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Sex Bias in Autism

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Abstract

Autism is a neurodevelopmental disorder with unknown exact etiology. Interestingly, it affects males more than females in a striking ratio (4:1), respectively. This biased ratio served as a clue to search about the factors that are sex linked and hence sex hormones and X chromosomes were good candidates. Although understanding the basic sex dimorphism in male and female brains is essential to understand autism pathology. Theories regarding the biased sex ratio in autism have been raised, and some have been supported by evidence from human studies. Furthermore, sex-linked genetic dysregulation has also been reported in autism. In this chapter, an overview of what is known about sex bias in autism is reviewed, emphasizing the importance of carrying on in uncoding the sex bias in autism.

Keywords: autism, androgens, digit ratio, RORA gene, X chromosome

1. Introduction

The exact etiology of autism is unknown and no single cause has been identified yet, but the disease is highly heritable and the heterogeneity in clinical presentation is thought to be due to different etiologies, complicates genetic and molecular analysis [1]. The studies strongly suggest a genetic basis of the disease with a complex mode of inheritance. However, autism occurs more in boys than girls in a 4:1 male to female ratio [2]. This bias toward the male gender caught the scientist's attention to search about the factors that are sex linked or gender specific.

2. Sex differences in the brain

Although there is an individual variation in human brain morphometry, it is known that male and female brains are different, both anatomically and physiologically. Male brain has

a larger cerebrum than female brain, and more white matter than gray matter, respectively [3, 4]. Despite the larger total volume of white matter in men, the ratio of corpus callosum to total cerebral volume is smaller in them [4]. This ratio is consistent with the finding that the increased size of the brain predicts decreased interhemispheric connectivity [5] and that larger brain comes with smaller corpus callosum [6]. Moreover, the male amygdala experiences an extended period of growth during childhood and men have a larger amygdala compared to women.

At micro-architectural level, male cerebral cortex has more neurons than that of female's and those neurons are densely packed with the exception of some areas of the brain [7]. This dense packing of neurons in male brain is associated with more intrahemispheric white matter projecting from these neurons suggesting, indirectly, a pattern of increased local connectivity and decreased interhemispheric connectivity in the male brain [8, 9].

Physiologically, and consistent with the above-mentioned observations, the language-related activation in female brain is more bilateral, suggesting a greater interhemispheric connectivity in females than males [10–12]. This sexual dimorphism of brain structure maybe related to sex chromosomes, hormonal effects, and environmental factors or as a result of a combination of all these factors. These morphological differences have a profound effect on brain physiology and substantiate one of the main hypotheses for etiology of autism as it will be discussed later.

3. Role of sex hormones in developing brain

Androgens including testosterone act on the developing brain to produce sex differences at the level of neuroanatomy and brain function. For more understanding of the role of sex hormones in brain development, scientists have divided its actions into prenatal and postnatal effects and described it via two main distinct mechanisms.

In the prenatal period, a very critical period of brain development especially in the first trimester, sex hormones have a permanent organizational effect. The organization mechanism is defined as a developmental mechanism in which steroids act on the brain during critical periods of brain development to mediate permanent sexually dimorphic differentiation of brain morphology, giving rise to male and female sexual behaviors and physiology in adulthood. On the other hand, in the postnatal period, sex hormones have a transient activational effect. Activation mechanism is defined as the acute effect of gonadal hormones on the fully developed nervous system, and it is responsible for maintaining sex specific behaviors in adulthood; it is not permanent and is associated with changes in sex hormones levels, for example, hormonal changes during menstrual cycle [13, 14]. Most studies about organizational effect of sex steroids focused on the role played by androgens in masculinization of the brain, while little is known about the feminization of the brain, which was though initially to be a passive process that occurs in the absence of high levels of androgens. However, a growing recognition that it might be induced through early postnatal estrogens, although the exact mechanisms remain unclear [14].

4. Androgens

Androgens are steroid hormones, principally testosterone and 5 α -dihydrotestosterone, synthesized and secreted into the blood stream in the form of testosterone. Once testosterone reaches its target tissues, it is either metabolized by an aromatase into estradiol, which performs its action by binding to estrogen receptors (ER α , ER β or GPER) or metabolized by 5 α -reductase into dihydrotestosterone (DHT) in majority of the male sexual organs. Testosterone can perform direct actions prior to any metabolic processing via binding to androgen receptors (ARs), a member of nuclear receptor super family. Once it binds to its receptors, AR enters the nucleus, binds the DNA and affects the transcription. Binding of estradiol to its receptors affects the transcription similarly.

