

# A Clinical and Laboratory Evaluation of Methionine Cycle-Transsulfuration and Androgen Pathway Markers in Children with Autistic Disorders

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## Key Words

Precocious puberty · Pervasive developmental delay · Sex steroid hormones · Sexual development

## Abstract

**Background/Aims:** The prevalence of autism spectrum disorders (ASDs) is 1 in 300 children in the US. ASDs are characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movement patterns, and sensory dysfunction. Pre-pubertal age children with ASDs were assessed for metabolites in the methionine cycle-transsulfuration and androgen pathways, and for present physical development/behaviors indicative of hyperandrogenicity. **Methods:** The Institutional Review Board of the Institute for Chronic Illnesses (Office for Human Research Protections, US Department of Health and Human Services IRB number: IRB00005375) approved the present study. Sixteen consecutive pre-pubertal age children ( $\leq 11$  years old; mean

$\pm$  SD:  $5.9 \pm 2.1$  years old) with previously diagnosed ASDs that presented to the Genetic Centers of America for outpatient care were evaluated. **Results:** Significantly ( $p < 0.01$ ) increased levels of serum/plasma dehydroepiandrosterone and serum total testosterone relative to the age- and sex-specific normal laboratory reference ranges were observed. Conversely, serum follicle-stimulating hormone levels were significantly ( $p < 0.01$ ) decreased. Plasma-reduced glutathione ( $p < 0.01$ ), plasma cysteine ( $p < 0.01$ ), plasma methionine ( $p < 0.01$ ), serum cystathionine ( $p < 0.05$ ), and serum homocysteine ( $p < 0.01$ ) were all significantly decreased. **Conclusion:** The results suggest a possible cyclical interaction between the methionine cycle-transsulfuration and androgen pathways in some children with ASDs.

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## Introduction

It has been estimated by the US Centers for Disease Control and Prevention that the prevalence of autism spectrum disorders (ASDs) is 1 in 300 children in the US [1]. ASDs are characterized by impairments in social relatedness and communication, repetitive behaviors, abnormal movement patterns, and sensory dysfunction [2]. In addition, autistic individuals have an increased preva-

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**Table 1.** A summary of clinical findings/behaviors of hyperandrogenicity among the children with autism spectrum disorders examined in the present study

Patient	Age years	Sex	Race	Clinical symptoms
1	8	M	Caucasian	Masturbation, growth spurt, aggressive behavior, body hair
2	6	M	Caucasian	Masturbation, Tanner stage 2.5, aggressive behavior, body hair
3	4	M	Hispanic	Masturbation
4	4	M	Caucasian	Masturbation, growth spurt
5	6	M	Hispanic	Masturbation, body hair, growth spurt
6	9	M	Caucasian	Facial hair, aggressive behavior, masturbation
7	3	M	Black	None
8	10	F	Caucasian	Body hair, facial hair, genital development, masturbation, growth spurt
9	3	M	Black	Body hair
10	6	M	Caucasian	Body hair, facial hair, genital development, interest in female sexual organs, growth spurt, masturbation
11	8	M	Caucasian	Masturbation, growth spurt, aggressive behavior
12	5	M	Caucasian	Aggressive behavior
13	7	M	Caucasian	Aggressive behavior, growth spurt
14	5	M	Caucasian	Aggressive behavior
15	7	M	Caucasian	Masturbation, interest in female sexual organs, aggressive behaviors, growth spurt
16	4	F	Black	Aggressive behavior, central pattern baldness

lence of gastrointestinal disease and dysbiosis [3], autoimmune disease [4], and mental retardation [5]. Autistic disorders also affect many more males than females, occurring at a ratio of at least 3:1.

It has previously been suggested as a medical hypothesis that some ASDs, in fact, may result from interactions between the methionine cycle-transsulfuration and androgen pathways [6]. A clinical and laboratory assessment of pre-pubertal age children with ASDs for metabolites in the methionine cycle-transsulfuration and androgen pathways and present physical development/behaviors indicative of hyperandrogenicity was undertaken in the present study.

