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ReviewArticle

Prenatal Screening of Maternal Immune Antigen Biomarkers Linked to Microglial Regulation of Brain Development May Predict Autism Risk

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Abstract

Recent discoveries of the connections between the maternal immune system [IS] and prenatal brain development suggest that routine prenatal screening for chronic disorders associated with IS dysfunction may be useful in identifying women at heightened risk for giving birth to a child with autism. Epidemiological studies have shown that the incidence of IS disorders, including systemic lupus erythematosus [SLE], rheumatoid arthritis [RA] and chronic obesity in combination with insulin-resistant diabetes, has increased significantly over the past several decades and that pregnant women with these conditions are at increased risk for having a child with autism. For this reason, physiological parameters associated with these prenatal conditions that can be detected before onset or at early stages of disease may serve as biomarkers for increased autism risk.

A physiological relationship between maternal IS dysfunction and impaired embryonic/fetal brain development may be defined by critical neuro developmental functions of brain microglia that are responsive to both neural and immunological stimuli. Impaired regulation of the developmentally versus immunologically defined functions of brain microglia may represent a primary cause of the neurological impairments characteristic of ASD. This critical cause/effect relationship provides the rationale for autism risk factor assessment using biomarkers associated with chronic immune conditions that impair the neuro developmental functions of microglia as a consequence of their inappropriate immunological activation. Moreover, the connection between abnormal IS function and impaired neural development suggests preventive approaches that can be used to decrease the overall risk for ASD in children born to mothers with these conditions.

ABBREVIATIONS

MIA: Maternal Immune Activation; ASD: Autism Spectrum Disorder; RA: Rheumatoid arthritis; SLE: Systemic Lupus Erythromatosus; CNS: Central Nervous System; QTE: Quantitative Threshold Exposure; ID: Intellectual Disability; IS: Immune System

INTRODUCTION

Relationship between Autism and Maternal Immune System [IS] Activation in Pregnancy

An incomplete, but suggestive, description of autism is of an inflammatory disorder that disrupts neural development beginning at the earliest stages of embryogenesis and continuing until 3-4 years post-partum see Figure (1). Maternal inflammation linked to an increased risk of some cases of autism may result from autoimmune dysfunction, immune responses to infectious disease during pregnancy or metabolic syndromes linked to chronic obesity/gestational diabetes see Table (1).

Long-term data from a 20 year research study in Denmark has provided significant evidence for the notion that maternal infection may trigger inflammatory responses that disrupt neural signal pathways critical to central nervous system [CNS] maturation [1]. The researchers found that hospitalization during the first trimester of pregnancy due to viral infection such as influenza increased the likelihood of having a child who developed autism

Figure 1 Interconnected relationship between genes, the immune system, and the environment in brain development.

Table 1: Risk factors weighted based on documented association with autism.

Maternal/Prenatal Risk Factors: Quantitative Association with ASD	
Risk factor *	**Calculated ASD risk** **
* (15%)Pre-natal obesity BMI>35	3
*Maternal obesity and PGDM	3.91
*Maternal obesity and GDM	3.04
*Prenatal auto-immune disorders	-
I family member	1.9
3 family members	5.5

Abbreviations: *% Significantly increased over past 30 years
**Numbers show n–fold increase above rates observed in the absence of stated risk factor ND=no data.
ASD: Autism Spectrum Disorder

spectrum disorder [ASD] by 3-fold. Moreover, bacterial infections in the second trimester correlated with a 40% increased risk of having a child with autism. Maternal inflammatory reactions to infectious disease rather than tissue destruction resulting from the infectious disease agent itself are believed to be responsible for the increased risk for ASD. For example, research has shown that inducing inflammatory responses in pregnant mice in the absence of an infectious disease agent was associated with abnormal behavior in newborn mice [2].

Autoimmune disorders also contribute to increased risk of giving birth to a child with autism. Research studies suggest that maternal antibodies associated with inflammatory responses linked to autoimmune disorders or infectious disease may bind to the developing fetal brain to impair normal growth [1,3]. This research study, conducted in Denmark of 700,000 births over a period of 10 years, produced data showing that maternal rheumatoid arthritis was associated with an 80% increased risk for having a child with autism [1]. Maternal celiac disease [occurring at an incidence rate of approximately 1.35%] was found to increase the risk by 350%. When the amniotic fluid was analyzed at the time of birth, the presence of inflammatory molecules correlated with the later development of autism [3].

