND FETAL BRAIN DEVELOPMENT

Brain-reactive antibodies have the potential to alter brain development *in utero* (53), resulting in damage that can be persistent (54) and lead to neurodevelopmental and neuropsychiatric disorders in the offspring. It has been proposed that AD-related impaired B cell tolerance in women can lead to the production of these antibodies. Indeed, ADs are more prevalent in mothers of children with neurodevelopmental and neuropsychiatric disorders including ASD (Table 1). A study by our group showed that mothers of children with ASD who had brain-reactive antibodies were significantly more likely to harbor anti-nuclear antibodies (53%) than those lacking brain-reactive antibodies (anti-nuclear antibody = 13.4%) and unselected women of child-bearing age (anti-nuclear antibody = 15%), suggesting a maternal predisposition to producing auto-antibodies. We also observed a significantly greater prevalence of AD in mothers of children with ASD who were also positive for brain-reactive antibodies [rheumatoid arthritis (RA) = 3.86%; SLE = 2.22%] compared to those lacking brain-reactive antibodies (RA = 1.45%; SLE = 0.16%) (67).

The BBB is immature during fetal development, presenting a uniquely vulnerable temporal window when antibodies can enter the CNS (2) while critical neurodevelopmental events are taking place. The antibodies that access the fetal brain are produced by the mother and are transported by the neonatal Fc receptor (FcRn) across the placenta into fetal circulation starting on week 13 of human gestation (68–70). Once maternal brain-reactive antibodies access the fetal circulation they may result in pathology if the timing of antigen expression also coincides with the period when the fetal BBB is permeable to IgG. Presence of maternal brain-reactive antibodies during pregnancy alone is, therefore, not enough to result in pathology, perhaps contributing to the fact that some mothers of neurotypically developing (NT) children also have anti-brain antibodies.

When assessing the potential for maternal brain-reactive antibodies to cause neurodevelopmental disorders we must also be mindful that the women harboring these antibodies will not necessarily present with neurological deficits resulting from antibody exposure as their BBB is likely to be intact or the impact of the antibody may be developmentally determined. Moreover, the deficits caused by *in utero* antibody exposure may be transient or long-lasting, and consequently, not all children born to mothers with brain-reactive antibodies will present post-natally with detectable symptoms. Transient insults may be

compensated for by plasticity mechanisms in the brain during gestation or post-natally. Furthermore, it is possible for deficits to be present only while the pathogenic antibody has access to the brain parenchyma, which is limited after birth by the maturation of the BBB (2) and the disappearance of maternal antibodies in the circulation of newborn infants (71). Lastly, symptomatology from the neurodevelopmental effects of maternal brain-reactive antibodies may appear later in life or may become apparent only if other stressors are present (72).

## MATERNAL BRAIN-REACTIVE ANTIBODIES AND ASD

ASD are a group of neurodevelopmental conditions characterized by impaired communication and social interactions, repetitive behaviors, and restricted interests or activities (DSM-V) (73). They are four times more likely to be diagnosed in males compared to females. Both prevalence and incidence of ASD are increasing, with a current estimate of 1 in 59 children being affected (74). The etiology of ASD is not completely understood; hundreds of genes have been associated with ASD (75, 76) but these account for just 10–20% of the diagnosed cases (77). Furthermore, twin studies indicate that only 37% of the susceptibility to ASD is due to genetic heritability (78). These data suggest that environmental factors also play an important role in determining the susceptibility to ASD. Maternal brain-reactive antibodies present *in utero* represent a potential environmental risk factor for ASD. Several groups have identified brain-reactive antibodies in mothers of children with ASD which are either absent or found at lower frequency in mothers of unaffected children (Table 2). Dalton et al. (79) showed that, when injected into pregnant mice, brain-reactive serum from a mother of a child with autism and a child with severe specific language disorder led to decreased exploration, deficient motor coordination, and altered cerebellar metabolites in the offspring compared to the offspring of mice given sera from mothers of NT children. In a study in which blood samples were collected mid-pregnancy, Croen et al. (80) suggested a direct pathogenic role for the antibodies. A significantly higher prevalence of reactivity to proteins from brain lysates of 39 and 73 kDa was detected in mothers of children with ASD compared to the general population control group. Moreover, this pattern of reactivity was seen in mothers of children with early onset ASD.

