Biotechnology Section

Gender Bias in Autism Spectrum Disorders- A Review

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ABSTRACT

Autism Spectrum Disorders (ASDs) have been one of the baffling mysteries of human mind. It is known that ASDs have a significant male predominance. Moreover, the differences between male and female brain that have been explored by many researchers point towards the role of hormones specifically steroid sex hormones during the developmental stages in functional development and coordination in brain structures. There is an abundance of receptors for steroid hormones such as testosterone, oestrogen, progesterone in brain. We have reviewed several research papers that indicate the involvement of steroid hormones acting through various pathways that affect cognitive, linguistic, social and emotional circuits which may culminate in manifestation of ASDs and the observed gender bias. These observations may also hold the key for a possible treatment or prevention of these types of disorders.

Keywords: Genetic predisposition, Oestrogen, Pervasive developmental disorders, Testosterone

INTRODUCTION

Autism is an umbrella term for a wide spectrum of disorders known as ASDs or Pervasive Developmental Disorders (PDDs) which are manifested as impairments in central nervous system that result in difficulty in speech, abnormal response to stimuli, poor eye contact, and repetitive behaviour in kids [Table/Fig-1] [1-5]. Recent surveys have suggested that about 60 per 10,000 kids suffer from PDD [6]. A significantly high prevalence of ASD has been observed amongst males as compared to females. A male-to-female gender ratio of 4:1 has been reported for ASD [7]. The typical male to female ratio points to a genetic origin of autism. Studies show that male and female brains are different anatomically and functionally. These differences begin to develop prenatally and mature by adulthood. Sexual differentiation is mainly determined by chromosomal, genetic and hormonal factors. Many researchers believe that autism is caused by imbalance of steroid hormones in early stages of embryonic development. Neurosteroids such as testosterone, oestrogen, androgen, progesterone and oestradiol play an important role in development of cerebellum [8-14]. Scientific data obtained in a study proved that autistic offspring had extensively higher

concentrations of many steroid hormones in saliva as compared to the normal children [12].

In human beings, a female possess superior ability in language related skills whereas males suffer from a higher incidence of social and communicative difficulty. These observations have given birth to extreme male brain theory which proposes that higher testosterone exposures during prenatal and neonatal developmental stage have a profound effect on sexual dimorphism in human behaviour and ASDs [15]. In this study, an effort has been put forth to highlight the implications of hormone, genetic variation and differential brain growth as well as the effect of their intra-associated events on gender bias in manifestation of ASD.

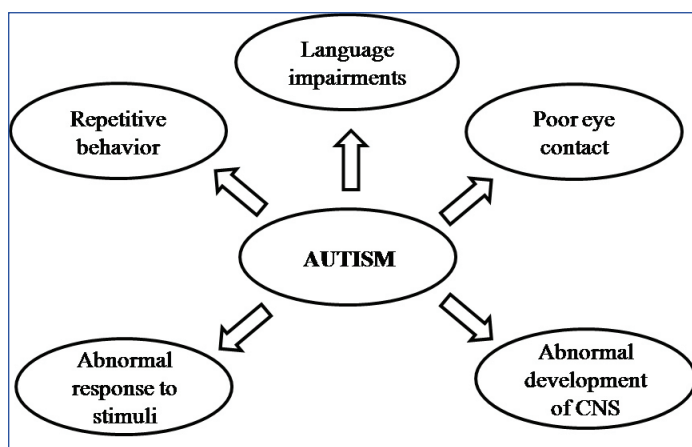### Causes and Risk Factors Associated with ASD

It has been suggested that ASD is caused by a complex interplay of genetic, environmental and biological factors [Table/Fig-2,3] [16-36].

Environmental Factors

It was thought that environmental factors trigger the expression of ASD for the subjects that possess the genetic predisposition. The role of environmental factors contributing to ASD is still inconclusive [37-42]. The environmental factors frequently studied in association with ASD were physiological (parental age, birth trauma), chemical factors and air pollutants. Studies that show association between exposure to heavy metals such as lead, mercury, cadmium and arsenic are incidence of ASD [43]. There are various environmental factors which may have a profound effect on neuronal development during pregnancy.