## Materials and Methods

The Institutional Review Board of the Institute for Chronic Illnesses (Office for Human Research Protections, US Department of Health and Human Services IRB number: IRB00005375) approved the present study. In this study, consecutive pre-pubertal age children ( $\leq 11$  years old) with previously diagnosed regressive ASDs (Autism or Pervasive Developmental Delay – Not Otherwise Specified) [7] that presented from November 2004 to November 2005 to the Genetic Centers of America for outpatient care were evaluated. A total of 16 patients with ASDs were identified (mean  $\pm$  SD:  $5.9 \pm 2.1$  years old). These patients had been determined to be

negative for Rett's Syndrome, Angelman/Prader-Willi Syndrome, Fragile-X Syndrome, chromosomal abnormalities, chromosomal sub-telomere abnormalities, inherited metabolic abnormalities (LabCorp, Inc.). The patients had no brain structural abnormalities (CT or MRI head scans) or adrenal abnormalities (abdominal ultrasounds). The patients were also tested and determined to be negative for polychlorinated biphenyls/chlorinated pesticide exposure, thyroid function abnormalities, kidney function abnormalities, and liver function abnormalities (LabCorp, Inc.). A clinical examination was undertaken for each patient to evaluate clinical symptoms/behaviors of hyperandrogenicity such as early growth spurt, increased body and facial hair, aggressive behavior, and early secondary sexual changes. Children were tested for androgen metabolites including serum total testosterone, serum/plasma dehydroepiandrosterone (DHEA), and serum follicle-stimulating hormone levels (LabCorp, Inc.) and for methionine cycle-transsulfuration pathway metabolites including plasma methionine, serum cystathionine, serum homocysteine (LabCorp, Inc.), plasma cysteine, and plasma reduced glutathione (Great Smokies Diagnostic Laboratory).

### Statistical Analyses

The laboratory results observed for methionine cycle-transsulfuration and androgen pathways were assessed by determining the mean  $\pm$  standard deviation among the children with ASDs examined in the present study. These values were then compared to the laboratory specific reference values. The two-tailed t test statistic was employed to determine statistical significance and a p-value  $< 0.05$  was considered significant.

**Table 2.** A summary of serum FSH, serum total testosterone, serum/plasma DHEA levels measured in children with autism spectrum disorders examined in the present study

Patient	Age years	Sex	Serum FSH mIU/ml (ref. range)	Serum total testosterone, ng/dl (ref. range)	Serum/plasma DHEA, ng/dl (ref. range)
1	8	M	1.2 (0.2–2.7)	25 (0–25)	NA
2	6	M	0.6 (0.2–2.7)	20 (0–20)	NA
3	4	M	NA	20 (0–10)	NA
4	4	M	0.3 (0.2–2.7) <sup>a</sup>	13 (0–10)	120 (26–72)
5	5	M	NA	23 (0–10)	NA
6	9	M	0.3 (0.2–2.7) <sup>a</sup>	24 (0–25)	284 (53–135)
7	3	M	0.4 (0.2–2.7)	14 (0–10)	107 (26–72)
8	10	F	0.8 (0.4–5.0) <sup>b</sup>	27 (0–30)	251 (234–529)
9	3	M	0.7 (0.2–2.7)	19 (0–10)	85 (26–72)
10	5	M	0.3 (0.2–2.7) <sup>a</sup>	17 (0–10)	118 (29–66) <sup>b</sup>
11	8	M	0.3 (0.2–2.7)	10 (0–25) <sup>a</sup>	181 (53–135)
12	5	M	0.3 (0.2–2.7)	14 (0–10)	100 (26–72)
13	7	M	0.3 (0.2–2.7) <sup>a</sup>	23 (0–20)	148 (29–66)
14	5	M	0.3 (0.2–2.7) <sup>a</sup>	14 (0–10)	NA
15	7	M	0.4 (0.2–2.7)	24 (0–20)	67 (29–66)
16	4	F	1.2 (0.4–6.6)	5 (0–10) <sup>c</sup>	94 (19–42)
Mean % of normal	–	–	35	256	222
p value <sup>d</sup>	–	–	<0.01	<0.01	<0.01

<sup>a</sup> Level less than detectable by the laboratory, it was assumed in the present study to be equal to the lowest level measurable by the laboratory.

<sup>b</sup> Last female age-dependent reference range established by the laboratory for children 7–9 years old.

<sup>c</sup> Levels less than detectable by the laboratory, it was assumed in the present study to be equal to the average level from the laboratory reference range.

<sup>d</sup> The t test statistic was employed to determine statistical significance.

NA = Not available.