Some of these maternal brain-reactive antibodies have been found to have antigenic specificity for proteins with potential neurodevelopmental roles including: lactate dehydrogenase A and B (LDH-A, LDH-B) (37 kDa band), Y-box bonding protein 1 (YBX1) (39 kDa band), stress-induced phosphoprotein 1 (STIP1) (upper 73 kDa band), collapsing response mediator protein 1 and 2 (CRMP1, CRMP2) (lower 70 kDa band), and guanine deaminase (GDA) (44 kDa band) (83, 84). Maternal reactivity to LDH alone or in combination with reactivity to CRMP1/CRMP2/STIP1 has been associated with a greater risk of ASD. Reactivity to LDH, STIP1, and CRMP1 together (the 37 and 73 kDa combined bands) was the most specific pattern for ASD

**TABLE 1** | Family history of autoimmune diseases and increased risk of neurodevelopmental/neuropsychiatric disorders.

	Associations with autoimmune disease (AD)	Prevalence/incidence of Neurodevelopmental/neuropsychiatric disorders (ND)		Family history of AD		ND sex bias linked to AD	References
		AD offspring	Control offspring	ND	NT		
Developmental problems	Maternal SLE	Hyperactivity = 13.1% Attention problems = 15.7% Reading difficulties = 21.6%	Hyperactivity = 1.3% Attention problems = 6% Reading difficulties = 9.3%			Male bias	(55)
Learning Disabilities (LD)	Increased risk of LD and maternal anti-Ro/La Abs [OR = 5.74 (95% CI, 1.39–23.74)] and SLE disease flares [OR = 9.43 (95% CI, 1.32–67.24)] during pregnancy	26%	7%			Male bias	(56)
	Maternal SLE and increased risk of impairments in learning and memory [OR = 3.45, 95% CI of OR (1.25, 9.09), $P = 0.02$ ]	54.9%	30.4%				(57)
Tourette Syndrome (TS)	Maternal AD and increased incidence of TS [IRR = 1.22 (95% CI, 1.01–1.48)]	2.25 per 10,000 person years	1.86 per 10,000 person years			Male bias	(58)
PDD	1st degree relative with a history of AD Family history of Hashimoto's thyroiditis and rheumatic fever			30.7%	11.9%		(59)
ADHD	Elevated maternal TPOAbs during pregnancy and increased risk of ADHD [OR = 1.77 (95% CI, 1.15–2.72)]						(60)
ASD	Maternal RA and increased incidence of ASD [IRR = 1.70 (95% CI, 1.07–2.54)] Maternal celiac disease and increased incidence of ASD [IRR = 2.97 (95% CI, 1.27–5.75)] Family history of type 1 diabetes and increased incidence of ASD [IRR = 1.78 (95% CI, 1.16–2.61)]						(61)
	AD in 1st degree relative and increased risk of ASD [OR 6.0] Maternal AD and increased risk of ASD [OR = 8.8]			1st degree relative AD = 21% Maternal AD = 16% RA = 46%	1st degree relative AD = 4% Maternal AD = 2% RA = 26%		(62)
	Maternal psoriasis and increased risk of ASD [OR = 2.7 (95% confidence interval, 1.3–5.8)]			Psoriasis = 2.7%	Psoriasis = 1.0%		(63)
	Family history of AD and increased risk of ASD [OR = 6, 95% CI, 2.5–14.1]			AD = 40%	AD = 10%		(64)
				Family history AD = 45%	Family history AD = 10%		(65)
	Maternal SLE and increased risk of ASD [OR = 2.19 (95% CI 1.09–4.39)]	1.4%	0.6%				(66)

AD, autoimmune disease; ND, Neurodevelopmental/neuropsychiatric disorders; NT, Neurotypical development; SLE, Systemic lupus erythematosus; LD, learning disabilities; Abs, antibodies; TS, Tourette Syndrome; PDD, Pervasive Developmental Disorders; ADHD, Attention deficit/hyperactivity disorder; TPOAbs, thyroid peroxidase antibodies; ASD, Autism Spectrum Disorder; RA, Rheumatoid arthritis.