During the male life, testosterone has three surges; the first is mediated via fetal testosterone (fT) between 4 and 6 weeks of gestation, which can almost reach pubertal levels. This early surge results in genitalia masculinization and sex dimorphic brain development in the mean of cell proliferation, death, migration, and synaptic formation [15]. The second surge occurs soon after birth called neonatal testosterone (nT) and drops by 4–6 months, during this surge, testosterone reaches pubertal levels and was found to be linked to gender-related typical and atypical behaviors and also contributes to normal growth of male-external genitalia [16]. The third surge is the pubertal one.

Testosterone is essential for the development of male secondary sexual characteristics, for general well-being, prevention of osteoporosis, and it has a direct effect on muscle development. It can easily cross the blood brain barrier and bind to AR, which are expressed in the cerebral cortex, cerebellum, mediobasal hypothalamus, amygdala, corpus callosum, and cingulate cortex [17, 18].

Testosterone in the brain was shown to affect the development of neurons as it prevents apoptosis, influences the neuronal connectivity, and alters the neurochemical profile. Both estradiol and testosterone modulate serotonergic and γ -aminobutyric acid neurotransmission. They increase the dendritic spines through the brain derived neurotrophic factor-5 [18].

DHEA and its DHEA-S are known as neurosteroids as they can undergo *de novo* synthesis in the CNS [19], but in humans, the blood represents the main source of DHEA and DHEA-S in the brain. Brain DHEA-S potentiates the action of glutamate and enhances the depolarization and calcium ion entry [20]. Interestingly, DHEA-S acts as antiglucocorticoid as it antagonizes the immunosuppressant and lympholytic actions of cortisol [21]. Subsequently, DHEA protects the hippocampus from the neurotoxic effect of glutamate analog and corticoids [22].

5. Sex-related theories of autism

Autism is a disease of male gender as the male to female ratio is 4:1 [23]. This bias toward the males served as a clue to search for a link between the gender and etiology of autism.

The clinical manifestation of the disease created many hypotheses that support this sex-linked relationship. The theories focusing on the sex ratio include the extreme male brain theory (EMB) of autism, which is an extension of the empathizing systemizing theory (E-S) and it links autism to prenatal exposure to high fetal testosterone [24]. The other theory is the X-linked imprinting theory of autism, which proposes the vulnerability of male to autism based on the fact that males have a single X chromosome [25].

6. The extreme male brain theory (EMB) of autism

Autism is a disease of male gender as the male to female ratio is 4:1 [23]. This bias toward the males served as a clue to search for a link between the gender and etiology of autism. The clinical manifestation of the disease created many hypotheses that support this sex-linked relationship.

E-S theory supported the clinical presentation of the disease and its strong association with the male gender. This theory proposed that females have stronger empathizing, which reflects the ability to identify the other mental status and respond to it with appropriate emotions. On the other hand, systemizing that is stronger in males reflects the drive to analyze a system in term of rules that govern the system [17]. A further extension of the E-S theory of autism is the EMB, which was proposed by Hans Asperger more than 60 years ago. EMB encodes that based on the E-S theory, females are stronger in sympathizing and males are stronger in systemizing, and thus, autism can be considered as an extreme of the normal male profile [24]. This psychological difference is a result of sex differences in the brain structure. Changes in normal brain anatomy have been established in children with autism. They have a larger than average head, which contains abnormally large brain. Cerebral cortex is enlarged with more white matter than the gray matter in autistics. Moreover, they have a greater growth of amygdala. All these changes reflect an exaggeration of normal male brain growth and describe the autistic brain as hypermasculinized brain, which goes along with extreme male brain theory of autism [26]. Studies in humans showed that prenatal exposure to high levels of fetal testosterone resulted in masculinization of the brain. This was first observed in girls with congenital adrenal hyperplasia (CAH), a congenital condition associated with abnormally high level of testosterone. Those girls showed autistic behavioral manifestations [27]. This observation led scientists to hypothesize that one cause of autism is a prenatal exposure to high levels of fetal testosterone.

