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Role of Fetal Alcohol Exposure on Molecular and Epigenetic Mechanisms of Autism

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INTRODUCTION

It is well established that during prenatal and early postnatal period brain development is very susceptible to injury caused by environmental factors. Although a number of definitions and improvements have been made in Autistic Spectrum Disorders **(ASD),** the etiological aspects remain unclear. Environmental risk factors such as alcohol and drugs of abuse acting during brain development have heterogeneous influences on the formation of various brain areas. Prenatal alcohol exposure can cause adverse alterations to the developing brain. Exposure to alcohol in utero is associated with behavioral problems including adaptive dysfunction, academic difficulties, and increased rates of psychiatric disorders. Cognitive impairment in executive function, learning and memory, language, visual-spatial ability, motor function, attention, and activity levels as well as hormonal abnormalities are well illustrated in the literature [1]. Pathological changes in the brain after fetal alcohol exposure are also diagnosed in individuals with ASD. It has been shown that young children with Fetal Alcohol Spectrum Disorder **(FASD)** show developmental delays in fine but not gross motor skills [2]. In addition, multiple similarities in motor dysfunctions in children with FASD or ASD have been established: poor hand/eye coordination [3], poor bimanual coordination [4], delayed motor reaction timing [5,6] etc.

A number of clinical studies have reported learning and memory deficits in children with heavy prenatal alcohol exposure as well as in individuals with autism. Poor inhibitory ability and poor performance have been found to be correlated with a task of inhibition control in children with prenatal alcohol exposure [7] as well as in children with ASD. Taken together, the data from

studies on fetal alcohol effects suggest that children exposed to ethanol during fetal development demonstrate a wide range of brain dysfunctions. In addition, the analysis on neurophysiological and behavioral changes in children with ASD illustrates similarity with brain abnormalities in individuals with FASD. Interestingly, a link between the pathogenetic mechanisms of FASDs and ASDs can be suggested. Despite some inconsistencies, the neuropsychological literature on children prenatally exposed to alcohol illustrates a pattern in which affected children have various brain functions similar to these of children with ASD. Moreover, the data from various studies on the mechanisms of pathophysiology of ASD and FASD demonstrate a cross talk in the molecular genetics and epigenetic bases of both disorders. This paper will provide a review of some molecular and epigenetic mechanisms of fetal alcohol exposure in brain dysfunctions characteristic for ASD.

Oxidative Stress Plays a Role in Pathogenesis of ASD and FASD

Autism spectrum disorders are complex neuro-developmental disorders that are proposed to be associated with oxidative stress which is induced by excessive Reactive Oxygen Species **(ROS)**. Oxidative stress is suggested as a potential mechanism in the development of autism after fetal alcohol exposure. Oxidative stress is a process of accumulation of ROS in cells with low antioxidant capacity. ROS are highly toxic and cause cellular damage with or without cell death via apoptosis or necrosis. It is well established that oxidative stress affects different brain regions, i.e., cerebellum, temporal and frontal cortices in autism [8,9,10,11]. The increase of oxidative stress levels may induce brain dysfunction associated with behavioral abnormalities, gastrointestinal disorders, and sleep disturbances that are present in autism. Loss of Purkinje and granule cells due to ROS and increased activities of Na+/K+- ATPase and Ca2+ /Mg2+-ATPase were detected in the cerebellum and in the frontal cortex of individuals with autism [12]. Patients with autistic spectrum disorders have diminished antioxidant resistance in plasma, due to decreased levels of antioxidant enzymes (superoxide dismutase and glutathione peroxidase), Glutathione **(GSH)** and vitamins (A,C and E) [13,14,15].

It has been shown that methylation capacity, sulfates levels, and the total glutathione levels are decreased in autism. On the other hand, both oxidized glutathione and the ratio of oxidized to reduced glutathione are increased in autism. In addition, the activity of glutathione peroxidase, superoxide dismutase, and catalase, as a part of the antioxidative stress system are decreased [13]. It is well known, that glutathione is involved in the neuroprotection against oxidative stress and neuro-inflammation in autism by supporting the anti-oxidative system. So, the question is whether substances which are depleting glutathione brain levels play a role in neurobiology of ASD. We have previously reported that chronic treatment with alcohol prenatally decreased the activity of two antioxidant enzymes-superoxide dismutase and catalase and the levels of glutathione in fetal developing brain neurons [16]. In addition, our data demonstrated that alcohol increased the apoptotic cell death of developing neurons by decreasing their antioxidant system.

Other studies demonstrated that chronic alcohol consumption depleted the levels of the key antioxidant Glutathione **(GSH) [**17,18].

Literature data reveal that in utero ethanol exposure elicits oxidative stress in the rat fetus [19]. Also, the neurotoxic action of alcohol during fetal development is well documented. There is substantial evidence for the adverse effect of fetal alcohol on different brain regions in the developing brain. Prenatal administration of ethanol reduces the number of cerebral granules and Purkinje neurons [20,21], hippocampal neurons [22,23], cortical neurons [24] and hypothalamic neurons [25,26]. It has been shown that ethanol induces oxidative stress and apoptosis in mouse embryos [27]. Our data also suggest that upon prenatal ethanol exposure, a large number of fetal neurons undergo cell death by an apoptotic process [28].