Genetics

Prominently, mutations in SHANK gene family (SHANK1, SHANK2 and SHANK3) are associated with the development of ASD. These genes encode for the scaffolding proteins located at the Postsynaptic Density (PSD) of glutamatergic synapses and are required for proper formation and functioning of the neural synapses [44]. Mutations in

[Table/Fig-1]: Autism and its effects.

Risk factors	Effects	Reference
Environmental		
Advanced Paternal age	The advanced maternal (≥35 years) or paternal (≥40 years) has been associated with higher incidence of the autism. However, higher risk is associated with advanced maternal age.	[16]
Air pollution	Maternal exposure to air pollution particularly, high concentration of fine particulate matter less than 2.5µ during pregnancy, particularly the third trimester, was associated with greater odds of a child having ASD.	[17]
Pesticide/ Insecticide exposure	Gestational exposure to pesticides such as organophosphates, insecticides such as pyrethroides and carbamates have been associated with greater incidence of ASD.	[18]
Heavy Metal and toxins	The accumulation of heavy metals viz. aluminum, lead, and mercury in ASD individuals. High intrauterine concentrations of heavy metals lead to developmental delays and deficits in motor performance and social behaviour.	[19,20]
Nutritional deficiency	Zinc (Zn^{2+}) deficiency results in irrecoverable impairment of learning and memory. Women taking folic acid supplementation during first trimester of pregnancy have been known to reduce the incidence of autism. Higher incidence of vitamin D deficiency has been reported amongst the infants with ASD. Vitamin A deficiency has been found to be prevalent amongst the ASD children.	[21-24]
Hormonal		
Testosterone	Brain arousal is enhanced in young male animals. Results in hypersensitisation of amygdala.	[8]
Progesterone	Low level adversely affects the development of the blood brain barrier. Affects the development of the amygdale (involved in social circuitry).	[25,26]
Oestrogen	Decrease in CYP19A1 could also to lead to less secretion of estradiol which results in high levels of testosterone. Lack of oestrogen causes hyperactivity of infundibular nucleus.	[11]
Genetic		
SHANK3	22q13.3 deletion in this gene is characterised by severe expressive language and speech delay, hypotonia, global developmental delay, and autistic behaviour. Mutations had an effect on dendritic spine morphology and synaptic transmission.	[27,28]
PTEN	Show widespread brain overgrowth and impaired social behaviour. Increases in cortical white matter and a distinctive cognitive profile in patients. Characterised by delayed language development, poor working memory and processing speed.	[29,30]
GABRB3 (GABAA receptor β3 subunit gene)	With the *Gabrb3 m*-/p+ mutation, which is predicted to increase inhibitory drive from Purkinje cells, CbN neurons in male mice upregulate their mGluR1/5 responses to synaptic stimulation, whereas females do not. Defect causes synaptic dysfunction. Alterations in phosphorylation may contribute to the pathophysiology of ASDs.	[31-33]
RELN	Haploin sufficiency cause cognitive impairment in rodents and also related to the higher brain functions. Mutations are associated with autosomal recessive lissencephaly with cerebellar hypoplasia. Epigenetic regulation results in behavioural alterations by the induction of LTP in them at PFC.	[34,35]
GRIN2B	Disruption leads to receptor trafficking defects, NMDAR hypofunction, and alteration of endogeneous modulator affinities.	[36]