Consistently, the Cambridge Fetal Testosterone project, in which a correlation between prenatal testosterone exposure and the development of behaviors related to autism was directly investigated, demonstrated an association between fetal testosterone, measured in women who had amniocentesis for other reasons, and the development of autistic traits in their children. Baron-Cohen and his colleagues had recently provided the first direct evidence of elevated fetal steroidogenic activity in autism through their longitudinal study [27, 28]. Moreover, this relationship was observed in both boys and girls denoting that this effect is due to the fetal testosterone rather than the sex of the child per se.

7. The X-linked imprinting theory of autism

Many clinical observations related to autism epidemiology raised the interest in finding a link between disease etiology and genetic mechanism. Strong familiarity and the higher incidence in siblings with a 3–5% recurrence rate (which accounts for 50–100-fold increase in autism risk compared to the general population) are among the main factors that suggested genetic basis for autism [29]. Moreover, the incidence of autism was found to be increased in monozygotic twins (60%), which indicate a strong genetic influence [30]. Epidemiological studies also found male relatives to male and female autistics to be affected more than female relatives. Accordingly, are females protected by a genetic mechanism? The X-linked imprinted hypothesis, hypothesize that, “the threshold for phenotypic expression of many autism related characteristics is influenced by an imprinted X-linked gene(s) that is protective in nature [25]. Imprinted genes are genes that are exclusively expressed from one parent (one allele only is expressed) [31]. They are known to play an important role in growth and development. Moreover, they affect neurodevelopment, brain function, and behavior [32, 33]. In case of the X-linked imprinted gene theory, the gene is expressed only on the X chromosome that is inherited from the father. Subsequently, as only females can carry paternal X chromosome, the threshold for phenotypic expression is higher in them compared to male [25].

8. Hyperandrogenism in autism

In the light of EMB theory of autism, numerous studies were conducted to investigate postnatal testosterone levels in autistic children. Although it has been demonstrated by some studies that children with autism have significantly elevated androgen levels [34, 35], other studies by Tordjman et al. [36] and Lutchmaya et al. [37] have not found significant differences in testosterone levels in autism. Moreover, postpubertal androgen levels were found to be lower than controls in autistic children [38]. We investigated the role of androgens in Saudi autistic children and our findings demonstrated a significant elevation of both total testosterone, which was increased by 53% in autism and free testosterone, which was 238% higher in autistic children, without significant differences in the sex hormone binding globulin (SHBG) levels between autistic group and control group. The significant increase of free testosterone in a magnitude higher than that of total testosterone is explained by the lack of concomitant increase in SHBG. Both total testosterone and free testosterone were positively and strongly correlated with each other and both of which had a positive strong correlation with DHEA, which was also significantly elevated in autistic children by 50% suggesting an adrenal source of the elevated androgens [35]. Significant elevation of those androgens has been reported in American autistic children in comparison with laboratory reference values by Geier's study, which reported 158% increase in total testosterone, 214% increase in serum free testosterone, and 192% increase in DHEA [34]. On the other hand, Croonenberghs and his colleagues reported a significantly lower testosterone levels in autistic children (12–18 years of age) compared to their age matched controls. This report does not conflict with the results of Geier's and Al-Zaid study for two reasons, first, the age group included in Croonenberghs's study was postpubertal, and second, the

decrease in testosterone level in this age group was explained by a greater negative feedback at the hypothalamic level. The preoptic region in the hypothalamic nucleus is androgen sensitive, and it is 2.5 times larger in males. The prenatal growth of this region is strongly determined by the presence of testosterone. Subsequently, too much testosterone early in life could influence androgen receptors or its sensitivity in this region. At a later age, namely during puberty, this could cause a negative feedback resulting in lower testosterone concentrations [38]. On the other hand, Tordjman et al. [36] and Lutchmaya et al. [37] reported a non-significant difference in testosterone level between autism group and their age matched control. However, it is important to note a significant limitation in the Tordjman's study, which was the remarkable heterogeneity in subjects involved in the study as it included prepubertal and postpubertal subjects.

