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Dehydroepiandrosterone sulfate, free testosterone, and sex hormone-binding globulin on susceptibility to attention-deficit/hyperactivity disorder

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ABSTRACT

The neuroendocrine system may affect the pathophysiology of gender differences in attention deficit/hyperactivity disorder (ADHD). This study examines whether the relationships among dehydroepiandrosterone sulfate (DHEA-S), free testosterone, or sex hormone-binding globulin (SHBG) and ADHD presentations exhibit gender differences. A total of 113 boys and 35 girls with ADHD (all drug naïve) and 46 and 26 healthy control boys and girls, respectively, were recruited. Blood samples were obtained to measure the serum levels of DHEA-S, free testosterone, and SHBG in each child. The Swanson, Nolan, and Pelham Scale for ADHD Version IV (SNAP-IV) was used to evaluate behavioral symptoms and the Wechsler Intelligence Scale for Children–Fourth Edition (WISC-IV) and the Conners' Continuous Performance Test (CPT) were utilized to assess neurocognitive functions. Patients with ADHD had lower DHEA-S levels than male and female healthy control subjects, and no significant differences were observed in free testosterone and SHBG levels between the patients and the controls. DHEA-S levels were negatively correlated with children's impulsivity performance in the CPT. SHBG levels were negatively correlated with ADHD behavior symptoms among boys. Free testosterone levels were not significantly correlated with either ADHD clinical symptoms or neuropsychological functions. We propose that DHEA-S serves as a potential biomarker of ADHD and is consistently involved in the pathogenesis of ADHD in both boys and girls. SHBG may be involved in behaviors associated with ADHD in boys. Additional studies with basic scientific measures are warranted to elucidate the relationship between androgen hormones and clinical presentations of ADHD.

1. Introduction

Attention deficit/hyperactivity disorder (ADHD), which is characterized by inattention, impulsivity, and hyperactivity, is a common neurodevelopmental disorder (Polanczyk et al., 2007). Epidemiological investigations have estimated an ADHD prevalence of 3–7% among school-age children (Polanczyk et al., 2014), with a male-dominant prevalence (with a boy-to-girl prevalence ratio in the range 2:1 to 9:1) (Rucklidge, 2010). Children with ADHD have a high risk of developing psychiatric comorbidities later in life (Franke et al., 2018), making early identification of, and effective treatment for, ADHD critical (Johnson et al., 2015). While various potential ADHD biomarkers have already been established (Faraone et al., 2014; Scassellati et al., 2012), the exact etiology of ADHD is unknown (Thome et al., 2012). Observations of gender differences have led many researchers to question the potential role of the neuroendocrine system in ADHD etiology (Davies, 2014; Golubchik et al., 2007; Martel et al., 2009; Strous et al., 2006).

Dehydroepiandrosterone sulfate (DHEA-S), the sulfated form of dehydroepiandrosterone (DHEA), is the most abundant steroid in the human body (Maninger et al., 2009). DHEA-S is produced by the adrenal cortex and in the brain; is associated with a number of physiological processes; and performs many vital neuropsychiatric functions (Perez-Neri et al., 2008). A previous study has established that DHEA/DHEA-S is partially responsible for the clinical characteristics of ADHD (Scassellati et al., 2012). Additionally, several studies have revealed that DHEA/DHEA-S is negatively correlated with the clinical severity of ADHD (Isik et al., 2018; Strous et al., 2001; van Goozen et al., 2000; Wang et al., 2011b), and that drug therapy may up-regulate peripheral DHEA/DHEA-S levels in children with ADHD (Lee

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Table 1
Comparisons of demographic data and psychopathology evaluations in boys and girls among patients with ADHD and healthy controls.