Approximately 23% of women of childbearing age in the U.S. are obese; moreover, obesity is increasingly recognized as an important cause of systemic inflammation. A clinical study based at Boston Medical Center between 1998-2014 correlated gestational obesity/diabetes with ASD occurrence in offspring. Of 2734 mother/child pairs enrolled in this study, 102 children were diagnosed with ASD [4]. The clinical data showed that chronic obesity and gestational or pre-gestational diabetes was associated with a four-fold increased risk of bearing a child with ASD. The individual associations of maternal obesity versus diabetes did not show this pattern of increased risk for ASD, suggesting that it is the combined association of obesity and insulin resistant diabetes that is most clearly linked to autism. Significantly, the combined obesity/diabetes clinical status in pregnant women also was associated with increased risk of other intellectual disability disorders, but not with other developmental disorders. The incidence of combined ASD and ID was higher than the incidence of ASD only in this group. Surprisingly, it is estimated that up to 1/3 of women of childbearing age may be have unrecognized diabetes; it is essential to include diagnostic screening for this disorder for women of child-bearing age.

Side headings/Subheadings

A Dual Role for Microglia in Synaptic Function and Neuroprotection: Implications for CNS Impairment: The duality of microglia structure/function relationships in CNS immune surveillance and neurodevelopment represents a critical connection between the immune system and the developing brain. Microglia originate in the yolk sac during embryogenesis and subsequently migrate to the neural tube, invading the primordial brain tissue as early as day 9 of embryogenesis; the window for further infiltration is closed around day 15 as the blood-brain barrier is put in place. The establishment of the blood brain barrier ensures that, under normal conditions, the repertory of neurological and immunological functions of microglia is generated from this original pool of myeloid cells in response to micro-environmental signals and interactions that shape the gene expression signature and morphogenetic characteristics of subsets of microglia at various locations within the brain parenchyma.

Microglia not only plays an important role in early developmental synaptic pattern formation, but also regulate CNS responses to immunological phenomena at all stages of life. The resident microglia are highly interactive with the tissue microenvironment of the brain, both responding to environmental signals of neural and/or immunological origin and directly contributing to synaptic pattern formation in the brain. Much current research is devoted to elucidating the gene expression pathways associated with their interchangeable and dualistic functions.

A critical neuro-developmental function for non-activated or 'resting' microglia involves the regulation of CNS synaptic connectivity patterning in the developing brain. With respect to their neuro-regulatory activities, Miyamoto et al. have shown that microglia directly contact dendrites, and, in association with calcium [Ca++] and actin filament recruitment, induce the formation of filopodia which create extensions to presynaptic terminals, thereby creating new synaptic junctions during brain development [5]. These dynamic processes or filopodial extensions protruding from microglial cells interact with synapses to regulate their activity in a developmentally associated fashion

[6-8]. The promotion of dendritic spine formation postnatally appears to be under the control of brain derived neurotrophic factor [BDNF], which may be released by microglial cells to effect synaptic modeling [9]. Microglia may engulf and destroy by phagocytosis neurons and their synaptic connections or, alternatively, generate associative dendritic contacts by micro-environmentally controlled mechanisms [10].

Microglia is also key players in orchestrating neuro-inflammatory responses of the innate immune system within the CNS. Research in mice [11] suggests that the developmental pathways of microglia mirror and reflect the stages of CNS development in a co-evolutionary interdependence. They have proposed that alterations of the brain microenvironment due to inflammatory processes may disrupt microglial gene expression profiles and, concomitantly, disrupt the normal patterns of brain development under their control. Increasing focus over the last decade on the role of neuro-inflammation in the genesis of neurodegenerative disorders such as Alzheimer's disease and multiple sclerosis has highlighted the destructive role of inflammatory responses of glial cells and their derivatives on neural structure and function. A less obvious, but equally insidious, consequence of the intricate relationship between the immune system [IS] and the developing central nervous system [CNS] is the potential for impaired brain development early in life as a consequence of neuro-inflammation. Recent research has produced intriguing evidence to suggest that microglia is critical to neuro developmental processes that, in their aberrant form, may be linked to autism.