In addition, high testosterone level in children was found to be associated with moodiness, low attachment, and low sociability in prepubertal ages [39], which are common observations in autistic children [40]. Interestingly, the main source of the elevated intrauterine fetal testosterone is not from maternal source, but it is from the fetus itself [14]; this suggests a genetic underlying problem in the fetus and also suggests that it is a lifelong condition. Moreover, the reported observation of low 2nd:4th digit ratio is not only in autistic children, but it is in their siblings and parents [41] suggesting also a genetic connection with autism. The genetic involvement of androgen dysregulation in autism has been reported, in a recent study, which linked a polymorphism in androgen receptor gene (SRD5A2) to autism in Slovak autistic children [42]. More recently, the discovered dysregulation of RORA gene in autism and its influence on the aromatase enzyme, a key regulator for sex hormone biosynthesis pathway, provides a possible broader explanation for the link between autism and hyperandrogenism [2]. Another explanation for the elevated androgens in autistic children was suggested by Geier and his colleagues who reported a significant abnormality in the DHEA synthesis pathway in autistic children. Normally, DHEA can either be converted into the storage molecule, dehydroepiandrosterone sulfate (DHEA-S), or into testosterone. In patients with autism, a decrease in transsulfuration metabolites was observed, resulting in a marked shift toward DHEA with a decrease in DHEA-S production and subsequent increase in testosterone [34] (**Figure 1**).

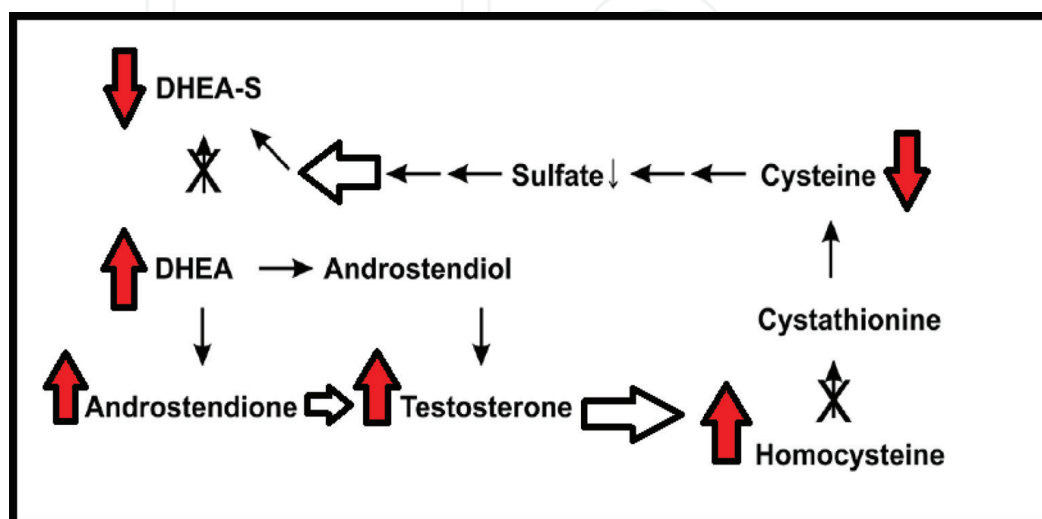


Figure 1. The potential interactions between the transsulfuration and androgen pathway in autism [34].

9. The 2nd:4th digit ratio as a promising screening tool in autism

The levels of androgens are not routinely assayed during pregnancy and direct measures of fetal testosterone concentrations are not usually available. To test the prenatal androgen theory of autism, indirect measures have been used in an attempt to investigate the consequences of fetal testosterone exposure. In 2001, Manning has proposed the 2nd:4th digit ratio as an indirect proxy to the prenatal androgen exposure. They studied the 2nd:4th digit ratio in 95 families with children diagnosed with autism and compared them to families with no autistic children. This study concluded that the highest 2nd:4th digit ratio was found in children without ASDs (autism spectrum disorders), lower in children with asperger syndrome and significantly lowest in children with autism [41]. We recently investigated the 2nd:4th digit in Saudi boys with autism and found it to be significantly lower in them compared to sex and age matched controls (**Figure 2**), and we concluded that 2nd:4th digit ratio could serve as a potential screening tool for autism [43]. This suggests that low 2nd:4th digit ratio is associated with an increased risk of autism. This finding is consistent with several other studies, which reported a lower ratio in British autistic patients [41], Thai patients [44] and Slovak patients [45]. A lower 2nd:4th digit ratio indicates the exposure of Saudi autistic children to high levels of fetal testosterone, as was substantiated by large amount of correlational evidence in both animal and human studies [46, 47]. Several theoretical observations may substantiate the connection between a lower 2nd:4th digit ratio and the increased fetal testosterone levels. First, the ratio is sexually dimorphic with males having a lower ratio compared to the females [48]. Second, the ratio is determined before birth, most probably by the 14th week of pregnancy, and it is not affected by later testosterone concentrations or fluctuations [46]. Third, the ratio was reported to correlate negatively with testosterone and positively with estradiol levels in adults [48]. Overall, this finding implicates increased fetal testosterone levels in the pathophysiology of autism.

