A prospective assessment of androgen levels in patients with autistic spectrum disorders: biochemical underpinnings and suggested therapies

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Abstract

Impairments in social relatedness and communication, repetitive behaviors, abnormal movement patterns, and sensory dysfunction characterize autism spectrum disorders (ASDs). Seventy consecutive patients with an ASD diagnosis (DSM-IV criteria, ≥ 6 years-old) who presented to the Genetic Centers of America for outpatient genetic/developmental evaluations from 2005–2007 were examined. Patients were evaluated using CLIA-approved Laboratory Cooperation of America (LabCorp) testing for: serum testosterone, serum free testosterone, % free testosterone, serum/plasma dehydroepiandrosterone (DHEA), androstendione, and follicle-stimulating hormone (FSH). Morning blood samples collected following an overnight fast, compared to the pertinent reference means, showed significantly increased relative mean levels for: serum testosterone (158%), serum free testosterone (214%), percent free testosterone (121%), DHEA (192%), and androstenedione (173%). By contrast, compared to the pertinent reference mean, the relative mean level of FSH (51%) was significantly decreased. Additionally, at least one of the androgen attributes examined exceeded its recognized laboratory age- and sex-specific reference range in 81.4% (57 of 70) of the patients examined. With respect to their age- and sex-specific reference ranges, females had significantly higher overall mean relative testosterone and relative free testosterone levels than males. Increased androgens in patients diagnosed with ASDs may involve cyclical interactions between the androgen and the transsulfuration pathways, particularly following mercury exposure. A review of therapies that have significantly improved clinical outcomes in ASD patients indicates they share commonality in helping lower androgens. Thus, androgens should be routinely clinically measured in patients with an ASD diagnosis and appropriate androgen-lowering therapies considered for those who have significantly elevated levels.

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INTRODUCTION

Autism spectrum disorders (ASDs) are prevalent neurodevelopmental disorders characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movement patterns, and sensory dysfunction [16]. Though symptoms of an ASD may be present from birth, they may also manifest between 12 and 24 months of age [56]. In addition, individuals with ASDs have an increased prevalence of gastrointestinal disease and dysbiosis [57], autoimmune disease [48], and mental retardation [8]. The US Centers for Disease Control and Prevention (CDC) recently reported ASDs may presently occur in as many as one in 150 children, and many more males than females have an ASD, with an overall male:female ratio of at least 3:1 [13].

The sex difference in the occurrence of ASD cases may reflect a male vulnerability to developing an ASD, a hypothesis supported by multiple lines of evidence. Baron-Cohen reported that individuals with an ASD tend to display a hypermasculine profile on many cognitive tasks [3]. Others have observed that individuals with an ASD also have lower-than-expected 2nd to 4th digit (2D:4D) ratios [28,38], which is correlated with higher ratios of fetal testosterone (FT) to fetal estrogen [36], as well as lower verbal and higher numerical intelligence [37]. Some neuroanatomical studies comparing the brains of individuals with and without an ASD reveal structural differences associated with high levels of FT, including hemispheric asymmetries [27]. Finally, girls with abnormally high FT levels as a result of congenital adrenal hyperplasia (CAH) have a higher number of autistic traits than their unaffected sisters [34]. Clinical examination of patients with an ASD has revealed that on average, girls with an ASD show a significant delay in the onset of menarche [35] (excess androgens have been linked to menstrual problems [12]) and are more likely to display elevated rates of testosterone-related disorders than neurotypical controls [28]. Other studies have shown elevated blood androgen metabolites in patients with an ASD in comparison with controls [20,50].

The present study is a moderate-scale clinical study, evaluating the current blood androgen levels of patients with an ASD.

MATERIALS AND METHODS

The Institutional Review Board (IRB) of the Institute for Chronic Illnesses (Office for Human Research Protections, US Department of Health and Human Services IRB number: IRB00005375) approved the present experimental study.