Evidence of Microglial Abnormalities in the Central Nervous System [CNS] Of Children with Autism: Postmortem analyses have documented abnormalities in microglial structure and localization in the autistic brain. Research by Minshew and Keller [12] showed enlarged microglia in 5/15 autopsied brains. Brain tissue from autistic patients has also revealed the presence of activated microglia and structural evidence of neuro-inflammation. Research by Morgan et al. [13], showed highly increased levels of activated microglia [5/13 cases] and moderately increased levels in 4/13 autistic males in the dorsolateral prefrontal cortex. Post-mortem studies by Tetrault et al. [14], similarly revealed increased microglial density in the fronto-insular and visual cortex in autistic brains. Moreover, studies of frozen brain tissue indicated active neuro-inflammatory processes in the cerebral cortex, cerebellum and white matter in the brain associated with increased microglial activation. This research revealed that the activated microglial cells showed process thickening and retraction, characteristic of neuro-inflammatory activation. Analysis of the cerebrospinal fluid of children with ASD has shown elevated levels of inflammatory cytokines linked to microglial activation [3, 15,16]. These data further suggested that the immunological dysfunction observed in the CNS of these patients arose within the brain tissue itself. It is important to note that microglial abnormalities associated with inflammation affect not only the developing brain *In utero,* but may also impact brain function and development long-term. Therapeutic approaches directed at decreasing the levels of neuroglial activation may help to reverse of mitigate this effect on brain function.

Structure/Function Abnormalities in Autistic Brain Caused By Microglial Dysfunction: Research suggests that the neuro-inflammatory processes resulting from abnormal levels microglial activation are important causative agents of the neurological damage associated with autism [17]. Moreover, microglial activation has also been linked to epileptic seizures, which occur in 20-30% of patients with ASD [18,19].

Research studies by Parkhurst et al. [20], have shown that genetically altered mice with low levels of CNS microglia have reduced learning capacity associated with depressed synapse formation. Their studies provided evidence that microglial number and functional integrity are required for synapse formation associated with learning processes. The authors further suggested that microglial activation in neurodegenerative disorders may negatively affect the supportive, regulatory neuro physiological functions of these cells.

Clinical studies on brain physiology have provided evidence of abnormal synaptic connectivity associated with autism, resulting in under-connected regions of the brain, particularly affecting the cortical regions. Loss of synaptic connectivity is a primary neurological deficit linked to ASD [12, 21-25]; moreover, there is evidence that under-connectivity is greatest in the cortical regions of the brain. Areas of the brain with reduced functional connectivity include the limbic cortex and the cerebellum [26-28]. Increased microglial density has been observed in the cerebellum of autistic brains which has been linked to reduced Purkinje fiber density and dysregulated connectivity to the cortical regions of the brain [29].

Systemic inflammatory processes triggered by circulating bacterial endotoxin/LPS have been shown to release IL-1β to trigger dysregulated microglial activation in the developing brain. Research on neuro-development in rats suggests that excessive maternal immune activation [MIA] of fetal microglia due to experimental endotoxemia results in the loss of neural precursor cells in the developing brain. Conversely, experimentally decreasing the number of activated microglia in the developing rat brain was observed to produce an increased number of neural progenitor cells, suggesting a role for brain microglia in regulating nerve cell density in the brain.