**TABLE 2** | Maternal brain-reactive antibodies linked to ASD.

Target	Reactivity	Phenotypic associations in humans	References
Cerebellar Purkinje cells and brainstem neurons	Adult rat and P1 mouse brains by IHC Binding to NB-1 cells (cells derived from a human neuroblastoma)	Serum obtained from a mother of a child with ASD and a child with a language disorder	(79)
LDH-A, LDH-B	37 kDa band on WB using human and rhesus macaque fetal brain proteins	ASD with behavioral regression Within individuals with ASD, 1. Abnormal sleep/wake cycle 2. Deficits in verbal and non-verbal language acquisition 3. Increased stereotypical behaviors on the ABC	(81–86)
STIP1 (target for upper band CRMP1, CRMP2 (target for lower band)	73 kDa band on WB using human and rhesus macaque fetal brain proteins	Within individuals with ASD, 1. Verbal language deficits 2. Delayed onset of social smile	(82–86)
LDH reactivity in combination with STIP1 or STIP1/CRMP1	37/73 kDa band combination on WB using human and rhesus macaque fetal brain proteins	1. Pattern only observed in mothers of children with ASD 2. ASD with behavioral regression 3. Greater total cerebral volume Within individuals with ASD, 4. Lower score for expressive language measured by MSEL 5. Increased stereotypical behaviors on the ABC	(81–83, 85–87)
YBX1	39 kDa band on WB using human and rhesus macaque fetal brain proteins	Early onset ASD	(80, 82–86)
YBX1 reactivity in combination with STIP1 or STIP1/CRMP1	39 kDa/73 kDa band combination on WB using human and rhesus macaque fetal brain proteins	1. Early onset ASD 2. Decreased motor skills scores measured with VABS 3. Increased irritability on the ABC compared to children with ASD born to mothers without these reactivities	(80, 82, 83, 85, 86)
Cypin or GDA	44 kDa band on WB using rhesus macaque fetal brain proteins		(83, 84)
Not identified	36 kDa/39 kDa band combination on WB with human fetal and rat embryonic brain proteins	ASD with developmental regression.	(88)
Not identified	Low molecular weight bands (~20–25 kDa) High molecular weight band (larger than 250 kDa) on WB using fetal rat brain proteins		(89)
Yo	Immunoblot with recombinant protein		(90)
Amphiphysin	Immunoblot with recombinant protein		(90)
Caspr2	Isolation and cloning of single human memory antigen-specific B cells Live cell-based assay using HEK-293 cells expressing tGFP-Caspr2 Binding to adult wild type but not to adult CNTNAP2 KO mouse brain by IHC		(91)

*P*, postnatal day; IHC, Immunohistochemistry; NB, neuroblastoma; ASD, Autism Spectrum Disorder; WB, Western blot; LDH-A, lactate dehydrogenase A; LDH-B, lactate dehydrogenase B; STIP1, stress-induced phosphoprotein 1; CRMP1, CRMP2, collapsing response mediator protein 1 and 2; MSEL, Mullen Scales of Early Learning; NT, Neurotypical development; YBX1, Y-box bonding protein 1; VABS, Vineland Adaptive Behavioral Scales; ABC, Aberrant Behavioral Checklist; GDA, Guanine deaminase; HEK, Human Embryonic Kidney cells; tGFP, Turbo green fluorescent protein; Caspr2, Contactin-associated protein-like 2; CNTNAP2, Contactin-associated protein-like 2 gene; KO, Knockout.

as it was detected in mothers of children with ASD but not in mothers of NT children (81, 83). Furthermore, presence of this antibody combination was associated with an increased risk of behavioral regression in ASD (81) and impairments in expressive language (82).

Animal studies have shown that exposure to maternal brain-reactive antibodies *in utero* can permanently alter the brain during development and cause sustained behavioral and cognitive deficits akin to those observed in ASD (Table 3). Martínez-Cerdeño et al. (96) and Ariza et al. (97) used a single

**TABLE 3 |** Animal studies demonstrating that *in utero* exposure to maternal brain-reactive antibodies can permanently alter the brain, leading to behavioral and cognitive deficits.

