ACTA NEUROBIOLOGIAE EXPERIMENTALIS

Review Acta Neurobiol Exp 2012, 72: 1–17

An evaluation of the role and treatment of elevated male hormones in autism spectrum disorders

David A. Geier[1], Janet K. Kern[1], Paul G. King[2], Lisa K. Sykes[2], and Mark R. Geier[3]*

[1] The Institute of Chronic Illnesses, Inc., Silver Spring, MD, USA [2] CoMeD, Inc., Silver Spring, MD, USA; [3] ASD Centers, LLC, Silver Spring, MD, USA, *mgeier@comcast.net

Autism, Asperger's syndrome (AS), and pervasive developmental disorder – not otherwise specified (PDD-NOS) compose the overall diagnostic category of autism spectrum disorder (ASD). Subjects diagnosed with an ASD have a male:female ratio of 4:1, and among subjects diagnosed with AS the male:female ratio is as high as 9:1. The purpose of this study was to examine evidence of the association between hyperandrogenism and autistic traits (ATs) among subjects diagnosed with an ASD, and to evaluate the effectiveness of anti-androgen therapy as a means to help treat ATs in subjects diagnosed with an ASD. Evidence of hyperandrogenism in subjects diagnosed with an ASD is supported by multiple studies in the areas of psychological framework, brain pathology, tissue culture, and pre- and postnatal androgen levels. Data from subjects diagnosed with other conditions associated with elevated androgens reveals many of these individuals have ATs. Finally, in a placebo-controlled trial of testosterone administration to neurotypical subjects, testosterone was found to increase ATs. In addition, a controlled trial of human transsexuals revealed a significant increase in ATs in female-to-male transsexuals and a decrease in ATs in male-to-female transsexuals. Data from multiple animals and human clinical trials suggest that anti-androgen medications have the ability to significantly reduce ATs in patients diagnosed with an ASD. In light of the robust association between hyperandrogenism and ASD, it is recommended subjects diagnosed with an ASD should undergo routine screening for elevated androgens, and appropriate treatment should be initiated for those with elevated androgens.

Key words: Autistic disorder, cyproterone acetate, leuprolide acetate, testosterone

INTRODUCTION

Autism, Asperger's syndrome (AS), and pervasive developmental disorder – not otherwise specified (PDD-NOS) are considered to lie on the same continuum, and compose the overall diagnostic category of autism spectrum disorder (ASD). The American Psychiatric Association's Diagnostic Statistical Manual of Mental Disorders, 4th Edition Text-Revised (DSM-IV-TR) is the main diagnostic reference used by mental health professionals and insurance providers in the United States to diagnose patients with an autistic disorder (American Psychiatric Association 2000). The diagnosis of an autistic disorder requires that at least six developmental and behavioral characteristics are apparent, that delays or abnormal functioning are identifiable in at least one of the following areas (with onset prior to age 3 years): social interaction, language as used in social communication, or symbolic or imaginative play, and finally, that the disturbance is not more easily attributed to Rett's Disorder or Childhood Disintegrative Disorder. The following is from the DSM-IV-TR criteria for Autistic Disorder: ''(1) qualitative impairment in social interaction, as manifested by at least two of the following: (a) marked impairment in the use of multiple nonverbal behaviors such as eye-to-eye gaze, facial expression, body postures, and gestures to regulate social interaction; (b) failure to develop peer relationships appropriate to development level; (c) a lack of spontaneous seeking to share enjoyment, interest, or achievements with other people (e.g., by a lack of showing, bringing, or pointing out objects of interest); (d) lack of social or emotional reciprocity. (2) qualitative impairments in communication as manifested by at least one of the following: (a) delay in, or total lack of, the development of spoken

Correspondence should be addressed to M.R. Geier
Email: mgeier@comcast.net

Received 13 November 2011, accepted 23 January 2012

language (not accompanied by an attempt to compensate through alterative modes of communication such as gesture or mime); (b) in individuals with adequate speech, marked impairment in the ability to initiate or sustain a conversation with others; (c) stereotyped and repetitive use of language or idiosyncratic language (d) lack of varied, spontaneous make-believe play or social imitative play appropriate to developmental level. (3) restricted repetitive and stereotyped patterns of behavior, interests, and activities, as manifested by at least one of the following: (a) encompassing preoccupation with one or more stereotyped and restricted patterns of interest that is abnormal either in intensity or focus; (b) apparently inflexible adherence to specific, nonfunctional routines or rituals; (c) stereotypes and repetitive motor mannerisms (e.g., hand or finger flapping or twisting, or complex whole-body movements); (d) persistent preoccupation with parts of objects."

Although a few children diagnosed with an ASD develop normal and even advanced skills in particular areas, most exhibit a wide range of profound behavioral problems and delayed or undeveloped skills. Further, a child diagnosed with an ASD may display a range of problem behaviors such as hyperactivity, poor attention, impulsivity, aggression, self injury, and tantrums. In addition, many frequently display unusual responses to sensory stimuli such as hypersensitivities to light or certain sounds, colors, smells, or touch and have a high threshold of pain (Geier et al. 2010). Therefore, in the absence of treatment, an ASD is, in general, a lifelong developmental disability that profoundly affects the way a person comprehends, communicates and relates to others (Geier et al. 2010).

Many clinical conditions occur in males more often than females, including autism, dyslexia, specific language impairment, attention-deficit hyperactivity disorder (ADHD), and early onset persistent antisocial behavior (Rutter et al. 2003). ASDs in particular have been described as an extreme manifestation of certain sexually dimorphic traits or as a consequence of an 'extreme male brain' (EMB) (Baron-Cohen 2002). The strong bias of subjects diagnosed with an ASD towards males has been well established (Fombonne 2005). Subjects diagnosed with an ASD have a sex ratio of 4:1 (male:female) across the full Intelligence Quotient (IQ) range, and the ratio is as high as 9:1 for AS (Scott et al. 2002), the subgroup in which individuals have intact IQ and language development.

The purpose of the present review is to examine evidence of hyperandrogenism and its association with clinical features of patients diagnosed with an ASD, and to examine evidence of the effectiveness of antiandrogen therapy as a means to help treat clinical features in patients diagnosed with an ASD.

AUTISM SPECTRUM DISORDER CLINICAL SYMPTOMS AND ANDROGEN LEVELS

Psychological framework

The EMB theory is an extension of the empathizing–systemizing (E–S) theory of typical psychological sex differences which proposes that females on average have a stronger drive to empathize (to identify another person's emotions and thoughts, and to respond to these with an appropriate emotion), while males have a stronger drive to systemize (to analyze or construct rule-based systems, whether mechanical, abstract, natural, etc.) (Baron-Cohen 2002). Evidence has been reported showing a female advantage in empathy and a male advantage in systemizing (Auyeung et al. 2009). Consistent with the EMB theory, individuals diagnosed with an ASD were shown to have a stronger drive to systemize but an impairment on tests of empathizing (Auyeung et al. 2009).

Brain pathology

Investigators hypothesized that fetal testosterone (fT) exposure facilitates the growth of certain areas in the right hemisphere of the brain while simultaneously inhibiting the growth of the same areas in the left hemisphere of the brain (Auyeung et al. 2009). In support of this hypothesis, studies investigating body asymmetry found that left-handedness and asymmetrical lateralization were associated both with being male and with autism (Auyeung et al. 2009). Analogies can also be drawn between sex differences in brain development and neuroanatomical characteristics found in autism. The typical male brain is heavier than the female brain, and individuals diagnosed with autism have heavier brains than typical males (Harden et al. 2001). The amygdala is also disproportionately large in boys compared to girls (Giedd et al. 1996) and children diagnosed with autism have enlarged amygdala (Hazlett et al. 2005). These findings are consistent with the EMB theory.

Molecular tissue culture studies

Researchers undertook a case-control study to understand the molecular basis of autism spectrum disorders based upon large-scale gene expression profiling (Hu et al. 2009). DNA microarray analyses were conducted on lymphoblastoid cell lines from over 20 sib pairs in which one sibling had a diagnosis of autism and the other was not affected, in order to identify biochemical and signaling pathways which are differentially regulated in cells from autistic and non-autistic siblings. Bioinformatics and gene ontological analyses of the data implicate genes which are involved especially at the level of androgenic hormones. Metabolic profiling of steroid hormones in lymphoblastoid cell lines from several pairs of siblings reveals higher levels of testosterone in the autistic sibling, which is consistent with the increased expression of two genes involved in the steroidogenesis pathway. Global gene expression profiling of cultured cells from ASD probands thus serves as a window to underlying metabolic and signaling deficits that may be relevant to the pathobiology of autism.