Subjects

In the present study, 70 consecutive patients (≥6 years-old) who prospectively presented to the Genetic Centers of America for outpatient genetic/developmental evaluations from 2005–2007 and who had an ASD diagnosis were examined. Each patient, prior to their presentation to the Genetic Centers of America, had been diagnosed with an ASD, based upon the criteria in the *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition* (DSM-IV). Table 1 summarizes the pertinent demographics of the patients with an ASD who were examined in the present study. Each patient underwent a genetic evaluation consistent with that recently described by the American College of Medical Genetics [46]. Patients were tested to rule-out brain structural abnormalities (CT or MRI head scans) and vision and hearing abnormalities. Additionally, laboratory testing was conducted on each patient, and all were determined to be negative for Fragile X Syndrome, chromosomal abnormalities (structural and numeric), subtelomere chromosome rearrangements, Angelman/Prader Willi Syndrome, urine organic acid abnormalities, Polychlorinated Biphenyl/pesticide exposure, and Rett Syndrome (CLIA-approved Laboratory Cooperation of America [LabCorp]).

Table 1. A summary of the patients with autistic disorders.

Descriptive Information	
Male / Female (ratio)	59 / 11 (5.4:1)
Mean Age in Years ± Std (range)	10.8 ± 4.1 (6–27)
Mean Date of Birth ± Std (range)	1995 ± 4.3 (1978–2000)
Race (n)	
White	82.8% (58)
Black	11.4% (8)
Indian	2.9% (2)
Oriental	2.9% (2)
Geographic Location [1] (n)	
Northeast	10% (7)
Southeast	54.3% (38)
Midwest	14.3% (10)
Mountain-Plain-Southern	14.3% (10)
West	7.1% (5)
Autism (n)	48.6% (34)
Autism Spectrum Disorders [2] (n)	51.4% (36)

Std = standard deviation
[1] Northeast = CT, NJ, NY, PA; Southeast = FL, KY, MD, NC, SC, TN, VA; Midwest = IA, IL; Mountain-Plain-Southern = KS, MO, TX, UT; West = CA.
[2] Autism spectrum disorders include patients diagnosed with pervasive developmental delay – not otherwise specified (PDD-NOS) and Asperger's disorder.

Evaluation

Patients with ASDs were tested for the following androgen attributes: serum testosterone, serum free testosterone, % free testosterone, serum/plasma dehydroepiandrosterone (DHEA), androstendione, and follicle-stimulating hormone (FSH) (LabCorp). These tests were performed on morning blood samples collected following an overnight fast.

Controls

In order to evaluate each of the androgen metabolites measured among the patients with ASDs examined in the present study, age- and sex-specific reference ranges for each test from LabCorp were utilized. Age- and sex-specific references ranges were available for each of the androgen metabolites examined.

Statistical Analyses

In the present study, the statistical package contained in Microsoft Excel 2002 and StatsDirect (Version 2.4.2) were utilized. For each patient, their androgen levels were evaluated in relation to the mean level from the age- and sex-specific reference range for each test, so as to convert each patient's measured test values into a percent of the mean value ([patient's laboratory value / mean level from the age- and sex-specific reference range] × 100=percent of the pertinent mean). For each type of androgen attribute examined, the individual results were then averaged to compute an overall average percent of the pertinent means, and the standard deviations for each attribute were calculated. Using the two-sample heteroscedastic t-test statistic, these "sex- and age- normalized" results from the patients with an ASD were then statistically compared to the corresponding data from the normal control populations that comprised the laboratory reference ranges. The null hypothesis was that there should be no difference in the normalized means between the patients with an ASD for each androgen attribute examined and the corresponding means from the control populations (derived from age- and sex-specific laboratory reference ranges). Additionally, the overall mean percent of the normalized mean observed for each androgen attribute examined was compared between male and female patients with an ASD in the present study, utilizing the Mann-Whitney U test statistic. The null hypothesis was that, between male and female patients with an ASD, there should be no difference between the overall normalized mean percent of average observed for each androgen attribute examined. For all the statistical tests in the present study, a two-tailed p-value < 0.05 was considered statistically significant.

