Recent Advances in Autism

Chapter 2

Metabolic and Immune Aspects of Sex Differences in Autism

Afaf El-Ansary[1,2,3,4*]; Naima Zayed[4]; Maha H Daghestani[1,5]; Abir Ben Bacha[3]

[1]Central laboratory, Female Centre for Scientific and Medical Studies, King Saud University, Saudi Arabia.

[2]Autism Research and Treatment Centre, Riyadh, Saudi Arabia.

[3]Member in Council for Nutritional and Environmental Medicine (CONEM) Norway and Head of CONEM Saudi Child Brain Research Group.

[4]Therapeutic Chemistry Department, National Research Centre, Dokki, Cairo, Egypt.

[5]Zoology Department, Science College, King Saud University

[6]Biochemistry Department, Science College, King Saud University

****Correspondence to: Afaf El-Ansary,** Central laboratory, Female Centre for Scientific and Medical Studies, King Saud University, Saudi Arabia*

Email: afafkelansary@gmail.com

Abstract

Autism as a Neurodevelopmental disorder is known to greatly affect males than females. It is very interesting to understand different mechanisms that might be related to male susceptibility or female protection such as sex hormones. Understanding the differences in vulnerability of both sexes' to develop impaired gut microbiota, oxidative stress, glutamate excitotoxicity, and neuro inflammation as etiological mechanisms of autism.

Here we highlight that females are better detoxifiers demonstrating much higher cytochrome P450 enzymatic activities and much lower oxidative markers. Additionally they are less vulnerable to develop glutamate excitotoxicity in case of the imbalanced GABA/glutamate ratio as etiological mechanism repeatedly recorded in autism. The estrogen-induced regulation of several pro inflammatory cytokines might help to understand the remarkable lower incidence of autism in females compared to males. Most interestingly is the relative abundance of Bifido bacterium as bile and tryptophan metabolizing bacteria in females compared to males. Clarifying

all these aspects of sex differences might help to suggest treatment strategies of autism.

Keywords: Autism; Oxidative stress; Glutamate excitotoxicity; Neuro inflammation; Gut microbiota

1. Introduction

Autism is a multifactorial neurodevelopmental disorder with multiple etiological mechanisms [1]. It is well documented that autism has been reported as more prevalent in males than females [2]. This sex/gender differences in prevalence has a great influence on clinical practice and research. Up to the world health organization, sex is defined as the biological and physiological characteristics that define men and women, while, gender refers to the socially constructed roles, behaviors, activities, and attributes that a given society considers suitable for men and women.

It is interesting to understand the sex differences in autism prevalence from Differential Liability Model point [3]. It is accepted that while female has specific protective factors, males has specific risk factors. Among these is the X chromosome gene protective effect or prenatal exposure to hormones as environmental factor [4,5]. These two mechanisms while shift females away from, they bring males closer to the threshold needed for clinical presentation of autism.

In relation to immune aspects of sex differences in autism, another environmental factor which might be of interest is the maternal immune activation. There is a relationship between vulnerability to develop autistic features and maternal–fetal autoantibodies which still needs to be understood in relation to the sex differences and occurrence of autism [6]. Animal studies show that microglial activation in the developing brain in response to maternal immune activation may be sex-specifically activated by prenatal sex hormones [7]. This implicates potential joint effects of hormonal and maternal immunologic factors in modulating sex-differential liability for autism [8].

There is also other gender-specific differences between boys and girls with autism. Adult females had changed lipid transport and metabolism proteins whereas, adult males showed predominant alteration in inflammatory signaling [9]. Earlier studies of intestinal disaccharidases in individual with autism observed that boys below 5 years of age had remarkably lower lactase activity than age-matching girls [10]. Using the valproic acid rodent model of autism demonstrated disturbed social interaction, increased expression of neuro inflammatory markers, deficits in the serotonergic system in brain and intestinal tissue, and increased levels of cecal butyrate in males as compared with females [11].

Glutamate excitotoxicity as potential mechanism repeatedly related to autism might help to understand this sex bias. It is well known that GABA signaling as regulator to ex-

citatory–inhibitory balance, greatly affect microglial activation as neuro-immune interaction [12,13,7].