Target	Animal model	Characteristics	References
Cerebellar Purkinje cells and brainstem neurons	Passive transfer: daily maternal serum injections into pregnant mice from E10 to E17	Decreased exploration Altered motor coordination Cerebellar metabolite abnormalities.	(79)
Unknown	Passive transfer: IV injections of pooled maternal IgG to pregnant rhesus macaque on gestation days 27, 41 and 55	Hyperactivity Increased stereotypies	(92)
LDH/STIP1/CRMP1 (antigens of molecular weights 37 and 73 kDa)	Passive transfer: single IV injection of purified maternal IgG into pregnant mice on E12	Delayed pre-weaning motor and sensory development. Increased number of USVs on P8 Males had a longer total USV duration on P8 Increased anxiety-like behaviors in males Slightly shorter social interaction in males	(93)
	Passive transfer: IV maternal IgG injection into rhesus macaque throughout pregnancy	Aberrant social behaviors Enlarged brain volume due to increases in white matter in male offspring.	(94)
	Single intraventricular maternal IgG injection into E14 mouse embryos	Increased repetitive behaviors measured as digging in the marble test and grooming Impaired social interactions	(95)
	Single intraventricular maternal IgG injection into E14 or E16 mouse embryos	Greater number of proliferating stem cells in the SVZ of the neocortex and ganglionic eminence Increased adult brain size and weight Increased adult cortical neuron some volume	(96)
	Single intraventricular maternal IgG injection into E14 mouse embryos	Decreased basal dendritic arborization in layer V pyramidal neurons of the frontal cortex Reduced the dendritic spine number and density in several brain regions	(97)
	Endogenous production: female mice were immunized prior to pregnancy with antigenic peptides recognized by anti-LDH/STIP1/CRMP1 antibodies.	Impaired social interactions Impaired social communication measured by USVs neonatally and as adults. Increased repetitive behaviors measured as grooming	(98)
Unknown	Passive transfer: daily IP injections of pooled maternal IgG to pregnant mice from E13 to E18.	Hyperactivity Increased anxiety Impaired social interactions Increased IL-12 levels on E16 and microglia activation on E18 fetal brains.	(99)
	Passive transfer: daily IP injections of pooled maternal IgG to pregnant mice from E13 to E18.	Greater cell proliferation in the SVZ and SGZ post-natally. Decreased cortical cell survival post-natally.	(100)
Caspr2	Passive transfer: single IV injection of anti-Caspr2 IgG to pregnant mice on E13.5	Male fetuses: 1. Thinner cortical plate 2. Fewer proliferating cells in the VZ 3. Reduced number of neurons in the entorhinal cortex Adult males: 1. Decreased number of GABAergic neurons in the hippocampus 2. Decreased dendritic arborization and spine density in CA1 pyramidal neurons 3. Increased stereotypic behaviors: increased digging measured as digging in the marble test 4. Impaired flexible learning 5. Impaired social interactions	(91)
DNA and NMDAR	Endogenous production: female mice were immunized prior to pregnancy with a peptide mimotope of DNA. Passive transfer: single IV injection of NMDAR reactive IgG on E14 to pregnant mice	Fetuses: 1. Increased cortical cell death and proliferation 2. Thinner cortical plate Adults: 1. Decreased cortical neuron size 2. Decreased cortical volume 3. Cognitive impairments in males	(101)
	Endogenous production: female mice were immunized prior to pregnancy with a peptide mimotope of DNA.	Increased female fetal death rate	(102)

*E*, Embryonic day; *IV*, intravenous; *LDH*, lactate dehydrogenase; *STIP1*, stress-induced phosphoprotein 1; *CRMP1*, collapsing response mediator protein 1; *SVZ*, subventricular zone; *USVs*, ultrasonic vocalizations; *P*, postnatal day; *IP*, intraperitoneal; *SGZ*, subgranular zone; *Caspr2*, Contactin-associated protein-like 2; *VZ*, ventricular zone; *NMDAR*, *N*-Methyl-*D*-aspartate receptor.

intraventricular embryonic injection model to assess the effects of brain-reactive antibodies recognizing LDH/STIP1/CRMP1 (antigens of molecular weights 37 and 73 kDa) on fetal brain development. These antibodies stimulated the proliferation of stem cells in the subventricular zone (SVZ) of the neocortex and ganglionic eminence, increased adult brain size and weight, and enlarged adult cortical neuron volume (96). Additionally, anti-LDH/STIP1/CRMP1 antibodies decreased basal dendritic arborization in layer V neurons of the frontal cortex and reduced the dendritic spine number and density in several brain regions (97). Complementing these studies, mice exposed *in utero* to maternal brain-reactive antibodies recognizing LDH/STIP1/CRMP1 showed ASD-like characteristics including increased anxiety-like behaviors (93), impaired social interactions (95), longer bouts of spontaneous grooming (95), increased digging (95), and delayed motor and sensory development (93).