Proposed Role Activated Immuno-Protective versus Neuro-Regulatory Microglia in Autism: Microglia may comprise a critical connection between maternal immune activation [MIA] and the dysregulated CNS development associated with autism. Research studies have shown that increased levels of microglial activation are associated with both schizophrenia and autism [2]. Inflammation due to infectious disease or autoimmunity has been demonstrated to induce the morphological transformation of "resting" microglia that display extensive filopodia projections to immunologically "activated" ameboid microglia that secrete pro-inflammatory cytokines [5]. Maternal inflammatory processes may trigger microglial activation to a pro-inflammatory state in response to these micro-environmental signals see Figure (2). This transition very likely involves the genetic switch from a gene expression profile directly involved in synaptic modeling in the developing brain to an inflammatory genetic signature. The morphogenetic transition of neuro-regulatory microglia to an immunologically activated state may deprive the developing

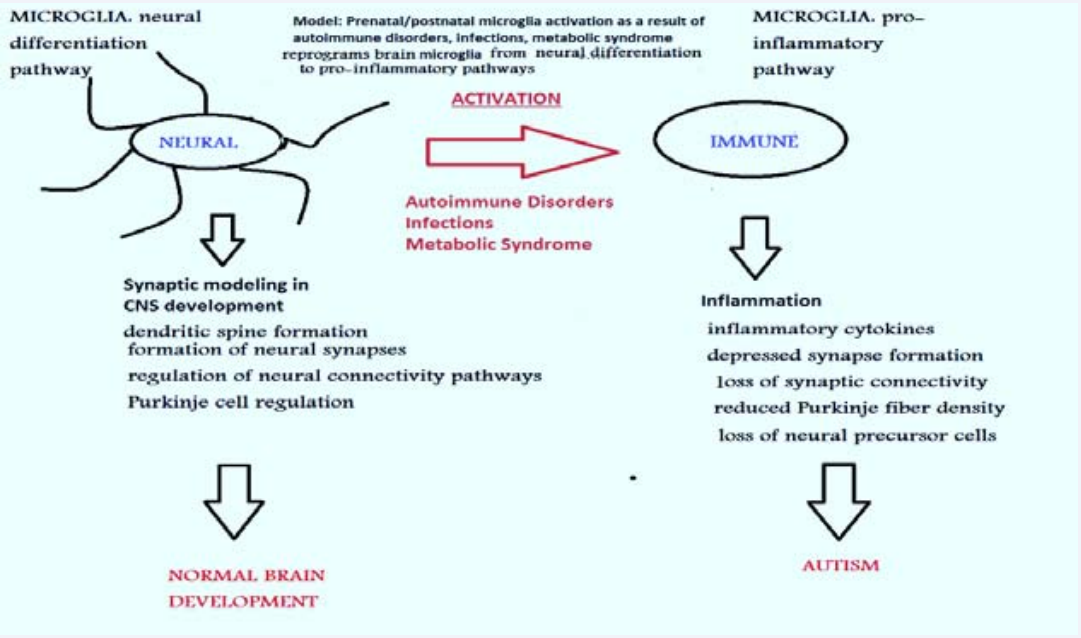

Figure 2 Critical role of microglia in brain development may be diverted to inflammatory functions in response to prenatal maternal IS activation.

brain of key regulatory signals for synaptic pattern formation while, at the same time, produce neurodegenerative activities associated with immunological functions of activated cells. This morphological response by microglia to inflammatory pathway activation may be directly associated with the disruption of synaptic modeling that may comprise the biological basis for the structural abnormalities in the brain that cause autism.

The role of activated microglia in autism does not appear to be primarily destructive as in neurodegenerative disorders such as multiple sclerosis. Rather, impaired neural synaptic signal connections associated with the function of neuro-regulatory microglia may be of central importance in the genesis of this disorder. An important connection between the IS and neuro developmental impairment may be the effects of IS activation on the morphogenetic transformation of microglia essential to neural development to neuro-inflammatory cells that have lost the capacity for neuro-synaptic modeling. This activation may transform the microglial cell from a cell that interacts with and molds synaptic function during critical stages of development to one whose activity is pro-inflammatory, with the concomitant loss of neuro-differentiation functions repressed by the genetic switch.

It is important to note that the disruptive inflammatory signal pathways that affect prenatal brain development are often maternal in origin; thus, the identification of maternal biomarkers linked to inflammatory diseases may thus represent an important clinical tool to identify individuals at risk for having a child with autism see (Figure 3).