This review is an attempt to understand the metabolic and immune aspects of sex differences in neurodevelopmental disorders giving more intention to autism as one of the most increased prevalence. This will be done through understanding factors affecting susceptibility to impairment of gut microbiota, oxidative stress, neuro inflammation, excitotoxicity as etiological mechanisms of autism.

2. Gut Microbiota and Sex Differences in Autism

Recently, Kushak and Winter [14] hypothesized that intestinal microbiota might play a critical role in the sex differences in individuals with autism. They considered three factors which include the intestinal microbiome; the intestinal metabolome; and gut-brain axis in humans and rodent model of autism. They hypothesize that microbiota is associated with autism prevalence in boys either directly or through microbial metabolites and/or epigenetic factors capable of regulating host gene expression through DNA methylation and/or histone modification [15].

Studies on animal models indicate that microbial exposure affects sex hormone levels and that both exert an effect on autoimmune diseases [16]. Transfer of gut bacteria from adult males to immature females altered the females' microbiota and leading to a remarkable elevation of testosterone. This help to suggest that composition of gut microbiota is capable of altering sex hormone levels and regulating the development of autoimmune disease. In an attempt to test this hypothesis, Takagishi et al. [17] examined the relationship between salivary testosterone levels and autistic traits in adults. No correlation was found between testosterone levels and autistic phenotypes in males and females. Moreover, White house et al. [18] observed that children with high levels of prenatal testosterone did not develop autism, indicating that prenatal testosterone alone may not be enough to be the trigger autistic features. Also, no relationship was found between early postnatal testosterone concentrations in saliva and traits associated with autism in young children (18-30-month-old) [19]. Jamnadass et al. [20] compared traits associated with autism in young adults with concentrations of testosterone, and DHEA, and estrogens in umbilical cord blood, but association with androgens or the androgen/estrogen ratio was completely absent. So, it can be suggested that elevated testosterone may not alone contribute to the development of autism, and thus sex differences in autism, can be due to elevated concentration of testosterone together with exposure to prenatal stress. Maternal stress that might activates the maternal immune system, exposure to high level of sex hormones, inflammatory agents, endocrine disrupting chemicals or other environmental factors that may induce epigenetic changes all together can explain the sex differences in autism [8,15].

de Theije et al. [11] reported a significant microbiota differences in valproic acid male rats compared to females. These differences were positively correlated with increased levels of cecal butyrate and ileal neutrophil infiltration and negatively correlated with intestinal levels of serotonin and social behavior scores [21]. The most direct demonstration of the effect of microbiota or its products on gender dimorphism was proved through a recent study done by Schaafsma et al. [22] in which they treated pregnant mice with lipopolysaccharide (LPS), components of the cell wall of Gram-negative bacteria known to activate maternal immune system. This treatment affected social responses in the newborn males but not females.

In relation to the effect of gender on intestinal microbiota in humans, a cross-sectional study was done on 230 healthy participants representing four European locations. Levels of Bacteroides-Prevotella group were found to be higher in males than in females [23]. In another study on 200 patients with gut infections, the genus Bacteroides was found to be much higher in females than males [24]. Based on this we can suggest that gender seems to affect the composition of gut microbiota in both animal models and humans.

Using gonadectomy, Org et al. [25] demonstrated that differences in gut microbiota composition between genders were clearly mediated at least in part by sex hormones. Moreover, they showed that testosterone treatment post gonadectomy prohibited the remarkable alterations in gut microbiota composition that were seen in untreated males. Interestingly, the hormonal status of male mice clearly affected the configuration of microbiota on chow and high fat diets, whereas in females this effect was more predominant in response to the high-fat diet. They also reported that hormonal changes powerfully disturb bile acid profiles and that significant sex-specific differences in bile acid profiles become more noticeable in response to a high-fat high-sugar diet. It has been shown that the rate of bile acid synthesis and bile acid pool sizes tend to be higher in females than in males [26]. Since bile acids have been shown to affect gut microbiota, it was suggested that, a possible mechanism for sex differences in bile acid composition might be contributed in the sex differences in autism [27]. This can find support in the recent study of Golubeva et al. [28] which demonstrated that reduction in the relative abundance of Bifidobacterium and Blautia as bile-metabolizing bacteria, is accompanying with deficient bile acid and tryptophan metabolism in the intestine, marked gastrointestinal dysfunction, together with impaired social interactions in BTBR mice.