A limitation of these animal studies is the timing of exposure to the maternal brain-reactive antibodies. Maternal brain-reactive antibodies associated with ASD in humans are likely to be present throughout the pregnancy. These studies did not simulate these conditions; they used a single injection into the pregnant mice at mid-gestation or a single intraventricular injection into the embryos. To address this, Jones et al. (98) completed a study in which female mice were immunized prior to pregnancy with antigenic peptides recognized by anti-LDH/STIP1/CRMP1 antibodies. The offspring mice exposed to endogenous maternal anti-LDH/STIP1/CRMP1 antibodies displayed fewer social interactions as juveniles and adults, increased repetitive behaviors/stereotypies assessed through the number and length of grooming bouts, and impaired social communication measured by ultrasonic vocalizations (USVs) neonatally and as adults.

In other studies identifying pathologic maternal brain-reactive antibodies, Singer et al. (88) found that, compared to mothers of NT children, mothers of children with ASD have a significantly higher prevalence of antibodies reactive to a 36 kDa protein present in rat embryonic and human fetal brain. They also noted a trend for a higher prevalence of antibodies recognizing human fetal proteins at 39 kDa in mothers of children with ASD compared to mothers of NT children. Presence of either of these reactivities was significantly associated with behavioral regression in children with ASD. Intraperitoneal administration of the purified maternal ASD-IgG to pregnant mice led to hyperactivity, increased anxiety, and shorter social interactions in the adult offspring relative to the offspring of pregnant mice given IgG from mothers of NT children or saline (99). Preliminary fetal brain studies suggested a role for microglia and IL-12 in the pathological mechanism of the ASD-IgG induced behavioral irregularities (99). Further assessment of the pathological mechanism of these antibodies showed greater cell proliferation in the subventricular and subgranular zones and decreased post-natal day (P)1-born cell density, suggesting reduced survival, in layers 2–4 of the frontal and parietal cortex (100). Due to the similarity in antigen size and the association with behavioral regression, it is possible that the samples from the Braunschweig and Singer studies contain antibodies that

recognize the same proteins at 36–39 and 73 kDa, and that these antibodies represent contributors to ASD risk in the general population. Determining the antigenicity of the antibodies identified by Singer et al. (88) will be necessary to resolve this question.

Animal studies of the pathogenic role of ASD-IgG have generally used IgG that was pooled from several mothers of children with ASD or endogenous polyclonal antibody following an immunization protocol. Thus, it has not been possible to identify the antibodies that are pathogenic from those that are not. Furthermore, specific ASD-like characteristics in the animal studies may result from exposure to distinct monoclonal antibodies. There is also likely to be a different proportion of potentially pathogenic antibodies in the pooled sample compared to the composition present in each of the mothers. As a result, the effects of antibodies at low concentration might be obscured by those at high concentration or they may not be detectable because the threshold concentration of antibody necessary to produce pathology may not have been reached. Moreover, the studies discussed above do not definitively identify the targeted antigen as it remains possible that the critical antibodies bind not only to the identified antigens (most of which are intracellular) but also cross-react with a neuronal membrane antigen. We addressed this concern by developing a protocol to generate monoclonal brain-reactive antibodies from mothers of children with ASD and a brain-reactive serology.

One of the monoclonal antibodies that we generated recognizes the extracellular domain of Caspr2, a protein encoded by the gene Contactin Associated Protein-Like 2 (CNTNAP2). Caspr2 is a cell-adhesion molecule expressed in the spines, dendrites, axons, and soma of neurons (103, 104). Both rare and common variants of CNTNAP2 have been linked to an increased risk of ASD or ASD-related endophenotypes including language delay and developmental language disorders (105–115). Furthermore, CNTNAP2 deficient mice exhibit ASD-like phenotypes including increased repetitive behaviors, and impaired communication and social interactions (116). The CNTNAP2 deficient mice also suffer from seizures, show neuronal migration abnormalities and have ectopic neurons in the corpus callosum (116), similar to cortical dysplasia-focal epilepsy (CDFE) syndrome, a syndromic form of ASD associated with mutant CNTNAP2 (114).