## Results

Table 1 summarizes evidence of clinical findings/behaviors of hyperandrogenicity in the children examined in the present study. It was observed that 15 of 16 children with ASDs examined (94%) had one or more clinical signs/symptoms of hyperandrogenicity including aggressive behavior, increased body hair and facial hair, early growth spurt, and early secondary sexual changes. Table 2 summarizes laboratory measured serum follicle-stimulating hormone, serum total testosterone, serum/plasma DHEA levels measured among the children examined. The children with ASDs examined had significantly increased levels of serum/plasma DHEA (mean = 222% of the laboratory normal reference,  $p < 0.01$ ) and serum total testosterone (mean = 256% of the laboratory normal reference,  $p < 0.01$ ) relative to the age- and sex-specific normal laboratory reference ranges; conversely, serum

follicle-stimulating hormone levels were significantly decreased (mean = 35% of the laboratory normal reference,  $p < 0.01$ ).

Table 3 summarizes the levels of plasma reduced glutathione (mean = 64% of the laboratory normal reference,  $p < 0.01$ ), plasma cysteine (mean = 81% of the laboratory normal reference,  $p < 0.01$ ), plasma methionine (mean = 56% of the laboratory normal reference,  $p < 0.01$ ), serum cystathionine (mean = 68% of the laboratory normal reference,  $p < 0.05$ ), and serum homocysteine (mean = 62% of the laboratory normal reference,  $p < 0.01$ ) observed in the children examined with ASDs. It was observed that there were significant reductions in the levels of each of the metabolites examined relative to their respective normal laboratory reference ranges.

**Table 3.** A summary of plasma reduced glutathione, plasma cysteine, plasma methionine, serum cystathione, and serum homocysteine observed in the children examined in the present study with autism spectrum disorders

Patient	Age years	Sex	Plasma reduced glutathione mg/dl (28–44)	Plasma cysteine mg/dl (2.70–4.30)	Serum cystathione, nmol/l (44–342)	Serum homocysteine, $\mu$ mol/l (5.1–13.9)	Plasma methionine $\mu$ mol/l (1.3–5.0)
1	9	M	20	2.72	83	5.0	1.7
2	6	M	20	2.58 <sup>a</sup>	NA	NA	NA
3	4	M	20	2.98	NA	NA	NA
4	4	M	18	2.47	NA	4.0 <sup>b</sup>	NA
5	5	M	28	2.79	NA	NA	NA
6	9	M	NA	NA	141	7.5	2.1
7	3	M	25	2.63	139	8.7	1.9
8	10	F	NA	NA	72	5.1	2.2
9	3	M	NA	NA	93	6.9	NA
10	3	M	27	3.09	111 <sup>c</sup>	5.1 <sup>c</sup>	NA
11	7	M	28	2.24	159 <sup>d</sup>	5.8 <sup>d</sup>	2.1 <sup>d</sup>
12	5	M	23	4.01	148	2.9	1.2
13	7	M	NA	NA	NA	NA	1.6
14	5	M	21	2.76	116	4.5	1.2
15	7	M	NA	NA	309	5.1	NA
16	7	F	NA	NA	88	9.9	1.9
Mean % of normal	–	–	64	81	68	62	56
p value <sup>e</sup>	–	–	<0.01	<0.01	<0.05	<0.01	<0.01

<sup>a</sup> The patient was 4 years old when this measurement was made.

<sup>b</sup> The patient was 2 years old when this measurement was made.

<sup>c</sup> The patient was 6 years old when this measurement was made.

<sup>d</sup> The patient was 6 years old when this measurement was made.

<sup>e</sup> The t test statistic was employed to determine statistical significance.

NA = Not available.