3. Sex Differences in Detoxification and Vulnerability to Oxidative Stress

A steroidal hormone known as 17β-estradiol (E2) is synthesized from cholesterol by ovary, and to a lesser extent, from testosterone in a reaction catalyzed by aromatase enzyme. It's well-known to exert a variety of actions related to neuronal signals and mitochondrial function. Recently, E2 has been reported as a neuro protectant which can protect neurons only under a specific condition rather than any condition [29-31]. Antioxidant activity is one of the

most recognized protective actions which is attributed to the phenolic moiety of E2's structure scavenges free radicals [32,33]. This antioxidant activity of E2 has been reported to play a role in a sex difference in oxidative stress. Oxidative damage to DNA and lipid is less in the liver mitochondria and synapsis of young adult female rats than male rats [34]. This sex difference was reduced by ovariectomy and ameliorated by E2 replacement [34], demonstrating that E2 may protect females from oxidative damage. Early sex differences in oxidative stress, a mechanism of injury associated with both reduced fetal growth, neurological, and cardiovascular diseases, are still not fully understood. Minghetti et al. [35] reported that, while no difference was reported between males and females in the total antioxidant capacity, the oxidative stress biomarker15-F2t-isoprostane was found to be significantly higher in plasma of males than females even in sex-like or unlike sex twins. This help to suggest that sex-based differences in oxidant injury vulnerability occurring early in life could represent a biological mechanism contributing to gender disparity later in life. This can be of great importance as sex difference factor in autism if we related to the previous study of El-Ansary and Al-Ayadhi [36] and Qasem et al. [37] in which they reported a significantly higher isoprostane as oxidative stress marker in plasma of male individuals with autism compared to control healthy subjects. In their study, elevated plasma levels of 8-isoprostane have also been correlated with anti-neural antibodies as a measure of autoimmunity as a pathological mechanism in autism [38,39]. Moreover, due to its role in platelet aggregation and vaso-constriction, isoprostanes can be related to the brain hypoperfusion as pathologic phenomenon in autistic children compared to controls. A correlation between 8-isoprostane and abnormal blood flow in autism was reported by Yao et al. [40]. Moreover, multiple neuroimaging studies have noted the relationship between oxidative stress and vascular homeostasis in the pathogenesis of autism, including the possible influence of 8-isoprostane.

The sexually dimorphic expression of the xenobiotic-metabolizing enzymes, cytochromes P450 (CYP) has been reported. Animal experiments, principally in rats, have revealed that expression of a number of these P450 enzymes is sex-dependent and regulated by GH secretory patterns. Multiple studies proved the importance of intact pituitary gland for gonadal hormones to regulate sex-dependent xenobiotic metabolism [41-43]. Growth hormone, a 191-amino acid protein hormone secreted by the anterior pituitary gland mediates the effects of sex hormones on liver drug and steroid metabolism. GH treatment of male, but not female rats suppressed total cytochrome P450 (CYP) enzymes as major players for phase I metabolism of drugs and toxicants [44,45]. Colby et al. [46] reported that GH mimic the effect of estradiol, and were manifested in testosterone-treated female rats, establishing a clear link between sex steroids and the responsiveness of hepatic xenobiotic metabolism to GH treatment [47,48]. Because children with autism demonstrate significantly higher levels of many growth-related hormones such as IGF-1, IGF-2, IGFBP-3 and GHBP together with significantly larger head circumferences and higher weights and BMIs compared to age and gender matching controls

[49], we can suggest that males are poor detoxifiers than females. Much higher GH in males can suppress CYP proteins as group of enzymes critically needed for class I detoxification. This suggestion can be supported through the recent work of Medhasi et al [50] in which they proved the genetic variation of cytochrome P450 metabolizing enzymes such as CYP2C9, CYP2C19, and CYP2D6 in autistic patients compared to healthy control subjects. The longer GH inter pulse interval that occurs in males, compared with females, is responsible for the sex differences in liver gene expression of detoxification enzymes and for the sexually dimorphic whole-body growth patterns that emerge at puberty [51].