Given the link between mutations in CNTNAP2 and ASD in human pedigrees and the presence of ASD-like phenotypes in CNTNAP2 deficient mice, we asked whether exposure to monoclonal anti-Caspr2 antibody (C6) *in utero* leads to ASD-like characteristics in mice (91). Indeed, *in utero* C6 exposure led to a thinner cortical plate and fewer proliferating cells in the ventricular zone, and to a reduction in the number of neurons in the entorhinal cortex and in the number of GABAergic neurons in the hippocampus of adults. We also observed decreased dendritic arborization and a reduced spine density in CA1 pyramidal neurons in adult mice exposed to C6 *in utero* when compared to the controls. Finally, these mice showed ASD-like behavioral abnormalities such as stereotypic behaviors, impaired flexible learning, and impaired social interactions. Interestingly, effects of C6 were only detected in male mice.

## MATERNAL BRAIN-REACTIVE ANTIBODIES AND SEX-BIAS

Neuropsychiatric conditions often display a bias for one sex over the other. Neuropsychiatric conditions diagnosed earlier in life are more frequently diagnosed in males while those manifesting during puberty or later in life show a female preponderance. For example, there is a male bias in ASD, ADHD, dyslexia, Tourette Syndrome, and learning disabilities [reviewed by (117)]. Conversely, anorexia nervosa and internalizing disorders such as depression (118) and anxiety are more prevalent in females (119). Neuropsychiatric conditions that are diagnosed earlier in life have been proposed to have a neurodevelopmental origin (119). Given the unique conditions during pregnancy that allow for the *in utero* environment including maternal antibodies and cytokine levels to influence development, it is reasonable that early-onset neuropsychiatric disorders are more frequent in children of mothers with AD (Table 1).

A sex-bias has indeed been described in multiple studies of the effects of maternal brain-reactive antibodies on development and behavior. For example, we observed a significant male bias for all the fetal brain developmental and adult behavioral effects of the C6 anti-Caspr2 antibody (91). Females exposed to C6 *in utero* were not affected while males developed disrupted brain anatomy and ASD-relevant behaviors. The animal studies of maternal anti-LDH/STIP1/CRMP antibodies have also noted a male-bias in ASD-like traits including impaired communication and social interactions (93, 98). Lastly, Wang et al. (102) observed a significantly higher rate of fetal death after embryonic day (E) 15 in female offspring exposed to SLE DNRAb compared to males. While females had a greater fetal death risk, males in this model were born with cognitive impairment (101). This difference between the sexes suggests that sex-dependent factors play a role in determining not only the impairments resulting from maternal brain-reactive antibody exposure but also the severity of the outcomes.

Sex chromosomes and gonadal hormones may influence the susceptibility to maternal brain-reactive antibodies. Sex chromosome genes contribute to sexual dimorphisms, including sex-specific patterns of brain development and function, independently from gonadal hormone influences (50, 120). For example, sex chromosomes have been implicated in the density of vasopressin fibers (121), the number of tyrosine hydroxylase expressing neurons (120, 122), social interactions (123, 124), aggression (125–127), and anxiety (128). Genes found in “sex specific” regions in the X and the Y chromosomes may account for some of these sexual dimorphisms. These genes are highly expressed in the brain (48, 50) and show expression, spatial, and temporal differences between sexes (50). Furthermore, sex differences may be in part explained by gene dosage as some genes on the X chromosome escape inactivation (129–131), and X chromosome imprinting can affect gene expression in the brain (132, 133). Indeed, X chromosome imprinting has been associated with social impairment in Turner syndrome (134). Higher expression of these sex chromosome genes may be protective if they encode the antigens recognized by the maternal brain-reactive antibodies when binding of the antibody leads to protein internalization and partial loss of function but

is only pathogenic if protein expression falls below a certain threshold. Conversely, if antibody interaction with its cognate antigen induces cell signaling cascade activation or apoptosis, then the sex with higher expression would be at an increased risk for developing the antibody-induced phenotypes. Furthermore, temporal differences in gene expression between sexes may be important as antibody exposure must coincide with this period in order to cause pathology. Moreover, the effects of sex chromosome genes on the susceptibility to maternal brain-reactive antibodies may be indirect if the proteins encoded modulate the expression or activity of the proteins recognized by the antibodies. For instance, the DNRAb mediated female fetal loss may be in part due to sex chromosome genes acting as regulating factors and contributing to the higher expression of the NMDAR subunit NR2A in the female brainstem by E17 (102).