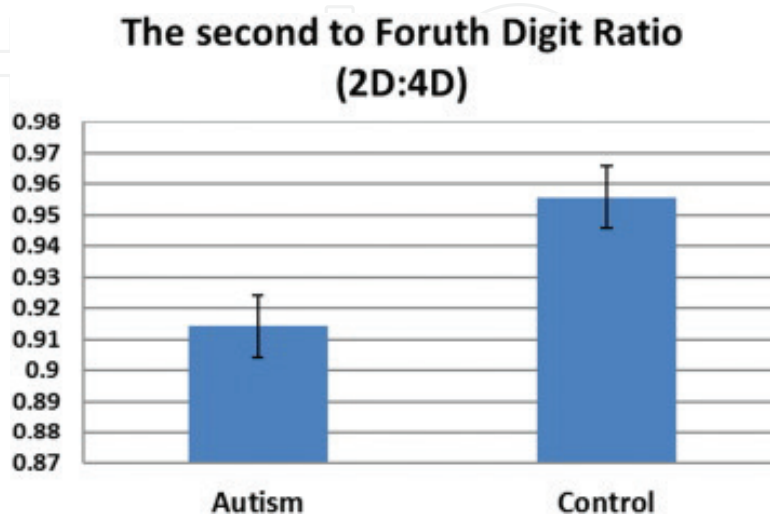


Figure 2. Bar chart demonstrating the second to fourth digit ratio (2D:4D) in the Saudi boys with autism and age and sex matched controls [43].

The significantly elevated levels of androgens in Saudi autistic children together with the significantly lower 2nd:4th digit ratio are consistent with the fetal androgen and extreme male brain theories of autism [24].

10. Sibling sex ratio in autism

It was hypothesized that the parental hormonal levels around the time of conception influence the sex ratios at birth [49]. Accordingly, high parental level of testosterone was suspected to be associated with the production of male offsprings.

In 2010, Mouridsen and his group showed that the sibling sex ratio in a group of 326 individuals with ASDs (245 males, 81 females) at the Danish university clinic was 0.585, which was significantly higher than that in the Danish live birth sex ratio over the same period. However, in autism childhood subclass of ASDs, the result reflected a non-significant difference in male proportion among siblings of autism than that in the Danish live birth sex ratio over the same period of time. Based on the published evidence, which indicates that the human sex ratio at birth is strikingly stable and it is controlled by the parental hormone levels around the time of conception [49], significantly higher male sex ratio was observed in the siblings of Danish autistic patients [50]. These findings indirectly support the EMB theory of autism. However, recently in 2015, Cheslack-Postava and his colleagues failed to find a link between autism and the number of sibling males in a large population-based study [51]. Thus, further studies of sibling sex ratio in autism need to be conducted on a large population scale in order to reach a clear conclusion.

11. The retinoic acid-related orphan receptor-alpha (RORA) gene and sex hormones in autism

A novel autism candidate gene has been identified, a hormone dependent transcription factor, which is the retinoic acid-related orphan receptor-alpha (RORA) [52]. The RORA gene was found to be differentially and reciprocally regulated by androgens and estrogens [2]. Both estradiol and dihydrotestosterone (DHT) enhance the binding of ER and AR, respectively, to the RORA gene promoter regions.

While the estrogen was found to enhance the RORA gene expression, the DHT had a reciprocal effect as it represses the expression of RORA gene. The target of the RORA gene expression is the aromatase enzyme responsible for estradiol biosynthesis. As aromatase is the key hydroxylating enzyme that converts androstenedione into estrone and testosterone into estradiol, it is therefore considered as a crucial protein in the regulation of the sex hormones levels in various tissues including the brain (**Figure 3**).