It is very interesting to correlate the suggested sex differences in cytochrome P450 with the recent alarming increase in the prevalence of autism. Chronic exposure to glyphosate as commonly used herbicide related to autism was found to induce disrupted gut microbiota, impaired sulfate transport, and suppression of the activity of the various members of cytochrome P450 (CYP) as important detoxifying enzymes [52]. With a much lower cytochrome P450 in males compared to females, male children can be more vulnerable to develop autistic traits due to poor detoxification capacity compared to females.

Male-specific genes can be classified based on their response to pituitary hormone ablation. Class I male-specific genes require GH for full expression, whereas class II male-specific genes are primarily regulated by the repressive actions of the female GH pattern in both rats and mice [53]. Rat CYP2C11 is a prototypic class I male-specific gene: it is induced after 4 weeks of age by the male, pulsatile GH secretion pattern, whereas a more continuous, female-like plasma GH profile abolishes CYP2C11 expression.

Once established, however, the different male- and female-dependent CYP isoform profiles are permanent and immutable. Das et al. [54] selectively blocked GH secretion in some newborn male rats, whereas giving concurrent physiologic replacement GH to other group of rats. The results demonstrate that adult male GH activation of the signal transduction pathway regulating expression of the principal CYP2C11 isoform is obligatorily dependent on perinatal GH imprinting, without which CYP2C11 and drug metabolism would be permanently and profoundly suppressed.

Studies carried out in rats and mice have established that sex-based differences in phase II detoxification including sulfotransferases [55,56], glutathione-S-transferases [55,57] and UDP-glucuronosyl transferases [55,58,59]. In addition to drugs and other xenobiotics, CYPs and other phase II detoxification enzymes metabolize endogenous sex steroids [60-63] suggesting that the sex-differentiation of drug metabolism reflects a need for sex-specific steroid metabolism. The extent to which sex differences in detoxification occur in human liver is largely unknown, but it could be suggested as a contributing factor in the sex-differences prevalence of autism [64].

Toxoplasma gondii as a parasite that infects about a third of human population usually cause significant morbidity in immunocompromised individuals such as autistics [65]. T. gondii uses sulfated proteoglycans for host cell invasion and sulfated sugars on the surface of host cells may functions as key parasite receptors. Patients with autism have impaired sulfation and sulfoxidation. The impaired sulfation of dehydroepiandrosterone (DHEA) to DHEA-S affected normal development of various brain functions because DHEA-S inhibited vascular neuro inflammation in autistic individuals probably caused by cerebral toxoplasmosis [65].

To associate the impaired sulfation of DHEA to inactive DHEA-S with the sex differences in autism, it is very interesting to know that decreased sulfation seen in autism results in less of the DHEA is converted into DHEA-S [66] and so more is available to be converted to testosterone. Elevated levels of androgen in individuals with autism as well as confirming the role of impaired sulfation in inducing the elevated level of testosterone [67,68]. In turn, the increased levels of testosterone seem to contribute to the sex-differences in the pathology of autism. Testosterone has been found to decrease cystathione β-synthase (CBS) activity, decrease GSH concentrations, and increase susceptibility to oxidative stress [68]. This can be considered as an important factor which may explain the sex differences in autism.

STAT5b homodimers mediate GH pulse-regulated gene expression, whereas STAT5a-STAT5b heterodimers regulate the expression of some female-specific GH-regulated genes. Ninety percent of the investigated sex-dependent detoxification genes were unaffected by the loss of STAT5b in female liver, where STAT5b activity is much lower than in males [69], and where STAT5a is required for expression of a subset of female-specific genes [70]. Thus, in male liver, STAT5b maintains male liver gene expression through its stimulatory effects on male-specific genes and its inhibitory effects on female-specific genes. These effects of STAT5b are likely to involve a combination of direct and indirect regulatory mechanisms.