Identified Biomarkers for Maternal Autoimmune Disorders That May Be of Predictive Value for Autism Risk:

Systemic Lupus Erythromatosis [SLE]: Systemic lupus erythromatosis [SLE] is an autoimmune disorder whose pathological manifestations include the hyper-activation of B and T cells. This results in the production of auto-antibodies that cause inflammation and damage to tissues and organ systems of the body. Due to the fact that there may be a long latency between immune system dysfunction and the appearance of symptoms, many individuals may not be aware that they are in the early stages of this disorder at the time of pregnancy [30]. Thus, the identification of biomarkers associated with preclinical IS changes associated with the later development of SLE is important in the diagnosis and treatment of this disorder as well as determining the potential ASD risk for the offspring of women with this preclinical condition see Table (2).

There are several auto-antibodies in clinical use for the diagnosis of SLE. Among the most important are anti-[ds] DNA and anti-Sm [anti-nuclear Smith] antibodies that are commonly used serum biomarkers for this autoimmune disorder [30]. Their reliable use, however, is limited by high specificity and low sensitivity binding parameters.

Newer diagnostic candidates include anti-Sm-D1, which recognizes poly ADP-ribose polymerase [PARP] proteomics [31]. In addition, anti-cmDNA antibodies are present at high levels in the serum of patients with SLE as is anti-Sjogren's Syndrome type B [SSB] antibodies, which is not only present in high titre but is one of the earliest detectable SLE biomarkers [32] and can frequently can be detected prior to the onset of clinical symptoms. The SSB nuclear antigen was first identified [along with SSA] in 1979 by Alspaugh and Madison in the sera of patients with primary Sjogren's syndrome [32]. Biswas et al. determined that the SBB antigen coincides with the cytoplasmic antigen La [33]. The SSB/La antigen is associated with neutrophil dysfunction in patients with SLE. The SSB antigen is a ribonucleo protein that regulates RNA polymerase III transcriptional activity.

A clinical study by Tang et al. showed that anti-SSB antibody

positivity was detected in 25.7% of patients tested [34]. The experimental group consisted of patients with a broad range of autoimmune disorders, including systemic lupus erythromatosus [SLE], Sjogren's syndrome [SS], rheumatoid arthritis [RA], progressive systemic sclerosis [PBS], and mixed connective tissue diseases [MCTD].SSB antibody positivity in this group was measured against control subjects who did not have autoimmune disorders. Moreover, when one excludes patients with rheumatoid arthritis or primary Sjogren's Syndrome from the diagnostic pool, the specificity of this antibody in identifying patients with SLE increased to approximately 96%. The anti-SSB antibodies can be detected on average 3.6 years prior to SLE diagnosis and approximately 2.8 years prior to the onset of disease symptomotology. This antibody test, therefore, represents an important predictive diagnostic tool for identifying pregnant women at risk for SLE. Moreover, the production of anti-SSB antibodies appears to be in part responsible for SLE pathogenesis [35]. This antibody can be reliably detected using the cost-effective method of line immunoassay [LIA].

Rheumatoid Arthritis: Rheumatoid Arthritis [RA] is a chronic autoimmune disorder with a global incidence rate of approximately 1%. The disorder involves joint inflammation and tissue destruction resulting from IS hyper-reactivity. RA serology is characterized by large numbers of circulating $CD4^+$ T cells and plasma cells, which appear to play a central role in disease pathology [36].

Identified biomarkers important in the diagnosis of RA include the erythrocyte sedimentation rate [ESR] and C-reactive protein levels [CRP], both of which are involved in the tissue destruction associated with RA. CRP is an important serological indicator of inflammation and increased levels can be detected

Table 2: Common biomarkers diagnostic for maternal autoimmune disorders that may be used to estimate risk for abnormal embryonic/fetal brain development.