Subsequently, investigators showed that male and female hormones differentially regulate the expression of a novel autism candidate gene, retinoic acid-related orphan receptor-alpha (RORA) in a neuronal cell line, SH-SY5Y (Sarachana et al. 2011). These investigators showed that one of the transcriptional targets of RORA is aromatase, which is a crucial enzyme in the biosynthesis of estrogen from testosterone. It is noteworthy that both RORA and aromatase proteins are decreased in the frontal cortex of autistic subjects, and that the level of aromatase protein is strongly correlated with the level of RORA protein in the brain tissues. Therefore, these investigators proposed that the reduction of RORA observed in subjects diagnosed with autism is exacerbated by a negative feedback mechanism involving decreased aromatase level, which further causes accumulation of its substrate, testosterone, and reduction of its product, estradiol. Testosterone and estradiol respectively exhibit negative and positive feedback regulation of RORA expression. Thus, a deficiency in RORA in autistic brain is expected to be further aggravated by increased levels of testosterone due to suppression of aromatase, a transcriptional target of RORA.

These results provide a molecular mechanism whereby testosterone may be increased in some cases of autism and further support for the relevance of RORA as a candidate gene for autism. Indeed, RORA's likely involvement in the balance between male and female hormones in brain tissues through regulation of aromatase transcription, coupled with its critical roles in Purkinje cell differentiation and cerebellar development as well as in neuroprotection against inflammation and oxidative stress, explains at least some of the pathology observed in autism. However, these investigators noted that not all samples from individuals diagnosed with an ASD are deficient in RORA. On the other hand, the investigators observed reductions in RORA in brain tissues from both male and female subjects diagnosed with an ASD, suggesting that RORA deficiency is not gender-specific. Interestingly, RORA and the estrogen receptor (ER) share a consensus binding site on DNA (AGGTCA) and consequently common target genes. The existence of shared gene targets may explain why females, with higher levels of estrogens, are less susceptible to autism. That is, estrogens may protect females against autism not only by increasing the level of RORA expression, but also by inducing shared target genes of RORA through ER, thus compensating in part for RORA deficiency.

Prenatal androgens

The ratio between the length of the 2nd and 4th digit (2D:4D) is sexually dimorphic, being lower in males than in females, and may be a useful proxy measure for fT production in humans during the first trimester of gestation (Manning et al. 1998). Studies researching fetal hand development have observed the sex difference in 2D:4D ratio in fetuses between 9 and 40 weeks of gestation (Malas et al. 2006). The 2D:4D ratio has been found to be negatively associated with the ratio of fT to fetal estradiol (fE) (Lutchmaya et al. 2004). Lower (i.e., hyper-masculinized) digit ratios have been found in children diagnosed with an ASD compared to typically developing children (Manning et al. 2001, de Bruin et al. 2006, Milne et al. 2006, Noipayak 2009, Krajmer et al. 2011). This pattern was also found in the siblings and parents of children diagnosed with autism, suggesting genetically-based elevated fT levels in subjects diagnosed with autism (Manning et al. 2001). The observed 2D:4D ratio suggests children diagnosed with an ASD may have been exposed to higher than average levels of fT.

The direct manipulation of fT levels is not possible in humans for ethical reasons. The investigation of prenatal hormone exposure and its relation to development in humans has therefore been investigated in naturally occurring abnormal environments such as in individuals with congenital adrenal hyperplasia (CAH) which is a genetic disorder that causes excess adrenal androgen production beginning prenatally in both males and females (New 1998). Studies of individuals with CAH have generally found that girls with CAH show masculinization of performance in activities typically dominated by males such as spatial orientation, visualization, targeting, personality, cognitive abilities, aggression, and sexuality (Berenbaum and Resnick 1997, Hines et al. 2003, Auyeung et al. 2009). Results from one study of girls with CAH suggest that they exhibit more autistic traits (ATs), measured using the adult version of the Autism Spectrum Quotient (AQ), compared to their unaffected sisters (Knickmeyer et al. 2006).

Finally, in our clinical practice, we have apparently observed the first untreated male subject with a DNA-confirmed CAH mutation (homozygous for Val281Leu mutations), CAH-associated clinical and laboratory findings, and a concurrent diagnosis of an ASD. The patient presented as a 12 year-old, Caucasian male, with a history of development regression that involved losing all words, becoming socially withdrawn, and hand flapping at 12 months of age. The patient was subsequently diagnosed with severe PDD-NOS at 2.5 years of age, and was diagnosed with CAH when 11-years-old. The patient was also diagnosed with precocious puberty with early secondary sexual development including testicular enlargement, growth spurt with a significantly advanced bone age, and elevated levels of dehydroepiandrosterone (DHEA), testosterone, and 17-α-hydroxyporgesterone.

The relationship between fT exposure and postnatal development was also examined using measures of fT levels in amniotic fluid, obtained during amniocentesis performed for other clinical reasons. In animal models, the critical period for sexual differentiation of the brain occurs when differences in serum testosterone are highest between sexes (Smith and Hines 2000). The human fT surge is thought to occur between weeks 8 and 24 of gestation (Collaer and Hines 1995). A major advantage of amniocentesis for examining hormone-behavior relations is that it is typically performed during the second trimester of pregnancy, during a relatively narrow window of time (usually 14–20 weeks of gestation) that coincides with the serum testosterone peak period in male fetuses (Auyeung et al. 2009). Although the origins of hormones (and in particular androgens) found in amniotic fluid are not fully understood, the main source seems to be the fetus itself (Cohen-Bendahan et al. 2005). Hormones can enter the amniotic fluid in two ways: *via* diffusion through the fetal skin in early pregnancy and *via* fetal urine in later pregnancy (Judd et al. 1976, Schindler 1982). Thus, testosterone levels measured in amniotic fluid might be expected to be a good reflection of the levels in the fetus, providing an alternative to direct assay of fetal serum (Finegan et al. 1989).

It has been found that in typically developing children whose mothers had undergone amniocentesis, fT levels show a positive association with systemizing, and a negative association with empathizing. Furthermore, fT was shown to significantly correlate with characteristics of subjects diagnosed with an ASD in typically developing children (Auyeung et al. 2009). For example, results from the Cambridge fT Project showed that in typically developing children whose mothers had undergone amniocentesis, fT was inversely associated with frequency of eye-contact in males when 12-months old (Lutchmaya et al. 2002a), and inversely predicted vocabulary development in children between the ages 18 and 24 months (Lutchmaya et al. 2002b). At 4 years of age, high levels of fT were associated with poorer quality of social relationships and more narrow interests (Knickmeyer et al. 2005). At 8 years of age, fT was positively correlated with the child version of the Systemizing Quotient (SQ) (Auyeung et al. 2006) and negatively correlated with the child version of the Empathy Quotient (EQ) (Chapman et al. 2006). In addition, investigators observed a significant relationship between fT levels and the later development of ATs as measured by the Childhood Autism Spectrum Test (CAST) and the Autism Quotient (AQ)-Child among children 6–10 years of age (Auyeung et al. 2009). Similarly, other investigators observed a significant relationship between fT levels and ATs measured using the Quantitative Checklist for Autism in Toddlers (Q-CHAT) among typically developing toddlers between 18 to 24 months of age (Auyeung et al. 2010). These consistent findings across different ages in development suggest the association between fT levels and ATs is robust.

Postnatal androgens

A series of clinical studies have examined androgen metabolites in patients diagnosed with an ASD. These studies have revealed hormonal patterns consistent with significantly elevated androgen levels in patients diagnosed with an ASD relative to controls. For example, Tordjman and colleagues (1997) examined a case series of patients diagnosed with an ASD relative to controls, and found that 1 in 3 pre-pubertal age children diagnosed with an ASD had significantly increased plasma testosterone levels relative to age- and sex-matched controls. Similarly, in a case series of patients diagnosed with an ASD, other investigators found that patients had significantly increased DHEA and serum testosterone levels relative to age- and sex-specific normal laboratory reference ranges from the Laboratory Corporation of America (LabCorp) (Geier and Geier 2006a).

Subsequent, these investigators evaluated a moderate size cohort of patients diagnosed with an ASD for androgen metabolites relative to age- and sex-specific laboratory reference ranges from LabCorp (Geier and Geier 2007). The results of the study showed significantly increased relative mean levels for serum testosterone (158%), serum free testosterone (214%), percent free testosterone (121%), DHEA (192%), and androstenedione (173%) among patients diagnosed with an ASD in comparison to controls. In addition, on an individual test basis, greater than 20% of ASD patients tested had levels greater than the laboratory age- and sex-specific reference range upper limit values for each androgen attribute examined. On the whole, it was observed that 81.4% (57 of 70) patients diagnosed with an ASD had at least one androgen that was greater than the pertinent upper limit for their laboratory age- and sex-specific reference range.

More recently, other investigators stated that patients diagnosed with an ASD have been hypothesized to be an exaggeration of normal male low-empathizing and high-systemizing behaviors, and tested this hypothesis at the molecular level by performing comprehensive multi-analyte profiling of blood serum from adult subjects diagnosed with AS compared with controls (Schwarz et al. 2011). This led to identification of distinct sex-specific biomarker fingerprints for male and female subjects. The results showed that free testosterone levels were elevated in adults with AS in comparison to controls. The increases in testosterone levels in females diagnosed with AS were also paralleled by increases in the levels of luteinizing hormone (LH) in the same subjects. These investigators hypothesized that LH pulsatility may predispose or cause hyperandrogenism in female adolescents. These investigators concluded that their observations lend support to the androgen theory of ASDs.