[Table/Fig-2]: Autism related risk factors and their effects.
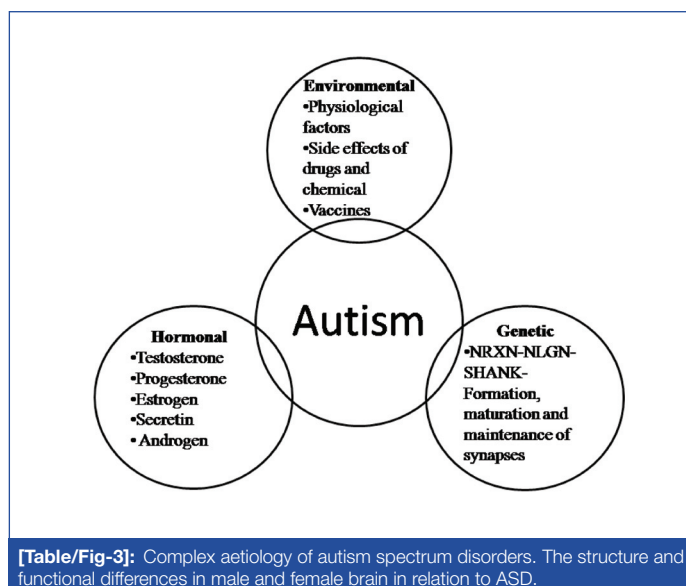
[Table/Fig-3]: Complex aetiology of autism spectrum disorders. The structure and functional differences in male and female brain in relation to ASD.

the genes encoding NRXN1, NLGN3, and NLGN4 in the patients of ASD have been reported recently [45]. The Neuexin – Neuroligin –Shank (NRXN-NLGN-SHANK) pathway plays a pivotal role in the formation, maturation and maintenance of synapses. However, the relationship between these mutations and ASD has not been established completely as these mutations have also been found in non symptomatic carriers in the same family. Moreover, same mutations can be associated with quite different phenotypes in different people [46].

Genes such as the SHANK family, Reelin (RELN), Human serotonin transporter (SLC6A4), Gamma-aminobutyric Acid Receptor (GABR), Neuroligin (NLGN), Human Oxytocin Receptor (OXTR), MET, SLC25A12, Glutamate Receptor Gene (GRIK1 or GluR6), Glyoxalase 1, PTEN, GRIN2B have been implicated in ASD. SHANK1 locus is on chromosome 19 and known to induce the enlargement of spine heads [44,47]. SHANK genes cause alterations in synaptic morphology, signaling and behaviour characteristics [48]. A hemizygous SHANK1 deletion results in male carriers but not female carriers to have ASD [44]. Loss of a functional copy of the SHANK3 gene can lead to the neurobehavioural manifestations of 22q13 deletion syndrome and or might give rise to ASD [49]. These genes affect the main parts of brain, social behaviour and many other things. Though several genes have been proven to be associated the aetiology is complex and it is hypothesised that there is a complex interplay between the environmental and genetic factors in causation of ASD.

Biological Factors

As discussed in the previous section, a strong male predominance is observed in ASD that has been baffling the scientific community for long. It has been suggested that the hormonal and immune processes may play a role in the male predominance in autism [50]. In Attention Deficit Hyperactivity Disorder (ADHD), an association with androgen receptor haplotypes has been discovered [51]. Sex steroid hormones such as testosterone, oestrogen and dihydrotestosterone are the hub of steroid hormones in charge for the organisation and maintenance of the sexual differentiation of the brain structures during premature development stages. Several studies carried out in different organisms demonstrate significant structural and behavioural dissimilarities in female and male brains. It has been documented that considerable differences exist in the distribution of the grey-white matter, callosal commissure, anterior commissure, cortex, hypothalamus, bed nucleus of the stria terminalis, limbic and chemical transmitter systems [52].

Sexually Dimorphic Regions of Brain and their Correlation with ASD

The males and females brains develop differently during adolescence and adulthood. It has been observed that male brains are optimised for intra-hemispheric and female brains for inter-hemispheric communication. The assessments of structure and function suggest that female brains are designed to facilitate communication between analytical and intuitive processing modes whereas male brains are structured to facilitate connectivity between perception and coordinated action [53]. Several functional Magnetic Resonance Imaging (fMRI) studies support the dominant concept that persons with ASD have poorer connectivity (or hypo-connectivity) between distant brain regions (such as the frontal and parietal lobes) and increased connectivity (or hyper-connectivity) between local brain regions (such as within the frontal lobe) [54-56]. This might be a reason for a greater incidence of ASD in male.