Maternal Autoimmune Disorder	Risk for ASD	Associated Biomarker	Predictive Value
Rheumatoid arthritis	80%	RF [rheumatoid factor]	Early predictive value; 60-70% positivity
		ESR [erythrocyte sedimentation rate]	Good predictive value when combined with anti-CRP
		Anti-CRP [C reactive protein]	Early predictive value, good combined value with ESR
		Anti-CCP [cyclic citrullinated peptide]	Very early predictive value
		Anti-PCA [parietal cell antibody]	High sensitivity [up to 97% predictive value]; 85% positivity
Systemic lupus erythromatosus	200%	Anti-SSB [Anti-Sjogren's Syndrome]	High sensitivity*;Up to 96% positivity; earliest pre-onset biomarker
		Anti-[ds]DNA	Low sensitivity; high positivity
		Anti-Sm [nuclear Smith protein]	Low sensitivity; high positivity
Obesity/Insulin-resistant diabetes	400%	Endotoxin/lipopolysaccharaide [LPS]	High sensitivity; high positivity [200% increase]
		CRP [C-reactive protein]	Low sensitivity, high positivity
		IL-6 [Interleukin-6]	Medium sensitivity, high positivity

Abbreviations: ASD: Autism Spectrum Disorder

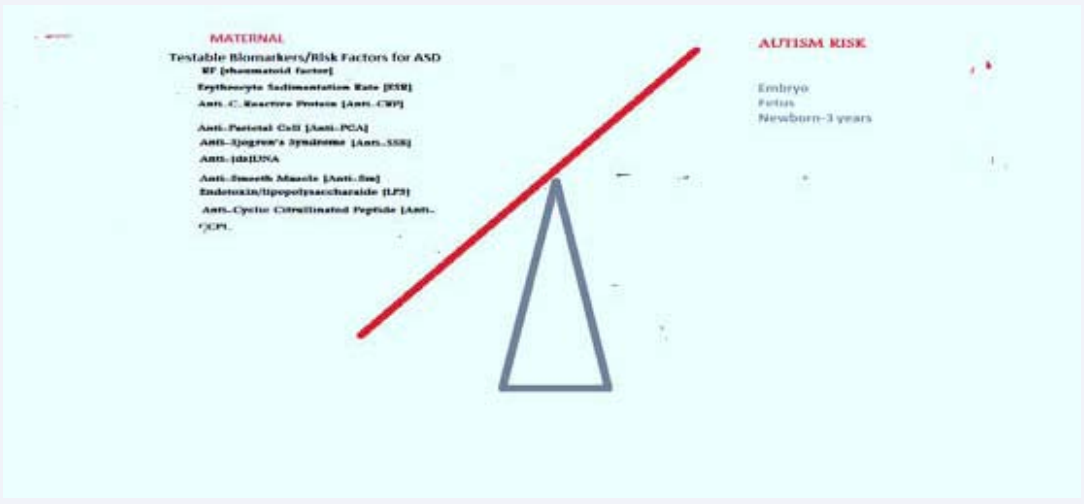

Figure 3 Testable maternal biomarkers that contribute to overall all risk for autism.

before the diagnosis of RA is apparent [37, 38]. ESR is another important biomarker for inflammation; the combined detection of CRP and ESR is a useful diagnostic indicator of RA [39] sees Table (2).

Elevated chemokine and cytokine levels are also seen in this disorder, even in unaffected first degree relatives, making these important potential biomarkers for early disease selection [40-42],

Additional biomarkers for RA in clinical use include rheumatoid factor [RF], anti-peri-nuclear factor [APF], anti-keratin antibodies [AKA], anti-filiggrin antibodies [AFA] and anti-cyclic citrullinated peptide antibodies [anti-CCP] [43, 44]. RF has shown limited specificity in primary care diagnosis of RA, while anti-PCA [parietal cell autoantibody] tests are more reliable in this regard. Approximately 60-70% of patients with RA test positive for RF; in contrast, 85% RA patients test positive for anti-PCA. Although RF is a reliable clinical biomarker, it is also detected in other auto-immune disorders such as SLE and Sjogren's syndrome. RF has been shown to have a positive predictive value [PPV] for RA between 0-5 years prior to the onset of symptoms in 88% of patients later diagnosed with RA. The PPV of anti-PCA initial positivity was shown to be very high, at 97%; in contrast, only 1.5% of healthy individuals who never developed RA showed a positive RF [20].