Other researchers undertook a study to determine whether there are significant differences in salivary levels and developmental patterns of steroid hormones between individuals diagnosed with autism and healthy children, in order to assess the possible roles they played in autism etiology (Majewska et al. 2010). Children of both sexes, 3−4 and 7−9 years of age, were recruited to the study. Children diagnosed with autism were compared to age- and sex-matched healthy children. Compared to controls, the children diagnosed with autism had significantly higher levels of many androgen steroids in saliva. The differences were more pronounced in males. Moreover, the investigators described that there were striking differences in patterns of developmental change for some steroids between the children diagnosed with autism and the control children, suggesting accelerated adrenarche in autism. The investigators concluded that their data indicates profoundly increased steroidogenesis in children diagnosed with autism.

Subsequent study by Takagishi and others (2010) examined salivary testosterone levels and autism spectrum quotient in adults. A total of 92 male and female adults participated in study. The subject's salivary testosterone level and score of Japanese version of the AQ were assessed to examine the relationship between salivary testosterone level and autistic traits in adults. These investigators observed a positive correlation between testosterone and AQ in a group of both sexes.

Other investigators recently reported on serum androgen levels among individuals diagnosed with an ASD (Ruta et al. 2011). These investigators examined androgen levels in a sample of subjects diagnosed with AS in comparison to age- and IQ-matched typical controls. Overall, androstenedione levels were observed to be significantly elevated in the ASD group in comparison to the controls.

In addition to observing correlation between patients diagnosed with an ASD and elevated postna-

tal androgen levels, a correlation also has been found between postnatal androgen levels in subjects with other conditions/exposures resulting in high androgen levels and ATs. For example, children diagnosed with premature adrenarche in comparison to children with on-time adrenarche revealed a significant increase in ATs, including: social withdrawal, social problems, problem behaviors, reduced language development, and information processing deficits (Dorn et al. 1999). In some instances we have observed in our clinical experience, boys with undescended testicles, who received testosterone treatments to resolve this condition, developed ATs and, in a few instances from our clinical experience, we have observed administration of testosterone treatments was associated with an individual being diagnosed with an ASD. Such clinical examples dramatically demonstrate the association between postnatal androgen levels and ATs.

Other investigators examined the impact of testosterone upon social interaction, first describing how humans automatically infer motives, intentions, and feelings from the bodily cues of others, especially cues from the eye region of their faces (van Honk et al. 2011). This cognitive empathic ability is one of the most important components of social interaction, and deficits in cognitive empathic ability are typically observed in subjects diagnosed with an ASD. These investigators evaluated the effects of testosterone administration on cognitive empathy in young adult females in a double-blind, placebo-controlled, crossover clinical trial. The results revealed that a single administration of testosterone in female subjects leads to significant impairment in the cognitive empathic ability to infer emotions, intentions, feelings, and other mental states from the eye region of the face. Moreover, the investigators observed that prenatal testosterone priming was crucial to effects following testosterone administration. These investigators concluded that testosterone may down-regulate social intelligence not only organizationally, by affecting fetal brain development, but also activationally, by impacting the brain's functioning in later in life. They described that in humans, the fetal period of prenatal development is considered critical for testosterone's effects on brain organization (between weeks 12 and 19 of gestation), whereas the hormone's activational effects come into prominence in adolescence and adulthood.

EVIDENCE SUPPORTING THE USEFULNESS OF ANTI-ANDROGEN MEDICATIONS TO TREAT AUTISM SPECTRUM DISORDER SYMPTOMS

In light of evidence revealing significantly elevated androgen levels in subjects diagnosed with an ASD, and a significant relationship between elevated androgens and ATs, it was previously hypothesized that treatments specifically targeting elevated androgen levels in subjects diagnosed with an ASD might significantly improve ATs (Geier and Geier 2005). The following are a series of studies examining the relationship between administration of anti-androgen medications and associated outcomes in ATs in animal model systems and in human clinical trials.

Animal models

Investigators reported that the correlation between neuronal mechanism of anxiety and the neuroanatomic expression/neuromodulatory role of gonadotropin-releasing hormone (GnRH) suggests a role for GnRH in the modulation of anxiety (Umathe et al. 2008c). Therefore, these investigators examined the influence of GnRH agonists and antagonist on the anxiety-like behavior of rats in the elevated plus-maze and social interaction tests. GnRH agonists, leuprolide [100 or 200 ng/rat, intracerebroventricularly (icv)] or 6-D-tryptophan luteinizing hormone-releasing hormone (d-Trp-6-LHRH) (400 ng/rat, icv), significantly increased the percentage of open arms entries, time spent in open arms, and time spent in social interaction. The observed anxiolytic effect of these agents was comparable with diazepam [0.5–1.0 mg/kg, intraperitoneally (ip)]. Treatment with a GnRH antagonist (pGlu-D-Phe-Trp-Ser-Tyr-D-Ala-Leu-Arg-Pro-Gly-NH2 (100 ng/rat, icv), significantly reduced the percentage of open arm indices and decreased time spent in social interaction, indicating an anxiogenic-like effect. These investigators concluded that GnRH has a putative role of in the control of anxiety, and this further adds to the importance of investigating the possible role of the hypothalamus-pituitary-gonadal axis in regulating the anxiety-related disorders arising out of hypothalamus-pituitary-adrenal axis dysregulation.

Other investigators described how ethanol inhibits the synthesis, content, and release of hypothalamic GnRH, and GnRH modulates the activity of several

neurotransmitters that experience adaptive changes from chronic exposure to ethanol, and GnRH in ethanol dependence (Umathe et al. 2008b). Hence, it was contemplated that GnRH agonist such as leuprolide may influence the behavioral consequences of withdrawing ethanol during a dependent state. These investigators produced ethanol dependence in mice by providing ethanol liquid diet for 10 days. Its withdrawal on day 11 led to physical signs of hyperexcitability with its peak at 6 h. Acute treatment with leuprolide (20 ng/mouse, icv), 10 min prior to peak, significantly attenuated hyperexcitability. Such an effect, occasioned by leuprolide, was evident even in castrated mice, even though castration significantly increased the hyperexcitability in the ethanol withdrawal state. Chronic treatment with leuprolide (10 ng/mouse, twice daily, icv) to day 10 significantly reduced the signs of hyperexcitability in the ethanol withdrawal state.

In another set of experiments, ethanol (2.4 g/kg, ip) was administered on days 1, 4, 7, 10 and 15, which caused gradual increase in locomotor activity, indicating ethanol-induced sensitization. Leuprolide (20 ng/mouse, icv), 10 min prior to the challenge dose of ethanol (2.4 g/kg, ip) on day 15 significantly attenuated the expression of sensitization to the hyperlocomotor effect of ethanol. Similarly, administration of leuprolide (20 ng/mouse, icv), 10 min prior to ethanol on days 1, 4, 7 and 10 not only reduced the gradual increase in locomotor activity, but also attenuated the sensitized locomotor response on day 15, indicating attenuation of the development of sensitization. Leuprolide per se did not affect physical signs and locomotor activity in the control group.

In addition, researchers reported that a characteristic behavior in alcohol abstinence among alcoholics is obsessive-compulsive behavior (OCD) (Umathe et al. 2008a). Ethanol is known to reduce hypothalamic synthesis, release, and mRNA expression of GnRH that modulates serotonergic, dopaminergic, and glutamatergic systems, which experience adaptive changes due to chronic exposure to ethanol. Such changes are also evident in OCD. Therefore, an investigation of the effect of ethanol-withdrawal on marble-burying behavior in mice was undertaken, particularly because this behavior simulates some aspects of obsessive-compulsive behavior. In addition, the influence of GnRH agonist was also studied in this investigation. An ethanol-withdrawal state was induced after its chronic administration, and marble-burying behavior was observed at 0, 6, 24, 48, and 96 h intervals. Further, the influence of leuprolide [50–600 μg/kg, subcutaneously (sc)] or fluoxetine (5–30 mg/kg, ip) was investigated on ethanol-withdrawal-induced changes in the marble-burying behavior. The results indicated that ethanol-withdrawal led to a gradual increase in marble-burying behavior up to 48 h, with a peak at 24 h interval. Administration of leuprolide (100–600 μg/kg, sc), 30 min prior to 24 h interval, dose-dependently reduced the expected ethanol-withdrawal-induced increase in marble-burying behavior, and this effect was comparable to that of fluoxetine (15 and 30 mg/kg, ip). Further, twice daily administration of leuprolide (50 μg/kg, sc), concomitant with ethanol, prevented the gradual increase in marble-burying behavior after ethanol-withdrawal, and this effect was comparable to fluoxetine (5 mg/kg, ip).