Broca's area (Brodmann's areas 44 and 45) is located in the ventral and posterior portions of the frontal lobe; it processes information coming from Wernicke's area (Brodmann's area 22) and converts it into a pattern for language production. In an autopsy analysis (10 men and 11 women free of neurological abnormalities), it was observed that the average volume of Broca's area in women was approximately 20% larger than in men. This difference may be associated with fact that the women possess better language skills than men [57]. Interestingly, Brun L et al., reported mis-folding within Broca's area as a distinctive feature of autistic disorder [58]. It is therefore expected that male subjects would suffer more sharply from ASD symptoms such as difficulty in communication.

The Bed Nucleus of Stria Terminalis (BNST) is a nucleus of the forebrain that receives projections from limbic system nuclei and sends projections to several hypothalamic and brainstem targeted areas. It may control behaviour responses to stress [59]. In a study involving 26 age-matched men and women, it was observed that the volume of the posteromedial region of the BNST is 2.5 times larger in men than that of in women. It was suggested that BNST may play a role in aggressive and sexual behaviours [60]. DeLorey TM et al., the expression of autism candidate gene Gamma Amino Butyric Acid A Receptor Beta 3 (GABRB3) subunit gene in mice heterozygous for the Gabrb3 disruption. They discovered reduction in *Gabrb3* gene expression in mouse model and more so in male mouse [61]. Gender related differences in cortical regions including sulcal and gyral thickness have been confirmed in several studies [62-64]. Atypical sulcal anatomy has been reported for young autistic children [65].

The limbic system is the seat of many emotions such as fear, anger and emotions related to sexual behaviour, memory and learning. The most prominent components of the limbic system are the thalamus, hypothalamus, hippocampus and the amygdale. Moreover, it is known to be a prominent gender dimorphic region. The large nuclei present in diencephalon region of thalamus help in sensory perception as well as in limbic and motor functions regulations. By using Positron Emission Tomography (PET), it was showed that women have larger thalamic nuclei. This study was done on 120 healthy subjects including 65 women and 55 men [66]. The volumetric measurements of right and left thalamic nuclei were performed by MRI in a case control study group consisting (age range: 9-43 years). The results observed no differences between these two groups for unadjusted thalamic volumes. However, the expected linear relationship between total brain volume and thalamic volume was not observed in the individuals with autism [67].

The hypothalamus is larger in men than in women [63]. The Interstitial Nuclei of the Anterior Hypothalamus (INAH)/Sexually Dimorphic Nucleus of the Preoptic Area (SDN-POA) volume is more than twice as large in young adult men as it is in women, and contains about twice as many cells in men [68]. Kurth F et al., observed decrease in grey matter in a section of the hypothalamus in subjects with autism. This region of hypothalamus has been associated with the synthesis of behaviour modulating hormones such as oxytocin and arginine vasopressin [69]. This supports the hypothesis of abnormal hormonal system in autism and has a potential for further investigation into therapeutic approaches.

Studies have shown that the hippocampus in women is larger than in men, which has been testified by two MRI studies. The amygdale, in contrast to the hippocampus, is larger in men. An MRI study of age group of 15 boys and 15 girls reported that the amygdale was larger in boys than in girls aged 7 to 11 years [70]. The amygdale is very important for masculinised social behaviour and which is affected by testosterone. If a female is exposed to a high dose of testosterone during the neonatal period, it resulted in masculinised social behaviour [71]. A larger right hippocampal volume than typically developing controls, even after controlling for total cerebral volume was observed in autistic children [72].