APF appears to react with keratohyalin granules in the buccal mucosa in patients with RA [45]. AKA reacts with keratinized tissue present only in patients with RA [approximately 60%] and can be used to identify these individuals from healthy controls [46]. In addition to RF, the presence of anti-CCP antibodies is a useful tool for early diagnosis of RA [47-49]. Anti-mutated citrullinated vimentin [MCV] antibodies comprise an additional biomarker for RA that is detectable in patients at very early stages of disease [50,51].

Combined Maternal Obesity/Gestational Diabetes: As in SLE and RI, the systemic immunological effects of maternal obesity/diabetes may also impinge on normal brain development due to their effects on brain microglia. Chronic obesity is associated with systemic inflammation, indicated by elevated levels of pro-inflammatory cytokines, C-reactive protein [CRP] and leukocyte activation [52] see Table (2). Long-term obesity thus triggers chronic inflammatory processes that are frequently complicated by the development of insulin-resistant Type-2 diabetes that further contributes to systemic metabolic dysfunction. Chronic inflammation of this type, sometimes referred to as "meta-inflammation", has been linked to the down regulation of Treg immune cells that normally function as important immune system regulators to control inflammatory processes in the body [53]. The insulin-resistant diabetes that frequently is associated with obesity in pregnancy appears to exacerbate the systemic inflammatory processes that may impair fetal brain development based on research suggesting that insulin receptor signaling is important in the regulation of synapse number, dendritic plasticity, and circuit function *In vivo* [54]. Obesity associated with insulin-resistant diabetes in pregnant women is associated with endotoxemia and placental inflammation [55]. Insulin resistance has been shown to produce metabolic alterations associated with increased levels of circulating endotoxins/lipopoly saccharides. A two-fold increase in the levels of endotoxins associated with increased levels of CRP and interleukin-6 [IL-6] has been documented in obese women [55]. Moreover, endotoxemia has been linked to chronic maternal obesity and gestational diabetes, a metabolic disturbance that may be linked to insulin resistance. It is striking that one of the clinical manifestations of insulin resistance associated with maternal obesity is an elevated level of circulating endodoxin/LPS. As maternal endotoxemia has been shown to activate fetal brain microglia in experimental systems, a direct link between this metabolic disorder and impaired brain development may converge upon the dysregulated activation of IS associated microglia.

The incidence rates for each of these autoimmune disorders: SLE, RA and obesity/type 2-diabetes is significant in women of child-bearing age; the link to development brain disorders *In utero* provides a powerful rationale for expert clinical management of these disorders during pregnancy. Moreover, biomarker testing for these disorders is necessitated by the fact that early stages may be asymptomatic and only identifiable by biomarker screening.

DISCUSSION & CONCLUSION

Conclusion: Can Maternal Autism Risk Factor Biomarker Assessment Be Used to Prevent Autism?

The physiological link between microglial dysfunction and impaired neural development in response to IS activation provides a cause/effect rationale for identifying immunological parameters associated with IS disorders epidemiologically linked to ASD. These biological parameters may thus comprise a category of IS biomarkers to identify women whose prenatal risk for having a child with autism is elevated and for whom a preventive plan to reduce overall risk factor exposure levels during embryonic, fetal and early postnatal brain development may be implemented to decrease the risk of ASD. In addition, the chronic nature of inflammatory processes may impose risk for brain deterioration in children once neurodevelopment is largely completed. Hence, there is a need for follow-up in high risk children to prevent the long-term effects of chronic inflammation on brain function.

The quantitative threshold exposure [QTE] hypothesis proposes that autism is a multi factorial disorder comprised of genetic and environmental risk factors that, in combinatorial fashion, determine whether their effects on IS function and CNS development are sufficiently disruptive to normal brain development to cause autism (see Crawford, 2015, 2016) [56,57]. This model suggests that no one risk factor is inevitably or solely responsible for most cases of ASD; rather, their quantitative threshold effects on prenatal and early postnatal brain development determine the onset of symptomatology based on their combined disruptive effects on brain development see Figure (4).