The microglia has a role in the pathogenesis of ASD as well as in FASD. Microglial activation and increased microglial density were observed in the dorsolateral prefrontal cortex in autism [29]. Tetreault NA et al. (2012) immunocytochemically identified microglia in Fronto-Insular **(FI)** and Visual Cortex **(VC)** in autopsy brains of well-phenotyped subjects with autism and matched controls [30]. In FI, individuals with autism had significantly more microglia compared to controls. In VC, microglial densities were also significantly greater in individuals with autism versus controls. The published data suggested that microglial immune cells had higher density throughout cerebral cortex in brains of people with autism due to increased activity.

The inflammatory response in CNS is mediated by the activated microglia and microglial cells play a central role in response to brain injuries and neurodegenerative processes. While some microglial functions are beneficial, recent studies suggest that the chronically activated microglia may be neurotoxic in various regions of brain [31]. In the healthy brain, the number of ramified or "resting" microglia equals that of neurons, and these cells contribute to the integration of the sensory systems [32]. Along with the astrocytes, they modulate important synaptic, metabolic and neurotrophic functions. However, microglia has been involved in brain–damage-induced neuroimmune responses [33,34].

A role for the microglia in ethanol's neurotoxic action in the adult brain has been identified [35-40]. Microglial cells are involved in the control of neuronal activities both in the adult and in the developing brain [41]. Additionally, microglial cells are quite mobile and they move from one area of the brain to another one, particularly during neurodegeneration [42,43,44]. They have been shown to penetrate into and scatter throughout the cortical grey and white matter as well as the diencephalon during the first 2 trimesters of gestation [45]. Microglial cells are activated in response to inflammatory stimulus [46]. They are able to produce Tumor Necrosis Factor **(TNF)**-α, which is a well-known proinflammatory substance. Also, microglial cells produce various other cytokines including Interleukin **(IL)**-1β, IL-6, Macrophage Inflammatory Protein **(MIP)**-1 α and MIP-2. Our published data indicate that ethanol treatment paradigm that induces apoptotic death of fetal MBH neurons increases the number of OX-6 stained microglia in a culture

model of fetal brain. We also showed that ethanol increased the level of chemokines macrophage inflammatory proteins, MIP-1 α and MIP-2 [47].

Cytokines and chemokines modulate brain function, as well as systemic and CNS responses to infection, injury, and inflammation [48]. In fact, cytokines, such as TNF- α, IL-1β, IL-6, and TGF-β family, are able to modulate neuronal activity [49] and IL-6 promotes oligodendrocyte survival [50]. Although, both IL-1β and IL-6 have been shown to cause neuronal death in many systems [51,52], IL-1β was ineffective in inducing cell death in cultured cortical neurons [53] and IL-6 produced no effect on the level of survival of motor neurons [54]. Ethanol induces TLR4/TLR2 association, triggering an inflammatory response in microglial cells [55]. These data propose that the neuro-immune crosstalk in different regions of the developing CNS may participate significantly in the pathogenesis of autism.

Effect of Alcohol on Neuro-Immune Interactions May Play a Role in the Pathogenesis of ASD

Abnormal immune function or responses during fetal life may lead to neuro developmental disorders including autism. Accumulating evidence suggests the role of immune dysfunctions in prenatal and perinatal onset of developmental abnormalities leading to autism. The neuro-immune-alcohol interactions are important in fetal alcohol brain disorders. Neuroimmune signaling in the brain affected by alcohol persists for long periods and can contribute to long-lasting changes in neurobiology. Various cellular compartments and signaling molecules involved in neuro-immune interactions play roles in mechanisms of ASD or FASD during fetal development. It is well documented that alcohol alters immune functions. Our data demonstrated that chronic alcohol intake decreased the activity of NK cells in rats [56]. Low activity of NK cells was detected in patients with ASD. Using *in vitro* experiments, it was demonstrated that ASD individuals had a reduced capacity of Natural Killer **(NK)** cells to kill K562 target cells (an immortalized myelogenous leukemia cell line) [57]. The increased level of TNF-α was found in cerebrospinal fluid of children with ASD (Chez MG). Increased expression levels of pro-inflammatory cytokines TNF-alpha and IL-6, and decreased Bcl2 expression in lymphoblasts, and decreased levels of TGF-β in plasma [58] and in serum [59] were found in autistic patients. Additionally, the elevations of many cytokines and chemokines are observed in the brain and cerebrospinal fluid of individuals with AD, as Interleukin **(IL)**-6, Transforming Growth Factor Beta 1 **(TGFβ1)**, C-C Motif Ligand 2 **(CCL2)** and CCL17 in the cerebellum [60,61,62]. Clinical studies indicated a link between dysfunctional immune activity and ASD, including maternal immune abnormalities during pregnancy [63,64]. Taken together, the data suggest that the immune dysfunctions caused by various substances during prenatal development may play a role in the pathogenesis of ASD. The immune abnormalities are proposed to be the link between fetal alcohol exposure and ASD. On one hand are the data indicating that alcohol affects microglial function in developing brain, and may cause apoptotic cell death, on the other hand are the published data, documenting increased number of individuals with ASD and immune abnormalities. Moreover,