Researchers report that corticotrophin-releasing factor (CRF) inhibits the release of GnRH (Umathe et al. 2008d). In addition to the endocrine effects, GnRH is reported to influence the behavior *via* its neuronal interactions. The investigators hypothesized that anxiety and depression produced by CRF could be also subsequent to the decrease in GnRH. Therefore, the investigators examined the influence of GnRH agonists on CRF or CRF antagonist-induced changes in the time spent socially in social interaction tests and in the time spent in immobility in forced swim test in mice, as the indices for anxiety and depression, respectively. Results indicated that GnRH agonists [leuprolide (20–80 ng/mouse, icv), or d-Trp-6-LHRH (40–160 ng/mouse, icv)] dose-dependently increased social interaction time and decreased immobility time, indicating an anxiolytic- and antidepressant-like effect, respectively. Administration of CRF (0.1 and 0.3 nmol/mouse, icv) produced opposite effects as that of GnRH agonist on these parameters. Further, it was seen that pretreatment with leuprolide (10 or 20 ng/mouse, icv) or d-Trp-6-LHRH (20 or 40 ng/mouse, icv) dose-dependently antagonized the effects of CRF (0.3 nmol/mouse, icv) in social interaction and forced swim test. CRF antagonist [alpha-Helical CRF (9-41), (1 or 10 nmol/mouse, icv)] was found to exhibit an anxiolytic- and antidepressant-like effect, and its sub-effective dose (0.1 nmol/mouse, icv), when administered along with sub-threshold dose of leuprolide (10 ng/mouse, icv), or d-Trp-6-LHRH (20 ng/mouse, icv) also produced significant anxiolytic- and antidepressant-like effect. These observations suggest a reciprocating role

for GnRH in modulating the CRF induced anxiogenic- and depressant-like effects.

Researchers stated that haloperidol, an antipsychotic agent, stimulates the release of GnRH, and this hormone is known to mimic some of the behavioral effects of haloperidol (Umathe et al. 2009). Hence, these investigators examined the contribution of GnRH in the behavioral effects of haloperidol. The studies revealed that haloperidol (0.15, 0.25 and 0.5 mg/kg, ip) and leuprolide (50, 100, 200 and 400 μg/kg, sc) dose-dependently inhibited conditioned avoidance response (CAR) in male Sprague-Dawley rats. In higher doses, haloperidol (0.5, 1 mg/kg, ip) and leuprolide (200, 400 μg/kg, sc) produced catalepsy in rats. Co-administration of sub-effective dose of leuprolide (50 or 100 μg/kg, sc) and haloperidol (0.15 or 0.5 mg/kg, ip) similarly inhibited CAR and induced catalepsy. Pre-treatment of rats with antide (GnRH antagonist; 10 μg/rat, sc) attenuated the inhibitory effect of both the agents on CAR; blocked leuprolide-induced catalepsy; and attenuated the intensity and reduced the duration of haloperidol-induced catalepsy.

Investigators described that leuprolide, dose dependently (100, 200, and 300 μg/kg, sc) inhibited marble-burying behavior in mice, which was comparable to that of fluoxetine (10 and 15 mg/kg, ip), a drug used in the treatment of OCD (Uday et al. 2007). Co-administration of sub-effective dose of leuprolide (50 μg/kg) and fluoxetine (5 mg/kg) significantly inhibited marble-burying-behavior. Pre-treatment with parachlorophenylalanine [300 mg/kg, ip (× 3 days)], a serotonin depleting agent, reversed the effect of fluoxetine, and partially attenuated the effect of leuprolide. Further, LHRH antagonist pre-treatment (2.5 μg/mouse, sc) completely blocked the effect of leuprolide and reduced the effect of fluoxetine. Motor activity remained unaffected after all treatments.

Further, investigators described that OCD is characterized by absurd, recurrent thoughts (obsessions) followed by certain stereotyped actions (compulsions) (Gaikward et al. 2010). OCD can impair all areas of brain functioning and produce devastating effects on patients and their families. Marble-burying behavior of mice is a well-accepted paradigm to screen anti-compulsive activity. These investigators evaluated the effect of ritanserin and leuprolide per se and in combination on marble-burying behavior of mice. The results showed that ritanserin (l, 2, and 20 mg/kg, ip) *per se* did not show any anti-compulsive effect. Leuprolide (200 and 300 μg/kg, sc) per se showed anti-compulsive effect, causing statistically significant inhibition of marble-burying behavior of mice. The prior treatment with ritanserin, 5HT(2A/2C) antagonist (20 mg /kg, ip), effectively blocked the inhibitory influence of leuprolide (300 μg/kg, sc) on marble burying behavior of mice, suggesting that leuprolide also requires serotonin to express its anti-compulsive effect. Further, the results also suggested that the effect of leuprolide appears to be mediated through 5HT(2A/2C) receptors.

In addition, researchers demonstrated that leuprolide acetate administration improved cognitive function in the Morris water maze and Y-maze tests (Bryan et al. 2010). Importantly, these investigators observed that pathways associated with improved cognition such as CaMKII and GluR1-Ser831 were up-regulated by leuprolide treatment. These investigators concluded that their results provide a means to help improve cognitive function in a state of cognitive decline.

Investigators described that self-injurious behavior (SIB) presents a serious problem in laboratory macaques that cannot be socially housed for scientific reasons and among institutionalized children and adults where it is often associated with different forms of brain dysfunction (Eaton et al. 1999). The investigators stated that limited success was observed in reducing SIB in macaques by enhancing their environment with enrichment devices. Psychotropic drugs also helped, but problems are associated with their use. Because sexual and aggressive behavioral problems in men have been treated with progestational drugs, these investigators tested the efficacy of cyproterone acetate (CA) (5–10 mg/kg/week) on reducing SIB in 8 singly housed, adult male rhesus macaques. The main findings were: (1) SIB and other atypical behaviors were significantly reduced during CA treatment; (2) serum testosterone was significantly reduced during CA treatment; (3) cerebral spinal fluid (CSF) levels of 5-hydroxyindoleacetic acid (5HIAA) and homovanillic acid (HVA), metabolites of serotonin and dopamine, respectively, declined significantly during CA treatment; (4) the duration of SIB positively correlated with levels of 5HIAA in CSF; but (5) sperm counts were not reduced during treatment. Thus, CA was an effective treatment (3 months) for adult male macaques whose behavioral problems include SIB. In summary, CA reduced SIB, overall aggression, serum testosterone, CSF 5HIAA, and CSF HVA. The study investigators hypothesized

that the progestin activity of CA represses the hypothalamic gonadal axis and decreases testosterone, which in turn decreases SIB. In addition, they speculated that the decrease in 5HIAA and HVA in CSF may have been caused by progestins decreasing the activity of monoamine oxidase (MAO). Therefore, the reduction of SIB may also be related to an increase in the availability of active monoamines in the CNS.

Finally, researchers recently hypothesized that GnRH plays a crucial role in controlling the extent of the brain's sex specificity and that changes in GnRH action during critical periods of brain development, such as puberty, will result in altered sex-specific behavioral and physiological patterns (Wojniusz et al. 2011). These investigators blocked puberty in half of the 48 same-sex Scottish mule Texel cross sheep twins with goserelin acetate, a GnRH analog, every 3 weeks, beginning just before puberty. To determine the effects of goserelin treatment on sex-specific behavior and emotion regulation in different social contexts, these investigators employed the food acquisition task (FAT) and measurement of heart rate variability (HRV).

These investigators observed that animals with blocked puberty displayed greater sex differences in relation to emotion and behavior regulation than their untreated twins. Treated males were more willing to move away from their companions compared to male controls and treated and untreated females. They were highly motivated by the prospect of obtaining "risky pellets," as shown by high correlations between FAT scores and "pellet runs." This exaggerated "maleness" could be interpreted as an expression of improved emotional control compared to their same-sex twins. One may speculate that they were able to better inhibit negative emotions, such as anxiety. In contrast to treated males, treated females tended to stay in the same place, as close as possible to their companions in the audience pen, and were much less prone to engage in food seeking. If they sought food, it was more often the nearby and visible hay than the remotely placed and out-of-sight pellets. This avoidance behavior might be interpreted as poor emotional control, leading to higher anxiety at the prospect of farther separation from the other animals.

The HRV results complemented behavioral findings. It was found that long-term treatment with goserelin affected cardiac vagal control in young sheep in a sex-specific manner. Treated males had significantly higher HRV (i.e., larger cardiac vagal influence) than untreated males and females. The HRV differences between treated males and females were significantly exaggerated compared to untreated animals. Larger cardiac vagal influence is thought to be related to better emotional control and a better ability to engage in adaptive behavior in various circumstances. It was previously shown in sheep that HRV differs with relation to positive and negative emotional states. Poor emotional control, e.g., in case of anxiety, is associated with lower HRV.