4. Glutamate Excitotoxicity in Relation to Sex of Autistic Patients

It is well known that both female and male fetuses produce androgens such as dehydroepiandrosterone [DHEA] in their adrenal glands [71], and placenta plays a critical role in the synthesis and conversion of sex steroids [72]. All sex hormones perform their function on their target tissues mainly through their receptors, and receptor binding to steroid response elements of a given gene, thereby influencing its expression. Additionally, non-genomic effects of sex hormones are mediated by less specific membrane receptors such as PKC, MAPK [73].

To understand the role of gonadal hormones in the sex differences in autism, it was interesting to find the relationship between sex hormones and glutamate excitotoxicity as one of the most repeatedly recorded etiological mechanism of autism. Although the hippocampus is not classically considered a sexually dimorphic structure, but it was found to be larger in males, has sex-specific hormonal regulation, and regulates behaviors which differ in males and

females [74-76]. Moreover, it also expresses androgens at very high levels throughout life and is extremely sensitive to excitatory stimuli such as GABA as the major excitatory neurotransmitter during early postnatal development, and glutamate as the most excitatory neurotransmitter [77]. It is not surprising to know that GABA is excitatory but cedes that role to glutamate around the end of the first week of life [78], which is still a time of normal developmental cell death in the hippocampus [77-79]. To understand the early excitatory character of GABA, it is very interesting to know that the chloride gradient is reversed relative to the basal gradient during prenatal and early postnatal brain development [80]. Due to the higher intracellular chloride concentrations, in early life, activation of GABA receptors induces depolarization via chloride efflux, leading to a significant but transient increase in intracellular calcium typical of normal excitation.

Sex determines neuron's response to the GABA excitatory- induced calcium influx with neurons derived from males suffering more cell death than those derived from females in primary culture [81,82]. Moreover, right after birth, pretreatment with dihydrotestosterone (DHT) exacerbates excitatory GABA-induced cell death [83]. After few days of birth, glutamate predominates as excitatory neurotransmitter [84].

5. Neuro-inflammation and Sex Differences in Autism

It is well documented that broad physiological and pathological consequences are related to the regulatory effect of estrogen of inflammatory responses. However, the molecular mechanism of estrogen regulation of inflammation is still poorly understood. Dai et al [85] reported that estrogen treatment inhibits NF-kB p65 and STAT-1 transcription factors and decrease the expression of inducible nitric (iNOS). Estrogen induces serine protease as key player in regulating inflammatory responses through the proteolysis of transcription factors such as NF-kB p65 and STAT-1 as well as in innate and adaptive immunity. Nevertheless, the underlying mechanism by which estrogen induces serine protease activity is unknown [86]. Given the increasing importance of immune tissue-derived iNOS, IFN-γ, and MCP-1 in health and disease, studies on estrogen-induced regulation of these proinflammatory molecules might offer a better understanding of the less incidence of autism in females compared to males.

Intrauterine maternal infections (IUI) during pregnancy increase the risk for development of certain mental health problems including schizophrenia and autism [87-89]. It was reported that, post LPS exposure, four pro-inflammatory (CXCL10, IL-1β, SOCS3andTNF-α) and three anti-inflammatory (IL-10, IL1ra, TGF-β) cytokines/chemokines were measured. For IL1b, TNF- α and SOCS3, a significant difference was observed between male and female offspring. While these pro-inflammatory markers were elevated in male offspring, they did not differentially affect in response to LPS exposure in females. This pattern is seen in IL-1b, CXLC10, TNF- a, and SOCS3 in hypothalamus (HYP), amygdala (AMYG), and prefrontal

cortex (PFC). On the other hand, there were no differences observed in CXCL10 or any of the anti-inflammatory transcripts [90].