considering that increased levels of anti- and pro-inflammatory cytokines and low activity of NK cells have been observed in ASD individuals [65] and that the chronic alcohol intake decreases the NK cell activity, proliferative capacity of T cells and increases the levels of pro-inflammatory cytokines, it is possible that alcohol is involved in the pathophysiology of ASD via modulation of immune system. We have previously shown that chronic alcohol administration suppresses NK cell cytolytic activity as well as IFN-γ production from splenocytes in rats [56,66]. Alcohol consumption is also known to decrease Concanavalin A **(ConA)**-induced production of various cytokines, including IL-2, IL-6, and IL-4 from splenocytes [67]. In our review [68] we illustrated that the models of alcohol abuse have identified significant frontal cortical degeneration and loss of hippocampal neurogenesis, consistent with neuroimmune activation pathology contributing to these alcohol-induced, long-lasting changes in the brain. Here, we postulate that the alcohol may play a role in the pathogenesis of ASD by affecting the neuro-immune regulation of developing brain.

Fetal Alcohol Exposure May Be Related to Epigenetic Changes Associated with ASD

It has been shown that the prenatal period of brain development is characterized by rapid and extensive changes in neuroplasticity and is prone to epigenetic modifications. Epigenetic alterations are involved in fetal alcohol toxicity in CNS. The term "epigenetics" refers to all heritable changes in gene expression caused by biochemical modifications in DNA [69]. Epigenetics refers to gene environmental interactions that are associated with alterations in gene expression and phenotype [70,71,72]. There are three main types of epigenetic modifications: DNA methylation, chromatin modifications, and non-coding RNA expression [73].

Epigenetic factors in ASDs are involved in molecular mechanisms of Rett syndrome etiology [74,75,76]. Rett syndrome is associated with mutations in the Methyl-Cpg-Binding Protein 2 **(MECP2)** gene encoding the transcriptional repressor MeCP2 [77], which are active in CNS regulation and neurodevelopment [78]. Decreased protein expression due to enhanced MeCP2 promoter methylation is demonstrated in autistic male brains [79]. MeCP2 binds to methylated DNA [80] and forms a complex with the enzyme HDAC1, and causes chromatin condensation and gene repression [81,82,83]. Studies have illustrated that aberrant methylation may inhibit the expression of MeCP2, and this might be linked to autism. Also, the aberrant promoter methylation at MeCP2 is found in brain DNA of female autistic patients [84]. Experimental data indicated changes in HMGN1 levels that might have induced histone modifications in the MeCP2 promoter region associated with autistic-like behaviour in mice. [85].

MeCP2 deficiency is associated with Brain-Derived Neurotrophic Factor **(BDNF)** dysregulation [86]. In addition, it was shown that BDNF expression is modulated by MeCP2 in an activity dependent manner [87]. Moreover, a recent study suggested a functional interconnection between 2 key epigenetic regulators, MeCP2 and SIRT1, that modulated MeCP2 binding to the BDNF promoter [88].

Another gene which is associated with the etiopathogenesis of ASDs is SHANK3 [89]. Epigenetic alterations are involved in the control of SHANK protein expression. Copy number variations or mutations of either of these proteins may be associated with ASDs [90,91]. For example, SHANK3 mutant mice demonstrated social abnormalities and stereotype behaviors, like those in ASD. It has also been demonstrated that SHANK3 variants are expressed in the developing rodent brain and their tissue-specific expression is regulated by DNA methylation of intragenic promoters [89].

Substantial evidence indicates alcohol-related gene modifications associated with disrupted neurodevelopment [92]. Epigenetic alterations after alcohol exposure may modulate gene methylation and affect migration and differentiation of neural stem cells.

The aberrant methylation in the brain of mice prenatally exposed to alcohol was associated with abnormal expression of various genes [93]. Furthermore, alcohol affects neurodevelopment by increased DNA methylation of genes regulating cell-cycle and differentiation [94]. Fetal alcohol exposure is linked to epigenetic modulations of key cellular processes and may affect neuroproliferation in a long-lasting manner. Decreased hippocampal volume and cellular density are observed in FASD models as well as in ASDs and are associated with behavioural and cognitive disruptions [95]. Neurodevelopmental disorders like attention deficits and ASDs have shared common traits with phenotypes in FASDs [96,97]. Taken together these data suggest that ethanol exposure early in development can lead to altered DNA methylation. Thus, it may play a key role in the long-term maintenance of altered gene expression of genes associated with cognitive and behavioral deficits in ASDs and FASDs.

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