These investigators concluded there is a need for personalized drugs with improved efficiency on sex-specific behavioral symptoms for many psychiatric and somatic disorders, and their findings suggest a new therapeutic use of gonadotropin modulation in preventing and treating cognitive impairment in patients with developmental cognitive disorders.

Human clinical trials

Investigators evaluated the effects of acute gonadal suppression on sexual function and behavior in a clinical trial conducted on eight normal men (Loosen et al. 1994). Administration of a potent GnRH antagonist reduced levels of serum testosterone, LH, and follicle-stimulating hormone (FSH). These effects coincided with significant reductions in outward-directed aggression, sexual function, sexual desire, anger, and anxiety.

A researcher undertook an investigation in order to further elucidate the possibility of using anti-androgenic agents in the treatment of OCD (Eriksson 2007). Six male patients, all suffering from therapy-resistant OCD, were included in a 48 weeks open-label trial of triptorelin, a long-acting GnRH-analogue. Every other week, the patients rated the severity of obsessive-compulsive disorder symptoms by means of a visual analogue scale. During the course of the trial, five out of six patients experienced a considerable improvement. The finding gives further support to the contention that anti-androgenic agents are effective in the treatment of obsessive-compulsive disorder.

Other investigators described the anti-aggressive effect of CA versus haloperidol in Alzheimer's disease (AD) (Huertas et al. 2007). These investigators described that AD is commonly accompanied by aggressive behavior, and that effective and safe anti-aggressive treatment is lacking. These investigators reported that 27 elderly patients diagnosed with AD

and associated aggressive behavior were recruited to the study. Each patient underwent a 15-day washout for psychotropics and then was randomly assigned to receive stable doses of either CA (100 mg/day) or haloperidol (2 mg/day) for 90 days. The trial was completed by 24 of the subjects (13 in CA group and 11 in the haloperidol group for 90 days), but three patients dropped out, all after adverse effects in the haloperidol group. The investigators observed that there was a significant increased in complete elimination of aggression in CA group (69.2%) in comparison to the haloperidol group (14.2%). These investigators concluded that CA showed significantly better efficacy and safety than haloperidol in controlling aggression.

Similarly, other researchers described CA treatment of 19 demented patients who developed severe aggressive behaviors or an agitation unresponsive to psychoactive drugs (even in association) or to environmental adaptation (Caparros-Lefebvre and Dewailly 2005). Seven patients were diagnosed with vascular dementia, 7 were diagnosed with AD, 2 were diagnosed with fronto-temporal degeneration, 2 were diagnosed with Huntington's disease, and 1 was diagnosed with probable diffuse Lewy Bodies Disease. Fifteen patients had prominent aggressive behavior and 4 had predominant aberrant motor behavior with aggressive behavior. It was observed that CA (50 to 100 mg, mean = 92.5 mg/day) improved significantly aggressive and impulsive behavior, but had no effect on aberrant motor behavior. When cyproterone was stopped, aggressive behaviors reappeared.

Furthermore, in support of the correlation between hormonal treatment status and ATs, investigators compared symptoms of ASD using AQ scores derived from five groups: (1) *n*=61 transmen (female-to-male transsexual people); (2) *n*=198 transwomen (male-to-female transsexual people); (3) *n*=76 typical males; (4) *n*=98 typical females; and (5) *n*=125 individuals with Asperger Syndrome (Jones et al. 2012). Transmen had a higher mean AQ than typical females, typical males and transwomen, but lower than individuals with AS. Transmen have more ATs and may have had difficulty socializing with female peers and thus found it easier to identify with male peer groups. The importance of these findings is that direct hormonal treatment with androgen therapy in transmen directly correlated with their development of symptoms of ASD.

In evaluating the effects of anti-androgen therapy administered to patients diagnosed with an ASD, investigators conducted a clinical trial of leuprolide acetate administration to 11 consecutive patients diagnosed with an ASD whose laboratory findings showed elevated androgen levels (Geier and Geier 2006b). Children were administered an intramuscular injection of 15 mg LUPRON DEPOT® every 28 days and supplemented with daily, subcutaneously injected LUPRON®, so that children were started on a dose of 50 μg of leuprolide acetate/kilogram bodyweight/day. Subjects were monitored as successive doses of leuprolide acetate were administered for persistent clinical/laboratory signs of increased androgens, and subjects were supplemented with additional subcutaneous injections of leuprolide acetate dosing and/or oral CA as clinically necessary. Subjects examined in the study were on the therapy for a minimum of 2 months and a maximum of 7 months. Laboratory testing was conducted on each subject at baseline and at approximately 3 months of treatment. In addition, a clinical examination was undertaken for each patient to evaluate clinical symptoms/behaviors of hyperandrogenemia such as early growth spurt, early secondary sexual changes, body and facial hair, and aggressive behaviors at baseline and at the end of the study period for each child. Autism Treatment Evaluation Checklist (ATEC) (Autism Research Institute, San Diego, CA) evaluations were conducted prior to beginning the protocol on each child and at the end of the study period for each child. The ATEC quantitatively evaluates (using a numeric scoring system) skills in a number of areas, including speech language/communication, sociability, sensory/cognitive awareness, and health/physical/behavior.

Among the subjects treated in the clinical trial, there was a significant overall improvement from the 70th percentile of severity (median baseline score = 87) at baseline to the 40–49th percentile of severity (median end of study period score = 63) at the end of the study. In the specific areas of sociability, cognitive awareness, and behavior, there were significant improvements among treated children when evaluating baseline measurements in comparison to those obtained at the end of the study period. Additionally, for specific subjects participating in the clinical trial having independent assessments by school evaluators, who were not aware of the treatment status of the child, there were significant improvements in general school skills mastered and in the frequency and severity of disrup-

tive/oppositional behavior at the end of the treatment period relative to baseline. When comparing the clinical examinations undertaken for each subject at baseline and at the end of the study period, significant reductions in clinical symptoms and the associated behaviors of hyperandrogenemia (such as early growth spurt, early secondary sexual changes, body and facial hair, and aggressive behaviors) were noted. Lab testing revealed a significant decrease in serum testosterone levels from a baseline median = 1.96 multiples of the mean (age- and sex-adjusted reference values) to an approximately 3 months median = 1.16 multiples of the mean (age- and sex-adjusted reference values). It was found that the treatment protocol employed did not significant adversely affect kidney, thyroid or liver function tests.

As a result, the investigators concluded, since their study employed therapeutic agents that were designed to lower androgen levels, and significant decreases in androgen levels were observed, the treatment protocol employed presents a novel method for helping to significantly reduce autistic-like behaviors. Furthermore, the investigators reported that in some of the subjects examined, significant autistic behavior improvements (i.e., better sleep patterns, improvements in attention and hyperactivity, and increased socialization) occurred within days of the administration of leurpolide acetate. Finally, the investigators concluded that leuprolide acetate administration significantly helped to ameliorate clinical symptoms/behaviors of hyperandrogenemia such as early growth spurt, early secondary sexual changes, body and facial hair, and aggressive behaviors that may be observed among some children diagnosed with an ASD.

Subsequently, investigators described their clinical experience following its administration to nearly 200 patients with an ASD diagnosis. Leuprolide acetate administration significantly lowered androgen levels and resulted in very significant overall clinical improvements in socialization, sensory/cognitive awareness, and health/physical/behavior skills, with few non-responders and minimal adverse clinical effects to the therapy. Leuprolide acetate administration also resulted in significant quantitative clinical ameliorations in hyperactivity/impulsivity, stereotypy, aggression, self injury, abnormal sexual behaviors, and/or irritability behaviors in many subjects diagnosed with an ASD (Geier and Geier 2007).

CONCERNS REGARDING THE CLINICAL SAFETY OF ANTI-ANDROGEN MEDICATIONS

In considering the in-use safety of anti-androgen medications, the medicines with the least side-effects and the most-characterized side-effect profiles, the medicines of choice, such as GnRH analogues and CA, have been on the market for many years. There are many individuals that have received these medications for many years to treat such conditions as prostate cancer (Benson 1993, Torri and Floriani 2005), female reproductive problems (Resimann et al. 2009), and premature puberty (Laron and Kauli 2000, Partsch and Sippell 2002) without serious adverse effects.

A review of the package insert for leuprolide acetate indicates leuprolide acetate injection is contraindicated in patients known to be hypersensitive to GnRH, GnRH agonist analogs, or any of the excipients in leuprolide acetate preparations. Anaphylactic reactions to synthetic GnRH or GnRH agonist analogues have been reported (MacLeod et al. 1987). Leuprolide acetate is contraindicated in women who are or may become pregnant while receiving the drug. Leuprolide acetate may cause fetal harm when administered to a pregnant woman.