RESULTS

Table 2 summarizes the androgen measurements made on the patients with ASDs examined in the present study. These patients had significantly increased overall relative mean levels of serum testosterone (158%), serum free testosterone (214%), % free testosterone (121%), DHEA (192%), and androstenedione (173%) in comparison to the laboratory age- and sex-specific reference ranges. Additionally, among these patients: 24.3% had serum testosterone, 42.9% had serum free testosterone, 35.7% had % free testosterone, 57.1% had DHEA, and 42.9% had androstenedione levels that were greater than their pertinent laboratory age- and sex-specific reference ranges' upper limits. Moreover, the patients with an ASD were found to have significantly decreased overall average relative levels of FSH (51%) in comparison to their pertinent laboratory age- and sex-specific reference ranges.

Figure 1 compares the overall percentage-of-mean results observed among male and female patients with an ASD examined in the present study. Based on the data, there were significantly increased overall range-relative levels of serum testosterone and serum free testosterone among females in comparison to males. Figure 2 evaluates the relationship between the pertinent individual percent-of-mean measures observed for serum testosterone, DHEA, and androstenedione, in comparison to the age of the patient.

Table 2. A summary of androgens measured among those with autism spectrum disorders.

Laboratory Test	% of Pertinent Mean ± Std	% > Reference Ranges' Upper Limit(n)	% < Reference Ranges' Lower Limit (n)
Serum Testosterone	158±122 **	24.3 (17)	0 (0)
Serum Free Testosterone	214±201 **	42.9 (30)	10 (7)
% Free Testosterone	121±47.6 *	35.7 (25)	12.9 (9)
DHEA	192±157 **	57.1 (40)	12.9 (9)
Androstenedione	173±99.5 **	42.9 (30)	2.9 (2)

The t-test statistic was utilized for statistical testing. Std = standard deviation, DHEA = dehydroepiandrosterone,
* p-value < 0.05; ** p-value < 0.01

DISCUSSION

This study presents the first intermediate-scale clinical evaluation of androgen levels in 70 consecutive patients with an ASD diagnosis. The patients examined who had an ASD were found to have overall significantly increased levels of serum testosterone, serum free testosterone, % free testosterone, DHEA, and androstenedione, on average, in comparison to their laboratory age- and sex-specific reference ranges. 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 in the present study. On the whole, it was observed that 81.4% (57 of 70) of ASD patients had at least one of the androgen markers, examined in the present study, that was greater than the pertinent upper limit for their laboratory age- and sex-specific reference range. Additionally, among those with an ASD, the female patients studied had a significantly increased overall relative percentage of mean levels for both serum testosterone and serum free testosterone, in comparison to male patients examined, in the present study.

To minimize the potential for selection bias, the patients with an ASD who were studied were simply consecutive patients who: prospectively presented for genetic/developmental evaluations at the Genetic Centers of America and were found to be free from known genetic abnormalities. To ensure this was the case, each of these patients was carefully evaluated to rule-out potential genetic and genetic-influenced biochemical factors as causal factors for their ASD. Thus, as a group, the patients studied represent a homogenous "genetically normal" population. Overall, the study's patients represent a geographically disperse population from across the US and, in general, their racial make up was similar to that of the US. Additionally, the male/female ratio observed among the ASD patients examined (5.4:1) is consistent with that observed in other ASD populations and indicates the population examined was not significantly gender skewed. Finally, most of the patients in this study had been previously diagnosed with either autism or another autism spectrum disorder. Hence, the present results provide findings that should, in general, be applicable to almost all who have an ASD diagnosis.

The laboratory tests employed in the present study were selected to minimize potential confounders associated with laboratory collection. To minimize sampling biases, all the laboratory specimens in the present study were collected at similar times (i.e. in the morning) and under similar conditions for the patients (i.e. following an overnight fast). The laboratory specimens were all sent to the same laboratory (i.e. LabCorp), and utilized the same age- and sex-specific reference ranges. LabCorp was utilized in the present study to ensure that the clinical laboratory used was available to many clinicians in the US.