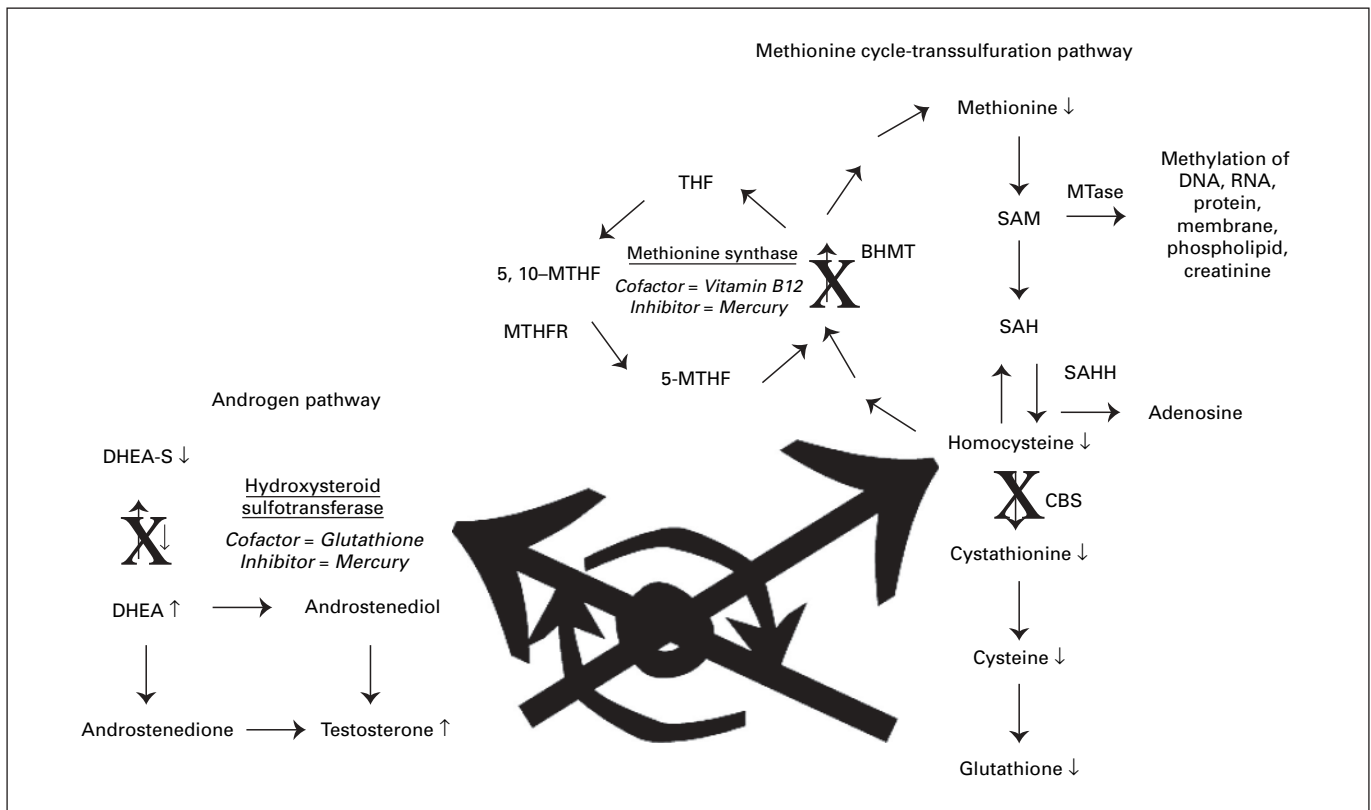
## Discussion

The results of the present study suggest that some children with ASDs may have significant biochemical abnormalities in their methionine cycle-transsulfuration and androgen pathways relative to normal children. Figure 1 summarizes the overall biochemical abnormalities observed in ASDs from the present clinical observations, and provides an overview for the hypothesized interaction between the methionine cycle-transsulfuration and androgen pathways.

In considering the distinctive abnormalities in the methionine cycle and transsulfuration pathways observed among children with ASDs, it was previously observed that some autistic children had significant decreases in their plasma levels of homocysteine, cystathionine, cysteine, and total glutathione in comparison to normal control children comparable to those observed in the present

study [8]. Additionally, it has been previously reported that autistic children had significant increases in their plasma levels of inactivated oxidized glutathione relative to controls. Overall, it was observed that there was an approximately 3-fold significant difference in the ratio of total glutathione to inactive oxidized glutathione in children with autistic disorders in comparison to normal children [8].

The observation of significant abnormalities in transsulfuration metabolites has a direct impact on the basis for the biochemical interaction between the transsulfuration and androgen pathways. The basis for the transsulfuration and androgen pathways to interact stems from the fact that a critical regulatory step in the androgen pathway involves the metabolite DHEA. DHEA is a key initial regulatory metabolite in the androgen synthesis pathway. It is at the DHEA location in the androgen synthesis pathway that, DHEA can either be converted further down the



**Fig. 1.** A summary of the clinical observations from the present study, and the hypothesized interactions between the methionine cycle-transsulfuration and androgen pathways. BHMT = Betaine homocysteine methyltransferase; CBS = cystathionine  $\beta$ -synthase; DHEA = dehydroepiandrosterone; DHEA-S = dehydroepiandrosterone-sulfate; 5-MTHF = 5-methyltetrahydrofolate; 5,10-MTHF = 5,10-methyltetrahydrofolate; MTase = methyltransferase; SAH = S-adenosylhomocysteine; SAHH = SAH hydrolase; SAM = S-adenosylmethionine; THF = tetrahydrofolate.

androgen pathway towards testosterone by being converted to androstenedione or androstenediol, or towards the normally favored storage molecule of DHEA-sulfate (DHEA-S). In examining the conversion step of DHEA to DHEA-S by the enzyme, hydroxysteroid sulfotransferase, this enzyme requires glutathione as a co-factor [9]. Since, some children with ASDs have significant decreases in their total glutathione levels and active reduced glutathione, and a significant increase in their inactive oxidized glutathione, there may be a marked shift toward DHEA, and subsequent metabolites in the androgen synthesis pathway.