Two-year carcinogenicity studies were conducted in rats and mice with leuprolide acetate. In rats, a dose-related increase of benign pituitary hyperplasia and benign pituitary adenomas was noted at 24 months when the drug was administered subcutaneously at high daily doses (0.6 to 4 mg/kg). There was a significant but not dose-related increase of pancreatic islet cell adenomas in females and of testicular interstitial cell adenomas in males (highest incidence in the low dose group). In mice, no pituitary abnormalities were observed at a dose as high as 60 mg/kg for two years. Patients were treated with leuprolide acetate for up to three years with doses as high as 10 mg/day and for two years with doses as high as 20 mg/day without demonstrable pituitary abnormalities, and patients treated with leuprolide acetate doses as high as 20 mg/day for up to two years caused no adverse effects differing from those observed with the receiving a 1 mg/day dose. Mutagenicity studies have been performed with leuprolide acetate using bacterial and mammalian systems. These studies provided no evidence of a mutagenic potential. Clinical and pharmacologic studies in adults ($\geq$ 18 years) with leuprolide acetate and

similar analogs have shown full reversibility of fertility suppression when the drug is discontinued after continuous administration for periods of up to 24 weeks. When administered on day 6 of pregnancy at test dosages of 0.00024, 0.0024, and 0.024 mg/kg to rabbits, leuprolide acetate produced a dose-related increase in major fetal abnormalities. Similar studies in rats failed to demonstrate an increase in major fetal malformations throughout gestation. There was increased fetal mortality and decreased fetal weights with the two higher doses of leuprolide acetate in rabbits and with the highest dose in rats. The effects on fetal mortality are expected consequences of the alterations in hormonal levels brought about by this drug. It is not known whether leuprolide acetate is excreted in human milk. Leuprolide acetate should not be used by nursing mothers.

A review of the package insert for CA indicates its administration is contraindicated in pregnancy, lactation, liver diseases, a history of jaundice or persistent itching during a previous pregnancy, a history of herpes of pregnancy, Dubin-Johnson syndrome, Rotor syndrome, previous or existing liver tumors, wasting diseases, depression, previous or existing thromboembolic processes, diabetes with vascular changes, and sickle-cell anemia.

Administration of high doses of CA during the hormone-sensitive differentiation phase of the genital organs (starting roughly on day 45 of gravidity) could cause feminization effects in male fetuses. Observation of male newborn children who had been exposed in the uterus to CA revealed no indications of feminization. Recognized first-line tests of genotoxicity gave negative results when conducted with CA. However, there is some evidence of genotoxicity as further tests showed that CA was capable of producing adducts with DNA (and an increase in DNA repair activity) in liver cells from rats and monkeys and also in freshly isolated human hepatocytes. This DNA adduct formation occurred at exposures that might be expected to occur in the recommended dose regimens for CA.

One *in vivo* consequence of CA treatment was the increased incidence of focal, possibly pre-neoplastic, liver lesions in which cellular enzymes were altered in female rats. The clinical relevance of these findings and how these findings relate to the risk of developing benign and malignant liver tumors in humans is presently unknown. Clinical experience to date would not support an increased incidence of hepatic tumors in man. Nor did investigations into the tumorigenicity of CA in rodents reveal any indication of a specific tumorigenic potential. However, it must be borne in mind that sexual steroids can promote the growth of certain hormone-dependent tissues and tumors. In rare cases benign, and in even rarer cases malignant, liver tumors leading in isolated cases to life-threatening intra-abdominal hemorrhage have been observed after the use of sex steroids to which the substance contained in CA also belongs. If severe upper abdominal complaints, liver enlargement or signs of intra-abdominal hemorrhage occur, a liver tumor should be included in the differential-diagnostic considerations.

Changes in body-weight and libido, a feeling of tension in the breasts, tiredness, diminished vitality, inner restlessness, and depressive moods may occur with CA administration. Disturbances of liver function, acute and fulminant hepatitis were reported with high-dose CA treatment. During treatment, liver function should be checked regularly. In diabetics, carbohydrate metabolism should also be monitored particularly carefully. A high-dosed treatment may reduce the function of the adrenal cortex, particularly the adrenocortical response to stress. It should be pointed out to patients whose occupation demands great concentration that CA can lead to tiredness and diminished vitality and can impair the ability to concentrate. CA should be used with caution in cardiovascular disease, ischemic heart disease, cerebrovascular disease, and hypertension. Hemoglobin and red cell counts may decrease on therapy with CA.

Several reviews have examined the relationship between administration of androgen deprivation therapy for the prostate in elderly men and the risk of metabolic syndrome, diabetes mellitus, and cardiovascular disease (Higano 2003, Kintzel et al. 2008). These investigators described changes in body composition, hyperlipidemia, insulin resistance, metabolic syndrome, and coronary syndrome are all reported adverse effects of anti-androgen therapy, which are consequences of reduced levels of circulating testosterone. These investigators described that pharmacists should provide counseling to these patients on primary disease prevention, and elderly men receiving anti-androgen therapy should be monitored routinely for signs and symptoms of metabolic syndrome, diabetes, and coronary artery disease, and healthy lifestyle practices should be encouraged, and physical therapy should be considered for these patients.

The American Academy of Pediatrics in 2009 issued a consensus statement describing that GnRH analogues are generally well tolerated in children and adolescents, and systemic complaints such as headaches or hot flashes occur occasionally but are usually short-term and do not interfere with therapy (Carel et al. 2009). It was also observed in previous long-term follow-up of individuals receiving GnRH analogue therapy in the treatment of premature puberty for many years that GnRH analogue administration was not associated with long-term reproductive dysfunction (normal ovarian function, normal menarche, etc.), impaired physical development (normal body mass index, normal body composition, normal bone mineral density, etc.) or reduced secretion of sex hormones in women and men (van der Sluis et al. 2002, Tanaka et al. 2005, Bertelloni and Mul 2008, Majiakou et al. 2010, Neely et al. 2010).

When considering the safety profile of anti-androgen medications in subjects diagnosed with an ASD, it is important to evaluate them in the context of currently used psychotropic medicines. For example, in comparative evaluations of the safety profile of anti-androgen medications such as CA *versus* haloperidol [a common psychiatric medicine used in the treatment of subjects diagnosed with an ASD (Malone and Waheed 2009)], serious adverse effects occurred much more frequently with haloperidol in comparison to CA therapy (Huertas et al. 2007). In addition, the package insert for haloperidol has a black box warning that elderly patients with dementia-related psychosis treated with antipsychotic drugs are at an increased risk of death. The packages insert cautions about the risk of sudden death, Torsades and QT interval prolongation, severe neurotoxcity manifesting as rigidity or inability to walk or talk, history of seizures, and increased body temperature. Finally, the package insert cautions that the safety of prolonged administration is not established.

As another example, risperidone, which has received an indication from the US Food and Administration for use in subjects diagnosed with an ASD, has a black box warning that elderly patients with dementia treated with atypical antipsychotic drugs are at an increased risk of death compared to placebo. The package insert also describes that risperidone should be used cautiously in patients diagnosed with a seizure disorder, who have had seizures in the past, or who have conditions that increase their risk for seizure. The package insert warns that risperidone has been associated with Neuroleptic Malignant Syndrome, a potentially fatal side effect, and Tardive Dyskinesia, a sometimes permanent side effect. The package insert also warns that risperidone administration may cause children to gain more weight than expected and can cause hyperprolactinemia, absence of menstrual period, breasts producing milk, the development of breasts by males, and the inability to achieve an erection. The package insert describes that use of risperidone was associated with high blood sugar levels, diabetes, and extrapyramidal symptoms. In addition, the package insert describes animal toxicity studies showing organ weight changes, including increases in spleen (with increased number of red blood cells) and pituitary weight and decreases in the weight of testes and prostate. Histopathology examination showed changes in the male and female genital tract, namely prostatic changes (fibrosis and clear basal cells), degenerative changes in the testicles, decreased glandular development of the uterus, and the absence of corpora lutea.

Finally, the purposed administration of anti-androgen medications to individuals diagnosed with an ASD is not intended to deprive the individual of their sexuality nor to alter their normal developmental trajectory, but rather to regularize a process that was proceeding in an abnormal fashion and producing adverse effects. Thus, the use of anti-androgen medications can safely improve the health of the patient and reduce ATs associated with abnormally elevated androgen levels.

CONCLUSIONS

The present review provides evidence for hyperandrogenism as a significant feature in subjects diagnosed with an ASD. Further, many investigations revealed a significant correlation between the clinical features of patients diagnosed with an ASD and hyperandrogenism. Finally, the present review presents data from animal models and human clinical trials demonstrating that anti-androgen medications have the ability to significantly improve clinical features of patient diagnosed with an ASD.

Anti-androgen therapy should be considered as an effective means to significantly help improve clinical features of patients diagnosed with an ASD. By directly targeting a biomedical indicator when it is elevated beyond the normal reference range, this therapy controls difficult ATs associated with high

testosterone such as self injurious behaviors and aggression, thus contributing to the quality of the patient's life and the normalcy of the patient's home life. Screening all individuals diagnosed with an ASD for elevated androgens, and assessing this elevation with regard to the individual's family, may produce not only treatment options for subjects diagnosed with an ASD but also an understanding of why these conditions occur and who may be at risk. Routine screening of all persons diagnosed with an ASD for elevated androgens is recommended as a standard part of the initial clinical assessment, and appropriate treatment should be initiated for those with elevated androgens (Geier and Geier 2008).