Sex hormones also influence brain development, aiding normal maturation of the fetal brain or altering normal development. Estrogen has neurotrophic and neuroprotective functions including modulation of neuronal apoptosis, migration, and spinogenesis and neurite growth (135–140). Estrogen administration during fetal development leads to masculinization of mouse neural pathways and behaviors (141, 142). Human fetal testosterone has been linked to narrow interests (143) and greater impairments in social skills and empathy in offspring (143–145). Additionally, treatment with estrogen reversed or mitigated some of the ASD-relevant behavioral phenotypes in animal models of ASD, importantly, CNTNAP2 mutant zebrafish (146) and Reeler heterozygous mice (147). As estrogen treatment showed a decrease in phenotypic behavior, these data suggest that estrogen may have a protective role in ASD and could therefore account for the lower prevalence in females. Overall, gonadal hormones modulate processes in brain development and maturation that could lead to compensation for or exacerbation of the pathologic effects of maternal brain-reactive antibodies. The specific effects of individual gonadal hormones are likely to depend on the neural pathways affected by the antibodies.

Given the sex-bias of neurodevelopmental and neuropsychiatric disorders in humans, understanding what causes maternal brain-reactive antibodies to affect preferentially one sex over the other will aid in our understanding of the pathological mechanisms of these conditions while also providing information that could lead to the discovery of new treatments. Of note, exposure to gonadal hormones *in utero* in litter-bearing animals differs from that in humans due to the “intrauterine position phenomenon.” Fetuses between two males will be exposed to higher levels of testosterone while fetuses between two females will be exposed to higher levels of estrogen [reviewed by (148)]. Consequently, post-natal sexually dimorphic characteristics including brain anatomy and behavior are influenced by fetal position [reviewed by (148)]. Nonetheless, the importance of gonadal hormones can be addressed in studies in which these are administered to neonates. Alternatively, treatment with gonadal hormone receptor agonists or antagonists, and the use of gonadal hormone receptor knockout mice can not only help us to identify which hormones play a role in the sex-bias that is observed but can also lead to the identification of the specific



molecular mechanisms involved. The “Four Core Genotypes” (FCG) mouse model offers the unique opportunity to isolate sex chromosome from gonadal hormone contributions to sex-bias. This mouse strain combines two mutations that allow for gonadal determination to be independent from sex chromosome complements. Specifically, the testes determining gene (*Sry*) was deleted from the Y chromosome ( $Y^-$ ) and a *Sry* transgene (*TgSry*) was inserted into autosomal chromosome 3, resulting in four genotypes: gonadal females with XX or  $XY^-$ , and gonadal males with  $XY^-$  *TgSry* or with XX *TgSry* (121, 149). This model has been informative in understanding the sex bias in autoimmune diseases such as SLE and multiple sclerosis (150).

## ASD AND THE MICROBIOME

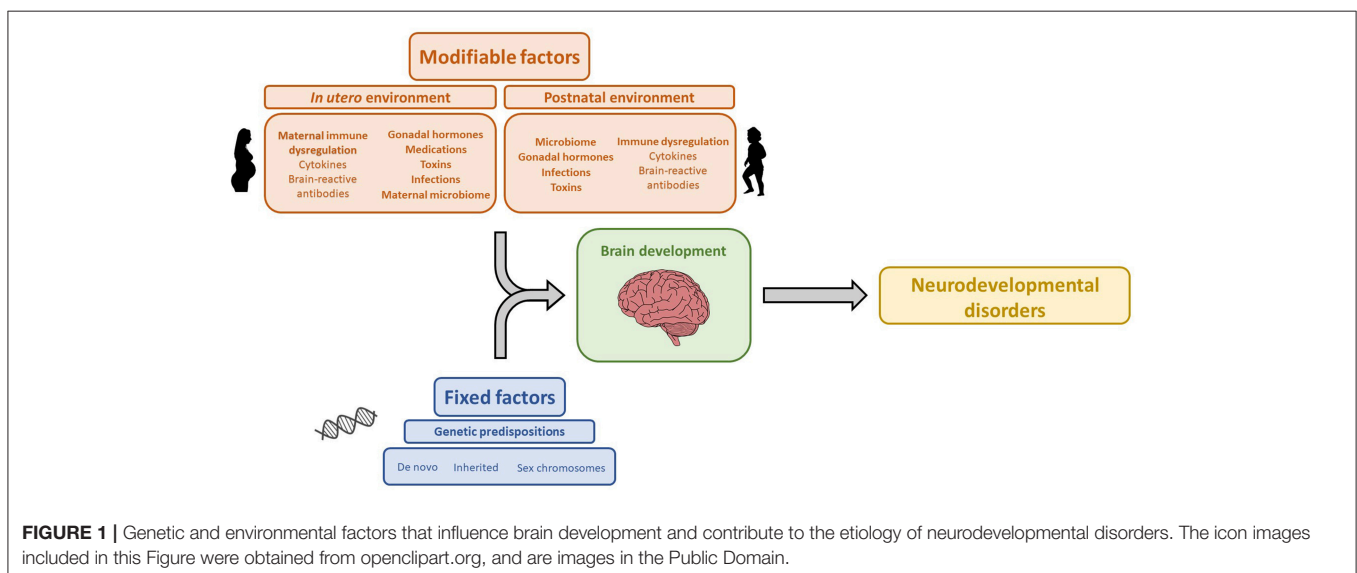
The gut microbiome is another environmental factor proposed to exert significant modulating effects on ASD susceptibility. Gastrointestinal (GI) disturbances, including increased intestinal permeability (151, 152) and inflammatory bowel disease (153), represent a common comorbidity in individuals with ASD (154, 155). Furthermore, there is a strong correlation between GI complaints and ASD symptom severity (156, 157). Given the effect of the gut microbiota on behavior [(158–164); reviewed by (165)], brain development [(3); reviewed by (165)] and brain gene expression in mice (158, 162–164, 166, 167), alterations of the gut microbiota may not only lead to an increase in the prevalence of GI disturbances in ASD but also promote ASD susceptibility. Indeed, individuals with ASD can have an altered gut microbiota composition (156, 168–173).