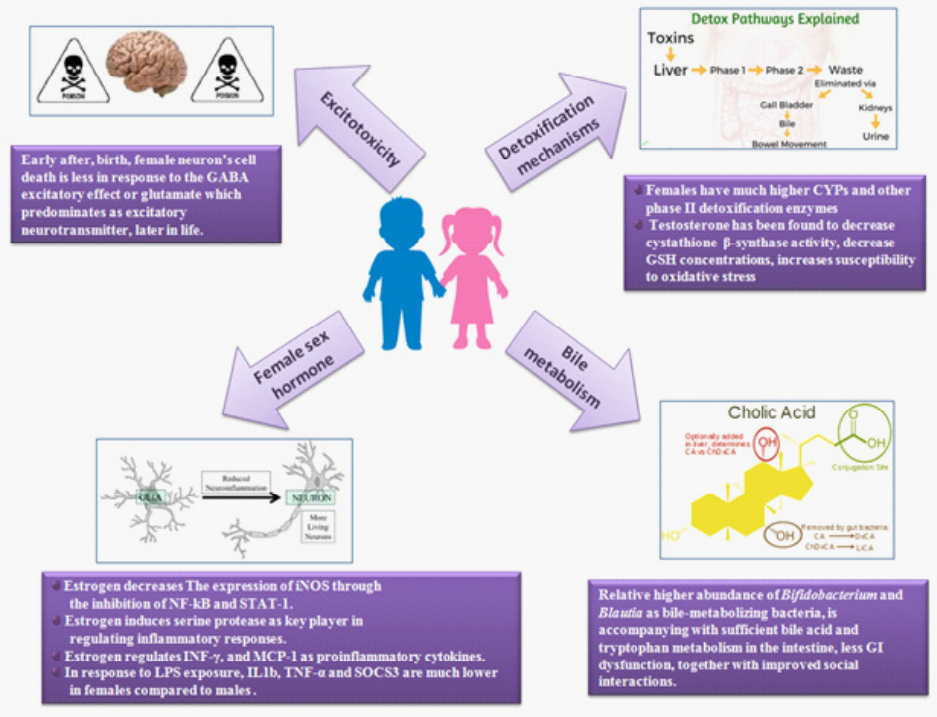

Figure 1: Summarizes the sex differences in detoxification, neuro inflammation, glutamate excitotoxicity, and bile metabolism as suggested reasons of less vulnerability of females to develop autistic phenotypes.

For IL1b, TNF- α and SOCS3, a significant difference was observed between male and female offspring. While these pro-inflammatory markers were elevated in male offspring, they did not differentially affect in response to LPS exposure in females. Regarding C3, there was a significant sex difference in the baseline levels, such that control females had significantly higher levels of C3 as compared to control males [90]. Complement-related proteins are known to play a critical role in synaptic pruning early during fetal brain development. Astrocyte-derived TGF-β specifically signals neurons to produce C1q [91]. Subsequently, C1q and C3 are expressed on weak synapses that are then targeted for phagocytosis and clearance by microglia, a process first reported in the developing visual system [92]. Beyond synaptic pruning, complement proteins may play a role in neurodevelopment. C1q and C3 expression are dynamically regulated in the cortex throughout development, with C1q expression increasing throughout the lifespan, and C3 expression is maximum in early development and decrease to a stable level later [93]. Mice with a global deletion of C1q show enhanced excitatory synaptic connections within the neocortex [94], supporting the idea that C1q is involved in preferentially pruning excitatory, but not inhibitory synapses during development. In contrast, work in the adult mouse cortex, has shown that C1q is selectively expressed only on GABA (inhibitory) neurons [93]. This might help to suggest that exposure to early life inflammation affects the expression of these complement-related transcripts, in such a manner that explain the sex differences in autism and support the vulnerability of males to develop glutamate excitotoxicity as an important etiological mechanism of this disorder.

In addition to the sex difference in immune response towards IUI, two neurotransmitters

were found to be significantly differed in males and females post IUI. Glutamate dehydrogenase1 (GAD1) mRNA transcripts as rate limiting enzyme in GABA synthesis, and dopamine type 2 receptor (DRD2) were remarkably increase in HYP and AMYG of male but not female offspring [90].

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