ACKNOWLEDGEMENT

We wish to thank Dr. Harold T. Pretorius for his endocrinology expertise in reviewing the present manuscript.

Funding

The study was financial supported by the non-profit Institute of Chronic Illnesses, Inc. and by the non-profit CoMeD, Inc. None of the organizations providing financial support for our study had any influence on data analyses or conclusions.

Potential conflict of interest statement

David Geier and Mark Geier have a patent pending for the treatment of autism spectrum disorders.

REFERENCES

American Psychiatric Association (2000) Diagnostic criteria for autistic disorder. In Diagnostic and statistical manual of mental disorders, Fourth edition—text revision (DSMIV-TR). American Psychiatric Association, Washington, DC, pp. 75.

Auyeung B, Baron-Cohen S, Chapman E, Knickmeyer RC, Taylor K, Hackett G (2006) Foetal testosterone and the child systemizing quotient. Eur J Endocrinol 155 :S123–S130.

Auyeung B, Baron-Cohen S, Ashwin E, Knickmeyer R, Taylor K, Hackett G (2009) Fetal testosterone and autistic traits. Br J Psychol 100: 1–22.

Auyeung B, Taylor K, Hackett G, Baron-Cohen S (2010) Foetal testosterone and autistic traits in 18 to 24-month-old children. Mol Autism 1: 11.

Baron-Cohen S (2002) The extreme male brain theory of autism. Trends Cogn Sci 6: 248–254.

Benson RC (1993) Total androgen blockade: the United States experience. Eur Urol 24 (Suppl 2): 72–76.

Berenbaum SA, Resnick SM (1997) Early androgen effects on aggression in children and adults with congenital adrenal hyperplasia. Psychoneuroendocrinology 22: 505–515.

Bertelloni S, Mul D (2008) Treatment of central precocious puberty by GnRH analogs: long-term outcome in men. Asian J Androl 10: 525–534.

Bryan KJ, Mudd JC, Richardson SL, Chang J, Lee HG, Zhu X, Smith MA, Casadesus G (2010) Down-regulation of serum gonadotropins is as effective as estrogen replacement at improving menopause-associated cognitive deficits. J Neurchem 112: 870–881.

Caparros-Lefebvre D, Dewailly D (2005) Preliminary pilot study of cyproterone acetate for the treatment of aggressive behavior associated with severe dementia. Rev Neurol (Pars) 161: 1071–1078.

Carel JC, Eugster EA, Rogol A, Ghizzoni L, Palmert MR, ESPE-LWPES GnRH Analogs Consensus Conference Group (2009) Consensus statement on the use of gonadotropin-releasing hormone analogs in children. Pediatrics 123: e752–e62.

Chapman E, Baron-Cohen S, Auyeung B, Knickmeyer R, Taylor K, Hackett G (2006) Fetal testosterone and empathy: evidence from the empathy quotient (EQ) and the 'Reading the mind in the eyes' test. Soc Neurosci 1: 135–148.

Cohen-Bendahan CC, van de Beek C, Berenbaum SA (2005) Prenatal sex hormone effects on child and adult sex-typed behavior: methods and findings. Neurosci Biobehav Rev 29: 353–384.

Collaer ML, Hines M (1995) Human behavioral sex differences: a role for gonadal hormones during early development? Psychol Bull 118: 55–107.

de Bruin EI, Veheij F, Wiegman T, Ferdinand RF (2006) Differences in finger length ratio between males with autism, pervasive developmental disorder-not otherwise specified, ADHD, and anxiety disorders. Dev Med Child Neurol 48: 962–965.

Dorn LD, Hitt SF, Rotenstein D (1999) Biopsychological and cognitive differences in children with premature vs on-time adrenarche. Arch Pediatr Adolesc Med 153: 137–146.

Eaton GG, Worlein JM, Kelley ST, Vijayaraghavan S, Hess DL, Axthelm MK, Bethea, CL (1999) Self-injurious behavior is decreased by cyproterone acetate in adult male rhesus (Macaca mulatta). Horm Behav 35: 195–203.

Eriksson T (2007) Anti-androgenic treatment of obsessive-compulsive disorder: an open-label clinical trial of the long-acting gonadotropin-releasing hormone analogue triporelin. Int Clin Psychopharmacol 22: 57–61.

Finegan JA, Bartleman B, Wong PY (1989) A window for the study of prenatal sex hormone influences on postnatal development. J Genet Psychol 150: 101–112.

Fombonne E (2005) The changing epidemiology of autism. J Appl Res Intellect Disabil 18: 281–294.

Gaikward U, Parle M, Jumar A, Gaikwad D (2010) Effect of ritanserin and leuprolide alone and combined on marble-burying behavior of mice. Acta Pol Pharm 67: 523–527.

Geier MR, Geier DA (2005) The potential importance of steroids in the treatment of autistic spectrum disorders and other disorders involving mercury toxicity. Med Hypotheses 64: 946–954.

Geier DA, Geier MR (2006a) A clinical and laboratory evaluation of methionine cycle-transsulfuration and androgen pathway markers in children with autistic disorders. Horm Res 66: 182–188.

Geier DA, Geier MR (2006b) A clinical trial of combined anti-androgen and anti-heavy metal therapy in autistic disorders. Neuro Endocrinol Lett 27: 833–838.

Geier DA, Geier MR (2007) A prospective assessment of androgen levels in patients with autistic spectrum disorders: biochemical underpinnings and suggested therapies. Neuro Endocrinol Lett 28: 565–573.

Geier DA, Geier MR (2008) Autism spectrum disorder-associated biomarkers for case evaluation and management by clinical geneticists. Expert Rev Mol Diagn 8: 671–674.

Geier DA, Kern JK, Geier MR (2010) The biological basis of autism spectrum disorders: understanding causation and treatment by clinical geneticists. Acta Neurobiol Exp (Wars) 70: 209–226.

Giedd JN, Snell JW, Lange N, Rajapakse JC, Casey BJ, Kozuch PL, Vaituzis AC, Vauss YC, Hamburger SD, Kaysen D, Rapoport JL (1996) Quantitative magnetic resonance imaging of human brain development: ages 4-18. Cereb Cortex 6: 551–560.

Harden AY, Minshew NJ, Mallikarjuhn M, Keshavan MS (2001) Brain volume in autism. J Child Neurol 16: 421–424.

Hazlett HC, Poe M, Gerig G, Smith RG, Provenzale J, Ross A, Gilmore J, Piven J (2005) Magnetic resonance imaging and head circumference study of brain size in autism: birth through age 2 years. Arch Gen Psychiatry 62: 1366–1376.

Higano CS (2003) Side effects of androgen deprivation therapy: monitoring and minimizing toxicity. Urology 61: 32–38.

Hines M, Fane BA, Pasterski VL, Matthews GA, Conway GS, Brook C (2003) Spatial ability following prenatal androgen abnormality: targeting and mental rotations performance in individuals with congential adrenal hyperplasia. Psychoneuroendocrinology 28: 1010–1026.

Hu VW, Nguyen A, Kim KS, Steinberg ME, Sarachana T, Scully MA, Soldin SJ, Luu T, Lee NH (2009) Gene expression profiling of lymphoblasts from autistic and nonaffected sib pairs: altered pathways in neuronal development and steroid biosynthesis. PLoS One 4: e5775.

Huertas D, Lopez-Ibor Alino JJ, Molina JD, Chamorro L, Balanza J, Jimenez MP, Hornillos M (2007) Antiaggressive effect of cyproterone versus haloperidol in Alzheimer's disease: a randomized double-blind pilot study. J Clin Psychiatry 68: 439–444.

Jones RM, Wheelwright S, Farrell K, Martin E, Green R, Di Ceglie D, Baron-Cohen S (2012) Brief report: female-to-male transsexual people and autistic traits. J Autism Dev Disord 42: 301–306

Judd HL, Robinson JD, Young PE, Jones OW (1976) Amniotic fluid testosterone levels in midpregnancy. Obstet Gynecol 48: 690–692.

Kintzel PE, Chase SL, Schultz LM, O'Rourke TJ (2008) Increased risk of metabolic syndrome, diabetes mellitus, and cardiovascular disease in men receiving androgen deprivation therapy for prostate cancer. Pharmacotherapy 28: 1511–1522.

Knickmeyer R, Baron-Cohen S, Raggatt P, Taylor K (2005) Foetal testosterone, social relationships, and restricted interests in children. J Child Psychol Psychiatry 46: 198–210.