Women also have a larger caudate nucleus as compared to men; this structure is located within the basal ganglia [73]. The caudate nucleus in children with autism has been observed to be enlarged. Increased size of the caudate nucleus in the basal ganglia has been associated with compulsive behaviours, difficulty with changes in routine, and stereotypical motor movements [74].

The Suprachiasmatic Nucleus (SCN) lies just above the optic chiasm; it receives direct retinal input and generates circadian rhythms. Vasopressin and Vasoactive Intestinal Polypeptide (VIP) nuclei are present in the different sub-divisions of the SCN. The VIP subnucleus of the SCN is significantly larger in young adult men than in women [75]. Interestingly, this sex difference may be reversed as the female sub-nucleus becomes larger after 40 years of age [76]. Abnormal sleep patterns have often been reported in patients with ASD [77-79] or Asperger syndrome [80-82].

Hormones that Affect Gender Bias in ASD

Many regions of brain demonstrate presence of sex hormone receptors such as androgens, oestrogen and progesterone. These hormones are known to effect the development of neuronal network, structural as well as cognitive development. Most of the psychological disorders exhibit marked difference in prevalence between the genders. For example, depression is more common in women while schizophrenia is prevalent in men. Recent research links these differences to hormones and their effect on sexually dimorphic structures in brain [83]. These are the clear indication for the involvement of sex hormones in creating a significant difference in prevalence of ASD in men and women. The immunocytochemical localisation of Oestrogen Receptors (ER) α, β and Androgen Receptors (ARs) has shown that there are indeed numerous targets for sex hormones in the adult human brain [11]. The findings that nuclear androgen and ER α and β staining in the SDN-POA was more intense in men than in women which supports the presence of sex differences in this nucleus [84,85]. In actuality, androgen and oestrogen pathways are metabolically linked together. It is possible that altered androgen and oestrogen levels could both lead, via different pathways, to alterations of cognitive, linguistic, social and emotional circuits. Testosterone affects development of callosal connections, resulting in differences in hemispheric specialisation between males and females [86].

Testosterone

Testosterone is a male sex hormone however, it is present in low concentration in women. In addition to gonads which are the primary producers of testosterone, it is also produced in brain [87]. De novo testosterone synthesis can occur starting from cholesterol or it could be produced from other steroids such as deoxycortisone or progesterone that are circulated along with blood. Testosterone after entering the cell cytoplasm binds to the nuclear ARs and gets transported to nucleus where it binds to hormone response element

on resulting in either stimulation of silencing of gene expression [88]. In young male animals, brain arousal is enhanced when testosterone binds to (ARs) of brainstem neurons. This results in hypersensitisation of amygdale in young male subjects. Arousal-related inputs regulate amygdaloid mechanisms for fear and anxiety, with resultant social avoidance. In sharp contrast to boys, girls do not have high levels of testosterone-facilitated arousal-causing inputs to the amygdala and also enjoy the protection afforded by oestrogenic hormones, oxytocin, and the oxytocin receptors. This theory suggests that original technologies applied to the molecular endocrinology of testosterone's actions through AR will offer new avenues of analysis into ASD [8]. Supported by the hypermasculine aspect, studied from the number of observation, a pathological intrauterine testosterone level is suggested to be responsible for the observed altered cerebral asymmetry and consecutive hemispheric dysfunction [9].

A second non genomic mechanism pathway for testosterone action has also been revealed in recent years. It binds to the membrane receptors leading to activation of second messengers. Moreover, testosterone can be converted to either oestradiol by aromatase or into dihydrotestosterone by reductase.