One potential limitation for the present study is that these patients with an ASD were assessed at a single clinic. There may be biasing selection factors that con-
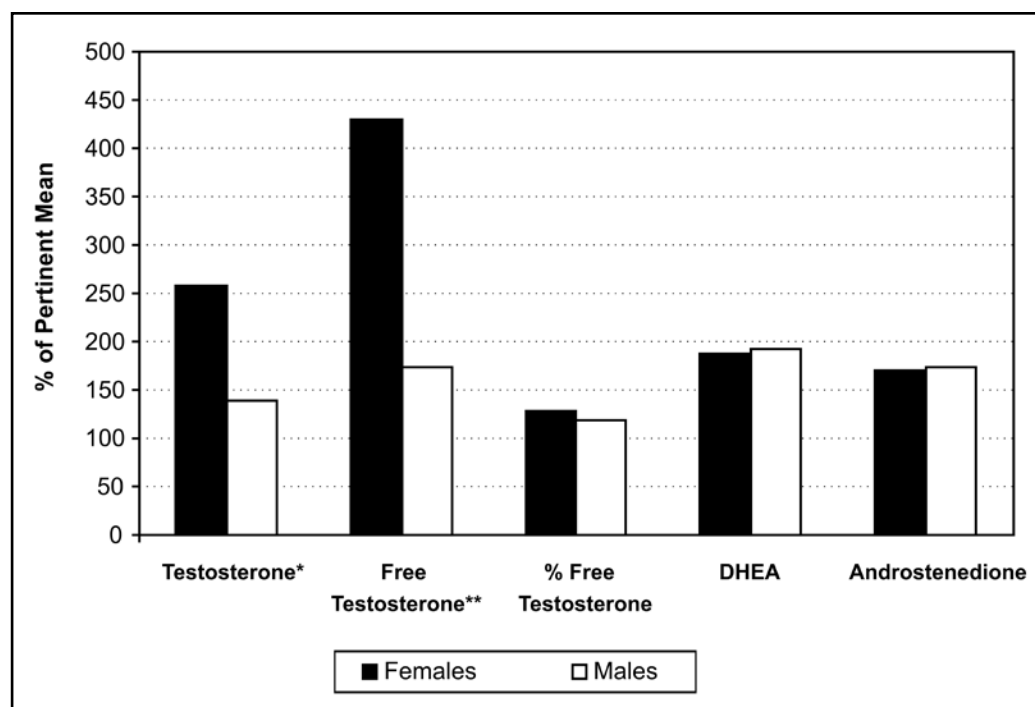

Figure 1. A comparison of blood androgen levels between males and females among those with autism spectrum disorders.
The Mann-Whitney U test statistic was utilized for statistical testing. DHEA = dehydroepiandrosterone
* p-value < 0.05; ** p-value < 0.01

tribute to whether a patient with an ASD presents to a given clinic. These biasing effects may be present in the data examined. However, given the robustness of the effects observed across the multiple different androgen attributes examined, and considering the fact that the patients studied were from across the US, these effects should have had a limited impact on the results observed in the present study. In addition, since the present study only assessed 70 patients with an ASD, it would be useful to examine an expanded cohort of patients with an ASD at different ASD clinics, in order to evaluate whether the results/observations of such a study in an expanded multiple-clinic population would be consistent with those of the present one. It would also be useful in future studies to compare the laboratory observations made in the present study with laboratory observations utilizing other laboratories. In so doing, it is imperative that any other laboratory employed for androgen testing should have age- and sex-specific reference ranges. Furthermore, given the wide variability observed across the age- and sex-specific reference ranges, studies which attempt to crudely categorize data, grouping together the overall age- and sex-ranges for the cases and the controls, may also fail to detect significant differences that are present in specific age- and sex-bands of data (i.e. the outliers in the data may mask the true differences in the populations).

Biochemical Underpinnings

The increased androgen metabolites observed in the present study do not appear to be the result of abnormalities in either the structure or the function of the pituitary. First, to rule-out pituitary structural anomalies that could account for overproduction of androgens, each patient with an ASD underwent a head CT or MRI scan. Second, to rule out pituitary function abnormalities, the study evaluated each patient's FSH level. These evaluations found that the FSH levels were significantly reduced in the study's patients who had an ASD, in comparison to their age- and sex-specific laboratory reference range mean values. This observation is consistent with previous studies that have shown significant reductions in FSH levels in patients with an ASD [20]. This suggests that the significantly increased level of androgens observed in patients with an ASD indicates that these patients' pituitaries are, if anything, attempting to down-regulate pituitary controlled androgen synthesis as they should be.