A deregulation of the RORA gene was found in autism and it was linked to an increase in androgen biosynthesis and to the higher levels of androgens in the lymphoblastoid cells observed in autistic children. The abnormality in the RORA gene in autism includes reduced expression and

increased methylation in lymphoblastoid cell lines and decreased expression of RORA gene in the brain of autistic children [52]. The reduction in the brain RORA gene in autistic children negatively impacts many physiological process regulated by the RORA gene, which includes differentiation of Purkinje cells [53], cerebral development [54, 55], neuronal protection against oxidative stress [56], suppression of inflammation [57], and regulation of circadian rhythm [58].

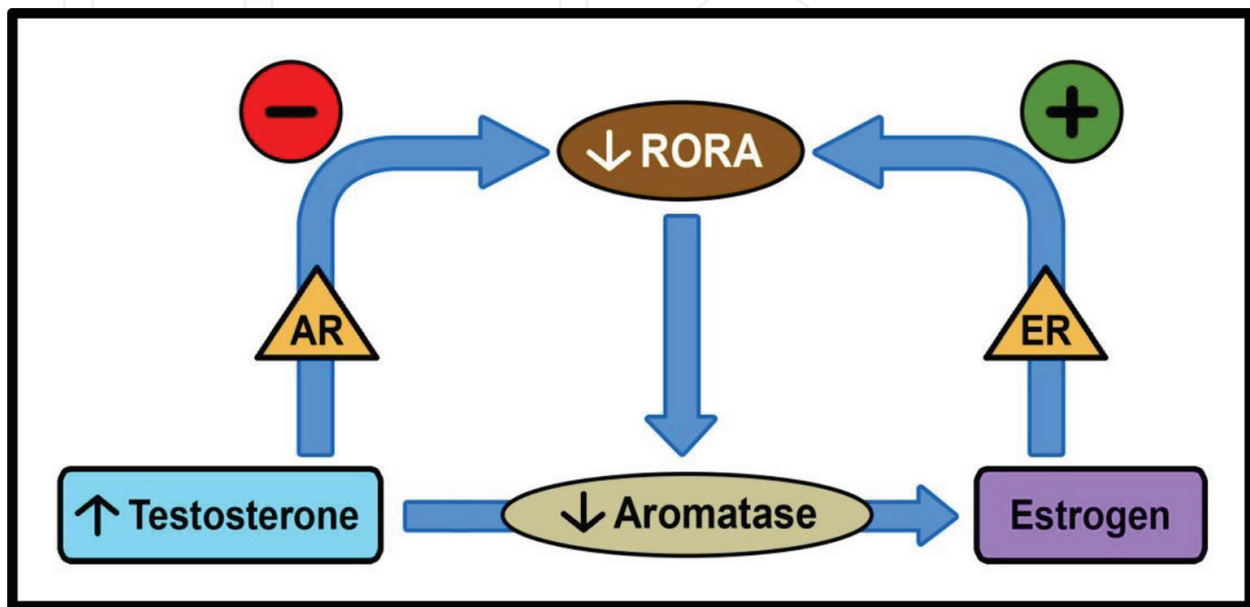


Figure 3. A model for the reciprocal hormonal effects on RORA gene [2].

The other aspect related to the abnormality in RORA gene in autism is its influence on the sex hormones level. Postmortem studies on autistic brains showed reduction in both RORA gene expression and aromatase in the frontal cortex and the cerebellum [52]. This reduction is exacerbated by negative feedback mechanisms as a result of decreased aromatase level with the subsequent accumulation of its substrate testosterone and reduced production of its product estradiol. The end result is a vicious cycle of increased testosterone and reduced estradiol [2]. This deregulation of the RORA gene in autism and its subsequent influence on the brain and the sex hormone levels in autistic children could provide a molecular explanation for the observed hyperandrogenism in children with autism.

12. Conclusion

In conclusion, autism has a striking male to female ratio, which acts as a code for autism etiology. Uncoding the sex bias in autism would aid dramatically in understanding the underlying mechanisms. Subsequently, early screening, treatment, and prevention measures could be conducted. Furthermore, sex-linked genetic and hormonal factors have been hypothesized in autism, and some evidences have been found. However, further direct investigations should be carried on.

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