If one accepts the premise of the QTE, then the quantitation of ASD risk factor parameters on a case-by case basis may permit a qualitative determination of overall maternal ASD risk. By identifying and attempting to quantify maternal autoimmune biomarkers implicated in ASD, individualized treatment/

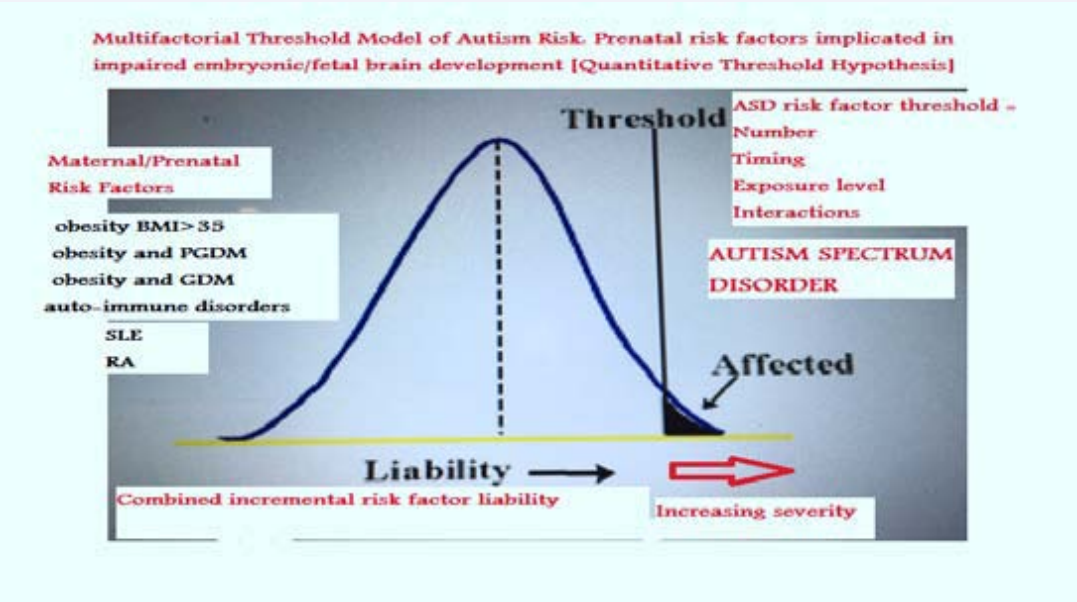

Figure 4 The Quantitative threshold hypothesis: multi-factorial risk factors contributing to ASD.

prevention plans can be designed to reduce the likelihood of its occurrence. Additional epidemiological and clinical research studies to identify the patterns of risk factor incidence associated with increased risk for ASD should permit the elaboration of quantitative assessment tools to generate more accurate risk profiles. Essential elements of the risk factor profile should include evaluations of medical and genetic family history followed by MIA antigen testing in pregnant women to assess physiological levels of critical biomarkers for ASD. Based on the results of these assessments, individuals would be placed in low risk versus elevated risk categories. Follow-up for at-risk individuals would include preventive approaches to reduce, monitor and clinically manage these maternal risk factors. The clinical management of chronic autoimmune disorders and obesity/diabetes by reducing inflammation and by adherence to medical treatment plans may reduce the pro-inflammatory MIA profile linked to ASD. Prompt treatment of infectious disease and avoidance of high risk exposure to viral and bacterial infections are also important management tools. The potential for folate supplementation to affect brain development *In utero* has been studied extensively [58]. While folate appears to play a role in early brain development, retrospective clinical research studies have not shown a demonstrable preventive effect. Moreover, this review and original research by Raghavan et al. [59], suggest that high levels of maternal folate may be be associated with increased risk of ASD. This latter study also showed that excess levels of vitamin B12, either alone or in combination with high folate my increase autism risk.

Clearly, this is an area that requires more study.

Post-natal biomarker assessments of infantsrn to at-risk mothers may be used to evaluate risk factors that may affect brain development in years 0-3 in order to identify infants for whom appropriate preventive strategies should be implemented. Post-natal risk assessment and ASD prevention will be the subject of a pending paper [S. Crawford, manuscript in preparation]. The goal of these assessment/preventive approaches is to minimize pre-natal and infant post-natal exposure to causative variables whose cumulative threshold effects may culminate in ASD.

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