In addition, abdominal ultrasounds were performed on a subset of the study's patients who had an ASD, to rule-out adrenal structural anomalies that might account for the increased androgen levels observed. These evaluations found no evidence of structural abnormalities in the adrenals.

Overall, it is apparent that the biochemical basis for abnormalities in the androgen synthesis pathway in those with an ASD may involve the regulator metabolite DHEA. DHEA can either be converted into the normally favored storage molecule, dehydroepiandrosterone-sul-
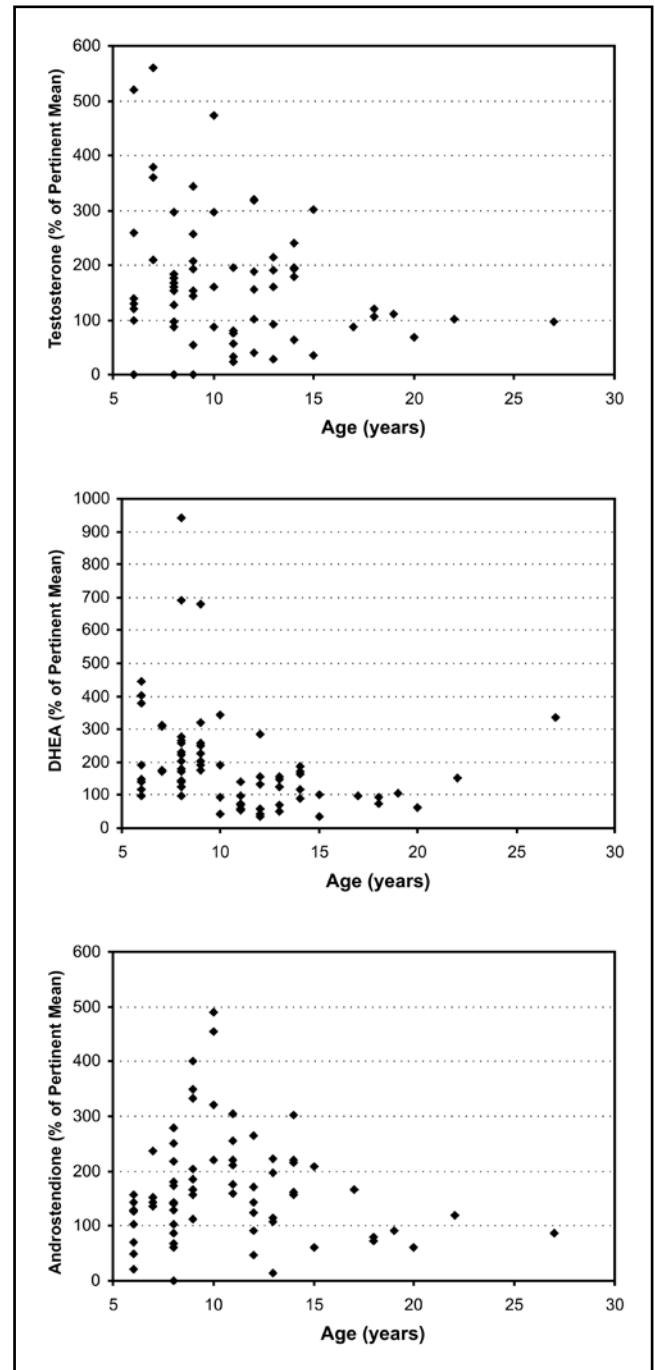

Figure 2. The age distribution of % of mean of various blood androgen measurements in patients with an autism spectrum disorder diagnosis.
DHEA = dehydroepiandrosterone

fate (DHEA-S), or further down the androgen pathway toward testosterone, it can be converted into androstenedione or androstenediol. The conversion of DHEA to DHEA-S by the enzyme hydroxysteroid sulfotransferase (HST) is dependent upon sulfation and is inhibited by inflammation [33,45].