The gut microbiota may act in concert with maternal brain-reactive antibodies, genetic variants and sex-specific factors to modulate ASD susceptibility pre- and post-natally. In the model of maternal immune activation (MIA), specific maternal gut microbiota are necessary for the development of MIA-associated

behavioral phenotypes and neurodevelopmental abnormalities. Segmented filamentous bacteria (SFB) -specific  $T_H17$  cells are stimulated by dendritic cells primed by poly (I:C) to produce high levels of IL-17a (174), a key cytokine for ASD-like phenotype induction in the MIA model (175, 176). Furthermore, during fetal development, metabolites produced by the maternal gut microbiota can alter BBB tight junction protein expression thus modifying BBB permeability. Offspring of germ-free mice have a decreased expression of occludin and claudin-5 which contributes to the increased BBB permeability observed from the fetal stage until adulthood (3). This increased BBB permeability makes the offspring more susceptible to the neurological effects of immune molecules including antibodies as these are then more likely to access the brain parenchyma (3). Finally, shifts in offspring microbiota composition can be induced by factors in the *in utero* environment and contribute to the development of ASD-like behavioral deficits. For example, the offspring of poly (I:C) treated mice have an altered gut microbiota diversity which leads to altered serum metabolites and increased IL-6 and gut permeability (177). Hsiao et al. (177) propose that the increased IL-6 expression alters tight junction protein expression, leading to increased gut permeability, and leaking of harmful metabolites into systemic circulation. Post-natal treatment of the offspring with *Bacteroides fragilis* reversed some of the ASD-like behavioral phenotypes and improved gut barrier permeability, possibly by restoring IL-6 expression, which in turn leads to a partial correction of tight junction protein expression (177).

## CONCLUSIONS

The association between maternal brain-reactive antibodies and the pathogenesis of neurodevelopmental disorders has been well-established by both epidemiologic and animal studies. Maternal autoimmune disease and brain-reactive antibodies have been shown to increase the risk of neurodevelopmental



disorders. Moreover, animal studies have shown that *in utero* exposure to maternal brain-reactive antibodies is sufficient to permanently alter brain anatomy and cause aberrant cognition or behavior mimicking certain neurodevelopmental syndromes. Specific neurodevelopmental disorders and the severity of symptomatology are likely determined by an interplay between genetics and environmental risk factors including maternal brain-reactive antibody, maternal cytokines, gonadal hormones, and the microbiome (Figure 1). As the prevalence of neurodevelopmental disorders has been significantly increasing (178), research on the *in utero* environment, including maternal brain-reactive antibodies, is of great biomedical importance. Identifying potentially pathogenic antibodies and understanding

their mechanisms of fetal brain injury provide an opportunity to detect and protect fetuses at risk.

## AUTHOR CONTRIBUTIONS

AG-G contributed to the concept and writing of the manuscript. BD contributed to the concept, writing, and reviewing of the manuscript.

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