Progesterone

Progesterone plays a role in establishing neuronal connectivity [89] and cortical circuitry [90]. Obstetrical complications and the brain changes, both are associated with autism due to low level of progesterone [13]. It has been found that if it is supplied to the foetus maternally, it does not only support pregnancy but also promotes brain development. A survey of mothers of autistic children (n=86) compared to mothers of naturally-developing children (n=88) regarding obstetrical histories, including five obstetrical risk factors indicative of low progesterone. This study recommends regular monitoring of progesterone levels in risk pregnancies. A theory suggested ensuring adequate levels of progesterone may decrease the likelihood of autism. The strongest of the low progesterone factors identified was the number of women giving birth to a child with autism who experienced contraceptive failure and this similar result found by Juul-Dam N et al., [13]. Low level of progesterone adversely affects the development of the blood brain barrier and has been implicated in autism [25]. Progesterone has a protective function by maintaining the proteins involved in Blood Brain Barrier (BBB) development and function [91]. The recent work suggests that much of the network pathology is due to axonal pathology, possibly myelination deficits [92-96] and myelination is highly dependent on progesterone [97,98]. Progesterone also affects development of the amygdala, a region also involved in social circuitry [26], here progesterone receptors are highly localised in developing male brains and affect development of the medial pre-optic area of the hypothalamus [99], a region rich in oxytocin-containing neurons involved in social behaviours [100]. At a cellular level, progesterone promotes neurogenesis [101], synaptogenesis and dendritic formation [102-107], again changes which have been proposed to underlie autism [108]. Although, the foetal brain has progesterone receptors, the foetus itself does not produce progesterone and the foetal brain is thus dependent on the maternal placenta supply for organising effects to take place. A male and female foetus will receive the same amount of progesterone from the mother; however the male foetal brain expresses progesterone receptors at critical periods, whereas the female brain has very few receptors [109]. Moreover, placentas are gender-specific and the placenta of a male foetus is more programmable and more liable to alter production of progesterone [110]. Thus, the male brain or the male-placenta may be more influenced by progesterone levels than the female brain, explaining the male preponderance in autism.

Oestrogen

Oestrogens are female sex hormones that include oestrone, oestradiol and oestriol produced in the ovaries, placenta, adrenal cortex and testes in males. Oestradiol induces the masculinisation characteristics in many cases [14]. Male and female hormones differentially control the expression in autistic candidates genes, Retinoic acid-related Orphan Receptor-Alpha (RORA) in a neuronal cell line, SH-SY5Y. RORA transcriptionally regulates aromatase, it is an enzyme that converts testosterone to oestrogen. Aromatase protein is significantly reduced in the frontal cortex of autistic patients with respect to sex- and age-matched controls, and is closely correlated with RORA protein levels in the brain. These consequences suggest that RORA may be under positive or negative control of male and female hormones, respectively, through one of its transcriptional targets, aromatase, moreover suggest a mechanism for introducing sex bias in autism. The level of RORA expression can be regulated by both male and female hormones through their particular receptors and that one of its transcriptional targets is CYP19A1 (aromatase gene). In which it has been proposed that the reduction of RORA observed in autism is exacerbated by a negative feedback mechanism involving decreased aromatase level, which further causes accumulation of testosterone and reduction of oestradiol. Deficiency in RORA in autistic brain is likely to be further enhanced by increased levels of testosterone due to repression of aromatase, a transcriptional target site of RORA. Moreover, it plays a critical role in purkinje cell differentiation and cerebellar development as well as in neuroprotection against inflammation and oxidative stress. These elucidate at least some of the pathology that has been observed in autism. However, the clarification proposes that not all samples from autistic individuals are lacking in RORA, as established by previous studies with lymphoblastoid cell lines and brain tissues. On the other side, decrease in RORA has been observed in brains of male as well as female subjects with ASD indicating that the effects are not specific to any one gender. Fascinatingly, RORA and ER allocate a consensus binding site on DNA (AGGTCA) and as a result target common genes. The shared gene targets may explain the lower incidence of ASD amongst the female subjects with higher levels of oestrogen. Increasing the levels of RORA expression, or by inducing shared target genes of RORA through ER, as a result compensate the part of RORA deficiency can protect females against autism [111]. Molecular alterations within the ER signaling pathway may have a say to the sex difference in ASD, but the extent of such abnormalities in the brain is not known. ER is involved in actions of reproductive organs and reproductive behaviour, whereas ER is known to arbitrate some of the effects of oestrogens on behaviours that are not particularly associated with reproduction, such as locomotors activity, fear responses, anxiety, and learning. The oestrogen-ER complex can bind directly to DNA via an oestrogen responsive element or become attached to a transcription factor. It recruits a variety of coregulators that result in the activation or repression of target genes by modifying chromatin structure. CYP19A1 expression is significantly lower in the brain of ASD subjects. Earlier studies have reported the expression of CYP19A1, the key enzyme required for oestrogen production in the cortex. In addition, CYP19A1 is enriched at synapses and localises to presynaptic structures in cortical neurons, signifying that brain-synthesised oestrogen and it has an important role in neuronal function. The decrease in CYP19A1 could lead to less production of oestradiol which results in increased levels of testosterone as observed in ASD subjects. Lack of oestrogens causes hyperactivity of infundibular nucleus [11].