Since, evaluations of the transsulfuration pathway in those with an ASD diagnosis has revealed significant decreases in transsulfuration metabolites including cys-
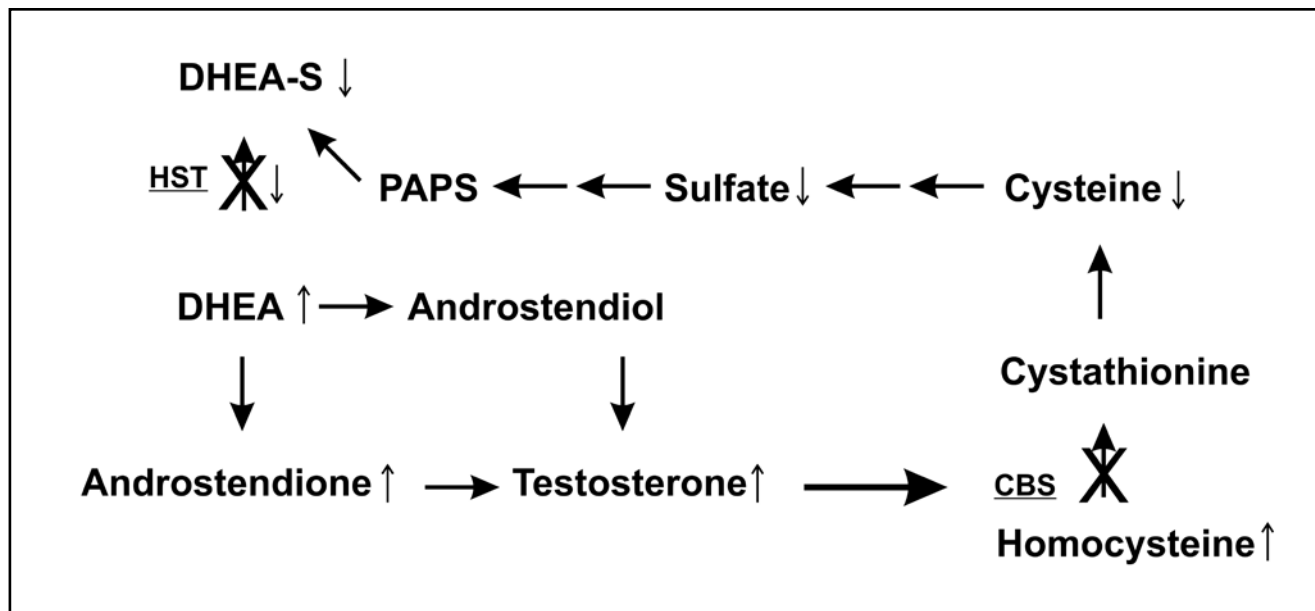
Figure 3. A summary of the potential interactions between the transsulfuration and androgen pathways in those with an autism spectrum disorder diagnosis.
HST = Hydroxysteroid sulfotransferase; PAPS = 3′-phosphoadenylyl sulfate; CBS = Cystathionine β-Synthase; DHEA-S = Dehydroepi-androsterone-sulfate; DHEA = Dehydroepiandrosterone.

teine [19,20,29,30], glutathione [19,20,29,30], and sulfate [54,55], impaired sulfation [1], as well as significant increases in common polymorphic variants known to modulate the transsulfuration pathway [29], there may be a marked shift toward DHEA, and subsequent metabolites in the androgen synthesis pathway. In addition, the presence of significant oxidative stress in those with an ASD diagnosis [15,32,39,40] (including pathologically confirmed inflammation [51]), and the apparent significant increase in mercury body-burden and toxicity in those with an ASD diagnosis [19,22,23,40] (mercury is a known inducer of pathologically confirmed inflammation [14]) may also lead to a significant overproduction of androgens in those with an ASD diagnosis. Previous studies in testicular tissue culture [18], animal models [52], and humans [5] have shown that low-dose exposure to mercury can induce significant increases in androgen levels. The apparent result, as demonstrated in these ASD cases, is a significantly increased DHEA level [20] (also observed in the present study) and a significantly lowered DHEA-S level [47], relative to controls.