Knickmeyer R, Baron-Cohen S, Fane BA, Wheelwright S, Mathews GA, Conway GS, Brook CG, Hines M (2006) Androgens and autistic traits: a study of individuals with congenital adrenal hyperplasia. Horm Behav 50: 148–153.

Krajmer P, Spajdel M, Kubranska A, Ostatnikova D (2011) 2D:4D finger ratio in Slovak autism spectrum population. Bratisl Lek Listy 112: 377–379.

Laron Z, Kauli R (2000) Experience with cyproterone acetate in the treatment of precocious puberty. J Pediatr Endocrinol Metab 13 (Suppl 1): 805–810.

Loosen PT, Purdon SE, Pavlou, SN (1994) Effects on behavior of modulation of gonadal function in men with gonadotropin-releasing hormone antagonists. Am J Psychiatry 151: 271–273.

Lutchmaya S, Baron Cohen S, Raggatt P (2002a) Foetal testosterone and eye contact in 12 month old infants. Infant Behav Dev 25: 327–335.

Lutchmaya S, Baron-Cohen S, Raggatt P (2002b) Foetal testosterone and vocabulary size in 18- and 24-months-old infants. Infant Behav Dev 24: 418–424.

Lutchmaya S, Baron-Cohen S, Raggatt P, Knickmeyer R, Manning JT (2004) 2nd to 4th digit ratios, fetal testosterone and estradiol. Early Hum Dev 77: 23–28.

MacLeod TL, Eisen A, Sussman GL (1987) Anaphylactic reaction to synthetic luteinizing hormone-releasing hormone. Fertil Steril 48: 500–502.

Majewska MD, Hill M, Urbanowicz E, Rok-Bojko P, Namyslowska I, Mierzejewski P (2010) Different levels of salivary steroids in autistic and health children. Eur. Neuropsychopharmacol 20(Suppl 3): S615–S616.

Majiakou MA, Manousaki D, Papadaki M, Hadjidakis D, Levidous G, Vakaki M, Papaefstathiou A, Lalioti N, Kanaka-Gantenbein C, Piaditis G, Chrousos GP, Dacou-Voutetakis C (2010) The efficacy and safety of gonadotropin-releasing hormone analog treatment in childhood and adolescence: a single center, long-term follow-up study. J Clin Endocrinol Metab 95: 109–117.

Malas MA, Dogan S, Evcil EH, Desdicioglu K (2006) Fetal development of the hand, digits and digit ratio (2D:4D). Early Hum Dev 82: 469–475.

Malone RP, Waheed A (2009) The role of antipsychotics in the management of behavioral symptoms in children and adolescents with autism. Drugs 69: 535–548.

Manning JT, Scutt D, Wilson J, Lewis-Jones DI (1998) The ratio of 2nd to 4th digit length: a predictor of sperum numbers and concentrations of testosterone, luteinizing hormone and oestrogen. Hum Reprod 13: 3000–3004.

Manning JT, Baron-Cohen S, Wheelwright S, Sanders G (2001) The 2nd to 4th digit ratio and autism. Dev Med Child Neurol 43: 160–164.

Milne E, White S, Campbell R, Swettenham J, Hansen P, Ramus F (2006) Motion and form coherence detection in autistic spectrum disorder: relationship to motor control and 2:4 digit ratio. J Autism Dev Disord 36: 225–237.

Neely EK, Lee PA, Bloch CA, Larsen L, Yang D, Mattia-Goldberg C, Chwalisz K (2010) Leuprolide acetate 1-month depot for central precocious puberty: hormonal suppression and recovery. Int J Pediatr Endocrinol 2010: 398639.

New MI (1998) Diagnosis and management of congenital adrenal hyperplasia. Annu Rev Med 49: 311–328.

Noipayak P (2009) The ratio of 2nd and 4th digit length in autistic children. J Med Assoc Thai 92: 1040–1045.

Partsch CJ, Sippell WG (2002) Treatment of central precocious puberty. Best Pract Res Clin Endocrinol Metab 16: 165–189.

Reismann P, Liko I, Igaz P, Patocs A, Racz K (2009) Pharmacological options for treatment of hyperandrogenic disorders. Mini Rev Med Chem 9: 1113–1126.

Ruta L, Ingudomnukul E, Taylor K, Chakrabati B, Baron-Cohen S (2011) Increased serum androstenedione in adults with autism spectrum conditions. Psychoneuroendocrinology 36: 1154–1163.

Rutter M, Caspi A, Moffitt TE (2003) Using sex differences in psychopathology to study causal mechanisms: unifying issues and research strategies. J Child Psychol Psychiatry 44: 1092–1115.

Sarachana T, Xu M, Wu RC, Hu VW (2011) Sex hormones in autism: androgens and estrogens differentially and reciprocally regulate RORA, a novel candidate gene for autism. PLoS One 6: e17116.

Schindler AE (1982) Hormones in human amniotic fluid. Monogr Endocrinol 21: 1–158.

Schwarz E, Guest PC, Rahmoune H, Wang L, Levin Y, Ingodomnukul E, Ruta L, Kent L, Spain M, Baron-Cohen S, Bahn S (2011) Sex-specific serum biomarker patterns in adults with Asperger's syndrome. Mol Psychiatry 16: 1213–1220.

Scott FJ, Baron-Cohen S, Bolton P, Brayne C (2002) Brief report: prevalence of autism spectrum conditions in children aged 5-11 years in Cambridgeshire, UK. Autism 6: 231–237.

Smith LL, Hines M (2000) Language lateralization and handedness in women prenatally exposed to diethylstilbestrol (DES). Psychoneuroendocrinology 25: 497–512.

Takagishi H, Takahashi T, Yamagishi T, Shinada M, Inukai K, Tanida S, Mifune N, Horita Y, Hashimoto H, Yang Y, Kameda T (2010) Salivary testosterone levels and autism spectrum quotient in adults. Neuro Endocrinol Lett 31: 837–841.

Tanaka T, Niimi H, Matsuo N, Fujieda K, Tachibana K, Ohyama K, Satoh M, Kugu K (2005) Results of long-term follow-up after treatment of central precocious puberty with leuprorelin acetate: evaluation of effectiveness of treatment and recovery of gonadal function. The TAP144-SP Japanese Study Group on Central Precocious Puberty. J Clin Endocrinol Metab 90: 1371–1376.

Tordjman S, Ferrari P, Sulmont V, Dume M, Roubertoux P (1997) Androgenic activity in autism. Am J Psychiatry 154: 1626–1627.

Torri V, Floriani I (2005) Cyproterone acetate in the therapy of prostate carcinoma. Arch Ital Urol Androl 77: 157–163.

Uday G, Pravinkumar B, Manish W, Sudhir U (2007) LHRH antagonist attenuates the effect of fluoxetine on marble-burying behavior in mice. Eur J Pharmacol 563: 155–159.

Umathe S, Bhutada P, Dixit P, Shende V (2008a) Increased marble-burying behavior in ethanol-withdrawal state: modulation by gonadotropin-releasing hormone agonist. Eur J Pharmacol 587: 175–180.

Umathe SN, Bhutada PS, Dixit PV, Jain NS (2008b) Leuprolide: a luteinizing hormone releasing agonist attenuated ethanol withdrawal syndrome and ethanol-induced locomotor sensitization in mice. Neuropeptides 42: 345–353.

Umathe SN, Bhutada PS, Jain NS, Dixit PV, Wanjari MM (2008c) Effects of central administration of gonadotropin-releasing hormone agonists and antagonist on elevated plus-maze and social interaction behavior in rats. Behav Pharmacol 19: 308–316.

Umathe SN, Bhutada PS, Jain NS, Shukla NR, Mundhaha YR, Dixit PV (2008d) Gonadotropin-releasing hormone agonist blocks anxiogenic-like and depressant-like effect of corticotrophin-releasing hormone in mice. Neuropeptides 42: 399–410.

Umathe SN, Wanjari MM, Manna SS, Jain NS (2009) A possible participation of gonadotropin-releasing hormone in the neuroleptic and cataleptic effect of haloperidol. Neuropeptides 43: 251–257.

van der Sluis IM, Boot AM, Krenning EP, Drop SL, de Muinck Keizer-Schrama SM (2002) Longitudinal follow-up of bone density and body composition in children with precocious or early puberty before, during and after cessation of GnRH agonist therapy. J Clin Endocrinol Metab 87: 506–512.

van Honk J, Schutter DJ, Bos PA, Kruijt AW, Lentjes EG, Baron-Cohen S (2011) Testosterone administration impairs cognitive empathy in women depending on second-to-fourth digit ratio. Proc Natl Acad Sci U S A 108: 3448–3452.

Wojniusz S, Vogele C, Ropstad E, Evans N, Robinson J, Sutterlin S, Erhard HW, Solbakk AK, Endestad T, Olberg DE, Haraldsen IR (2011) Prepubertal gonadotropin-releasing hormone analog leads to exaggerated behavioral and emotional sex differences in sheep. Horm Behav 59: 22–27.