The result, as demonstrated in the present study, is that some children with ASDs have significant increases in their levels of serum/plasma DHEA and serum total testosterone. In considering the results of the present study in the context of previous controlled clinical assessments of individuals with ASDs, it has recently been reported

that some autistics have significantly lower DHEA-S levels relative to controls [10]. It has been reported in a case series of male/female autistic children of pre- and pubertal ages that 33% had levels of plasma testosterone more than two standard deviations above the mean levels in age- and sex-matched controls [11]. It is also important to recognize that while significant increases in DHEA and testosterone have been observed in the autistic children examined in the present study, other androgen metabolites such as androstenediol, androstenedione, or dihydrotestosterone may be significantly elevated in some children with ASDs.

It would be expected since pre-pubertal children with ASDs were observed to have significant increases in androgen metabolites that significant increase in precocious puberty should be observed among some children with ASDs. In an assessment of an inpatient clinic of autistic children, it was previously observed that 33% showed

signs of precocious puberty [11]. Baron-Cohen et al. [12] have recently recognized that precocious puberty occurs among some boys with autistic disorders. Additionally, in epidemiological studies of children with neurodevelopmental disorders, it has been observed that children with neurodevelopmental disorders appeared to be more than 20-times more likely to have the diagnoses of precocious puberty than controls [13].

Furthermore, it has not only been shown that glutathione plays a critical role in the androgen synthesis pathway, but testosterone, and possibly other androgen metabolites, may have an influence on the methionine cycle-transsulfuration pathways. Giltay et al. [14, 15] in a series of animal studies have demonstrated that testosterone administration at least partially blocks the conversion of homocysteine to cystathionine, whereas estrogen administration had the opposite effect. Additionally, Vrbikova et al. [16] have shown in humans significant positive correlations between homocysteine and androstenedione levels and glutathione and DHEA-S levels. As a result, high testosterone levels, and possibly other androgen metabolites, would be expected to significantly reduce total glutathione levels, and cause a cyclical pattern of interaction to develop between the transsulfuration and androgen pathways.

In addition to the androgen block of the transsulfuration pathway at the homocysteine position, patients with ASDs may have a significant block in the methionine cycle at the homocysteine position. It was observed in the present study that the children examined with ASDs had significant reductions in their plasma methionine levels, and similar results have been previously published showing significant reduction in plasma methionine and S-adenosylmethionine levels in autistic children [8]. The basis for a block in methionine synthase, which recycles homocysteine to methionine, may stem from the fact that children with ASDs have been shown to have a significant increase in single nucleotide polymorphisms (SNPs) in the methylenetetrahydrofolate reductase (MTHFR) gene. It was reported that only 2% of autistic patients did not have at least one SNP in their MTHFR gene. The MTHFR gene is of significant importance in the folate pathway, and SNPs in the MTHFR gene have been shown to significantly reduce its functional capacity (i.e., 30–60%, depending upon the exact SNP pattern), and hence reduce the ability of methionine synthase to recycle homocysteine to methionine [17].

Some children with ASDs may have significant blocks in their transsulfuration and methionine cycle pathways. Children with ASDs are left with the primary functional

components of their transsulfuration-methionine cycle pathway that remain intact involving the enzyme S-adenosylhomocysteine hydrolase, which can reversibly convert S-adenosylhomocysteine to homocysteine and convert S-adenosylhomocysteine to adenosine. It has been previously reported that some autistic children have significantly elevated S-adenosylhomocysteine and adenosine levels in comparison to neurotypical controls [8]. It has been previously reported in another study that autistic children have significantly elevated homocysteine levels in comparison to controls [18] (i.e., both studies show a biochemical block isolating disruptions to the same location in the transsulfuration and methionine cycle pathways).

## Conclusion

The results of the present study suggest a new biochemical phenomenon that may involve the cyclical interaction between the methionine cycle-transsulfuration and androgen pathways in some children with ASDs. Furthermore, studies should also be undertaken to evaluate other cohorts in databases/registries, so as to compare the compatibility of the present results with other autistic populations.

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