Furthermore, it has been shown that testosterone, and possibly other androgen metabolites, may have a negative impact on the transsulfuration pathway. A series of animal studies demonstrated that testosterone administration at least partially blocks the conversion of homocysteine to cystathionine, whereas estrogen administration had the opposite affect [25,26]. Additionally, researchers have shown significant positive correlations between homocysteine and androstenedione levels and glutathione and DHEA-S levels in humans [53]. Thus, high levels of androgens are expected to block the transsulfuration

pathway. The apparent result, as demonstrated in those with an ASD diagnosis, is significantly increased homocysteine [42], *S*-adenosylhomocysteine (SAH) [29,30], or adenosine [29,30] levels, in comparison to controls.

In putting these pieces together, environmental exposures (particularly mercury exposure) that adversely effect HST and the transsulfuration pathway can cause a cyclical biochemical interaction pattern to develop between the transsulfuration and androgen pathways that directly correlate with the biochemistry observed in those with an ASD diagnosis. As expected, this interaction pattern and androgen elevations are consistent with the behavioral/physical traits associated with or defining those who have an ASD diagnosis. Figure 3 summarizes the overall potential interactions between the androgen and transsulfuration pathways in those having an ASD.

Additionally, HST was shown to be necessary for appropriate function of bile salts [43]. As a result, given the aforementioned abnormalities observed in patients diagnosed with an ASD, this interference with HST production may contribute to malabsorption and the high prevalence of gastrointestinal disease found in ASD cases. Furthermore, impaired sulfation may also play an important role in other common biochemical abnormalities found in ASD cases, involving neurotransmitters, peptides, glycosaminoglycans, amines, and/or phenols [54].

Suggested Therapies
In clinically considering the apparent biochemical abnormalities in the androgen pathway among those having an ASD diagnosis, some have suggested that therapies which address the steroid hormone pathways

Table 3. Examples of clinical outcomes observed in patients with autism spectrum disorders following leuprolide acetate (LUPRON®) therapy.

PATIENT	OBSERVATIONS
18 Yr-Old **Male** Caucasian Diagnosis: Autism	**Pre-Treatment:** ATEC: overall impairments = 80–89th percentile, speech/language/communication = 30–39th percentile, sensory/cognitive/awareness = 40–49th percentile, health/physical/behavior = 90–99th percentile, and sociability = 70–79th percentile. Extreme aggressive behaviors including being destructive, violent, and was reported to hit and injure himself and others. Patient has sexual behaviors (such as masturbation)
Dosing: 15 mg IM Depot (28 days) 0.2 mL SQ (everyday), gradually increased to 0.5 mL SQ (everyday)	**Treatment (Day 156):** ATEC: overall impairments = 30–39th percentile, speech/language/communication = 30–39th percentile, sensory/cognitive/awareness = 30–39th percentile, health/physical/behavior = 70–79th percentile, and sociability = 50–59th percentile. Parents and educators reported major improvements in attention, cognitive awareness, receptive language skills, and especially reduced level of aggressive behaviors. Reduction of self-mutation and physical violence towards others. Patient has had a significant reduction in his sexual behaviors (such as masturbation). The patient still suffers from mood swings and occasional sleep problems.
11 Yr-Old **Male** Caucasian Diagnosis: Autism	**Pre-Treatment:** ATEC: overall impairments = 80–89th percentile of severity), speech/language/communication = 70–79th percentile, sensory/cognitive/awareness = 50–59th percentile, health/physical/behavior = 80–89th percentile, and sociability = 60–69th percentile. Patient had a bone age consistent in age with a 14–15 Yr-Old. Patient has body hair (since 9 Yr-Old) and sexual behaviors (such as masturbation since 9 Yr-Old)
Dosing: 15 mg IM Depot (28 days) 0.4 mL SQ (everyday), gradually increased to 0.7 mL SQ (everyday)	**Treatment (Day 104):** ATEC: overall impairments = 40–49th percentile, speech/language/communications = 60–69th percentile, sensory/cognitive/awareness = 40–49th percentile, health/physical/behavior = 60–69th percentile, and sociability = 20–29th percentile. Parents and educators reported major improvements in attention, cognitive awareness, and receptive language skills. Patient has had a significant decrease in body hair and sexual behaviors (such as masturbation)
9 Yr-Old **Male** African American Diagnosis: PDD-NOS	**Pre-Treatment:** ATEC: overall impairments = 20–29th percentile of severity), speech/language/communication = 20–29th percentile, sensory/cognitive/awareness = 40–49th percentile, health/physical/behavior = 20–29th percentile, and sociability = 40–49th percentile. Patient has body and facial hair (since 5 Yr-Old), body odor (in the last yr), sexual behaviors (such as erections and advanced genital development)
Dosing: 15 mg IM Depot (28 days) 0.5 mL SQ, gradually increased to 0.7 mL SQ (everyday)	**Treatment (Day 58):** ATEC: overall impairments = 0–9th percentile, speech/language/communications = 0–9th percentile, sensory/cognitive/awareness = 0–9th percentile, health/physical/behavior = 0–9th percentile, and sociability = 0–9th percentile. Parents and educators reported major improvements in attention, cognitive awareness, and receptive language skills. Patient has been observed to take a very active interest in the world around him. Patient has had a significant decrease in body and facial hair, body odor, and sexual behaviors (such as erections and genital development).