Vasopressin

Vasopressin is androgen-dependent and of particular importance to male behaviour. Excess vasopressin or disruptions in the vasopressin system could contribute to the male vulnerability to ASD. On the other hand, protective processes mediated via oxytocin or the oxytocin receptor might help to elucidate the relatively rare occurrence of ASD in females. Disruptions in either oxytocin or vasopressin or their receptors

could result from genetic variation or epigenetic modifications of gene expression, especially during early development [112].

Further studies should inspect the mRNA and protein levels of sex hormone receptors in the brain samples from ASD and control subjects, and such information would be helpful for better perceptive of the relationship between oestrogen-related and testosterone-related signaling pathways in ASD.

Secretin

Secretin is a gastrointestinal hormone which was first represented as an effective treatment for ASD in 1998. Secretin hormone is a 27 amino-acid polypeptide produced in the intestine. It plays a role in gastrointestinal function. There is a postulated role of secretin in decreasing immune responses in the gut lumen. Secretin receptors have also been demonstrated in the brains of rats and pigs [113]. When injected intracerebrally, it has been observed that secretin decreased the locomotor activity of rats. However, there is still an uncertainty about the role of secretin in the human brain [114].

Secretin alters the activity of structures involved in behavioural breaking in of stress adaptation and visceral reflex reactions. The physiological effects of secretin on behavioural, endocrine, autonomic and sensory neuronal activation patterns, collectively, contribute to central c-fos activation. These studies can consent for further examination of secretin as a brain/gut stress regulatory hormone [115].

Studies provide the first direct immunocytochemical demonstration of secretinergic immunoreactivity in the forebrain and provides evidence that the hypothalamus, similar to the gut, which is capable of synthesising secretin. Secretin has a twin expression in the gut and brain secretin cells, as well as its overlapping central distribution with other stress-adaptation neurohormones, particularly oxytocin, indicate that it is a stress-sensitive hormone. Colchicine-induced an upregulation of secretin that signify secretin may be synthesised on command in the response to stress, a possible mechanism of actions that may underlie secretin's role in autism [116].

Williams K et al., carried out a systematic review of published reports on effectiveness of intravenous secretin therapy for autistic children. However, this survey was unable to establish any effectiveness of secretin in improving ASD related symptoms. Thus, it should not be recommended or administered as a treatment for ASD [117].

Studies on the exact aetiology and cure of ASDs have eluded the medical science for many decades. The studies point to a complex aetiology involving genetic, hormonal and environmental factors that affect during the neuronal development. Extensive experimental evidence based on state of art analytical tools would be required to prove the association conclusively. This would also give an insight to the cure or prevention of this complex developmental disorder.

CONCLUSION

In the present study, we have made a strong case for involvement of different hormones especially sex hormones to be involved in ASDs. These hormones have multiple receptors in different areas of brain and hence hormones and their receptors can prove to be targets for discovery of drugs that could treat autism.

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