Pervasive Developmental Delay – Not Otherwise Specified = PDD-NOS; Intramuscular = IM; Subcutaneous = SQ

The Autism Treatment Evaluation Checklist (ATEC) Form was developed by the Autism Research Institute (San Diego, California). The ATEC consists of 4 subtests: Speech/Language/Communication (14 items – scores can range from 0–28), Sociability (20 items – scores can range from 0–40), Sensory/Cognitive/Awareness (18 items – scores can range from 0–36), Health/Physical/Behavior (25 items – scores can range from 0–75), The Autism Research Institute calculates four subscale scores and a total score (total scores can range from 0–180) from the ATEC form. The scores are weighted according to the response and the corresponding subscale. The higher the subscale and total score, the more impaired the subject. The lower the subscale and total score, the less impaired the subject. The ATEC can also be used to monitor the effectiveness of treatment (such as the treatment regimens described herein) of a subject suffering from autism or an autism spectrum disorder.

in ASD cases may help to improve clinical outcomes [24]. Recently, several studies with drugs having known anti-androgen effects including: leuprolide acetate [49], cyproterone acetate [7], spironolactone [17], risperidone [58], haloperidol [58], and pioglitazone [6] were reported to have beneficial effects in ASDs. These studies noted that the therapies utilizing these drugs resulted in significant clinical ameliorations in hyperactivity/impulsivity, stereotypy, aggression, self injury, abnormal sexual behaviors, and/or irritability behaviors that frequently occur in those with an ASD diagnosis [2,9,10,21,31,44].

Furthermore, in our own clinical experience we have observed that leuprolide acetate (LUPRON®) administration to nearly 200 patients diagnosed with ASDs significantly lowered androgen levels and has 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. Table 3 summarizes three representative examples of patients with ASD diagnoses from different age groups that were drawn from our LUPRON® treated ASD patients.

SUMMARY

Notably, the aforementioned understanding of the biochemical interactions and regulation between the transsulfuration and androgen pathways represents a potentially new understanding of control mechanisms in living systems. The understanding that the transsulfuration and androgen pathways interact so as to help to regulate one another may have significant importance in a number of other important chronic diseases with biomarkers similar to those who have an ASD diagnosis [24], and it may also be of significant importance in understanding how the human body undergoes maturational transitions.

CONCLUSION

The present study provides the first intermediate-scale clinical evaluation of androgen levels in 70 consecutive patients with an ASD diagnosis who prospectively presented to the Genetic Centers of America. It was observed that these patients had overall significantly increased androgen metabolites, in comparison to their age- and sex-specific reference range means. In light of the fact that excessive androgens were so frequently found among the patients with an ASD diagnosis in this study, and, given how important androgens may be to the pathogenesis and clinical presentation of those with an ASD diagnosis, clinicians should routinely measure androgens in those who have an ASD diagnosis, and potential treatments for those having an ASD diagnosis should consider monitoring and normalizing androgen levels. It is clear that additional research should be conducted to evaluate the potential role for androgens in patients with an ASD diagnosis.

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