	Boys		Girls		Statistics	
	ADHD (n = 113)	Control (n = 46)	ADHD (n = 35)	Control (n = 26)	F	*P*
Demographic data						
Age, years	9.1 ± 2.3	9.3 ± 2.4	8.6 ± 2.3	9.2 ± 1.9	0.638	0.592
Height, cm	135.7 ± 15.0	136.4 ± 14.6	130.7 ± 14.3	136.8 ± 13.2	1.318	0.270
Body weight, kg	35.7 ± 14.7	33.8 ± 11.1	32.2 ± 14.2	34.0 ± 10.9	0.686	0.561
ADHD subtypes					5.845 [a]	0.024
Inattentive type	33 (29.2)	–	18 (51.4)	–		
H/I or combined type	80 (70.8)	–	17 (48.6)	–		
Comorbidities						
ODD or conduct disorder	30 (26.8)	–	4 (11.4)	–	3.537 [a]	0.044
Tic disorders	16 (14.3)	–	1 (2.9)	–	3.406 [a]	0.052
WISC-IV						
Full Scale Intelligence Quotient	98.1 ± 10.7	108.9 ± 14.5	96.5 ± 10.4	107.9 ± 11.9	13.830	< 0.001
Verbal Comprehension Index	101.1 ± 11.5	107.8 ± 12.4	100.4 ± 10.8	104.2 ± 10.5	4.312	0.006
Perceptual Reasoning Index	99.7 ± 12.4	110.2 ± 17.0	95.0 ± 11.8	109.9 ± 16.2	11.862	< 0.001
Working Memory Index	99.9 ± 12.9	108.2 ± 12.5	97.2 ± 11.9	107.7 ± 11.5	8.376	< 0.001
Processing Speed Index	93.4 ± 9.6	100.5 ± 11.6	95.5 ± 8.3	103.0 ± 11.9	9.550	< 0.001
Conners' CPT						
Confidence Index	65.7 ± 22.8	55.2 ± 18.8	59.6 ± 21.6	34.6 ± 19.4	15.266	< 0.001
Omission	60.1 ± 20.7	53.5 ± 16.5	60.9 ± 14.2	49.1 ± 6.8	3.949	0.009
Commission	49.7 ± 9.8	43.9 ± 11.1	48.5 ± 8.7	49.9 ± 8.2	4.122	0.007
Hit Reaction Time	55.7 ± 14.3	58.1 ± 11.2	58.3 ± 8.4	52.2 ± 10.9	1.630	0.184
Hit RT SE	58.7 ± 12.5	53.3 ± 10.6	59.0 ± 9.4	50.2 ± 10.0	5.845	0.001
Variability	58.0 ± 11.6	53.9 ± 9.0	57.7 ± 8.7	50.2 ± 10.3	4.935	0.002
Detectability	52.0 ± 8.7	46.4 ± 11.9	49.5 ± 8.7	50.9 ± 8.3	3.963	0.009
SNAP-IV						
SNAP-IV parent form (I)	16.4 ± 5.7	6.3 ± 6.3	17.4 ± 5.0	5.8 ± 5.5	51.900	< 0.001
SNAP-IV parent form (H)	14.9 ± 6.6	5.2 ± 5.5	12.9 ± 5.5	4.1 ± 5.7	39.111	< 0.001
SNAP-IV parent form (O)	12.2 ± 6.1	5.7 ± 5.3	10.7 ± 5.8	5.0 ± 5.2	19.348	< 0.001
SNAP-IV teacher form (I)	15.3 ± 5.4	5.2 ± 5.6	13.3 ± 7.0	4.5 ± 3.5	48.514	< 0.001
SNAP-IV teacher form (H)	13.1 ± 6.7	3.6 ± 3.9	7.6 ± 5.9	2.3 ± 2.6	41.578	< 0.001
SNAP-IV teacher form (O)	9.6 ± 6.2	2.4 ± 3.7	4.7 ± 4.5	1.4 ± 1.6	30.777	< 0.001

Notes: Data are expressed as mean ± SD or n (%); [a] Pearson Chi-square; H/I, hyperactive/impulsive type; ODD, oppositional defiant disorder; SNAP-IV, the Swanson, Nolan, and Pelham–Version IV Scale for ADHD; WISC-IV, the Wechsler Intelligence Scale for Children–Fourth Edition; CPT, Conners' Continuous Performance Test; Hit RT SE, Hit Reaction Time Standard Error; I, inattention scores; H, hyperactivity/impulsivity scores; O, oppositional scores.

et al., 2008; Maayan et al., 2003; Wang et al., 2011a, 2014). Genetic studies have also demonstrated that the *STS* gene, which slices the sulfate moiety off of steroid sulfates, may be involved in the underlying biological pathogenesis of ADHD (Davies et al., 2014; Wang et al., 2017a).

Testosterone is the primary sex hormone that is generated by the testicles and adrenal glands and is responsible for the proper development of male sexual characteristics (Albin and Norjavaara, 2013). Most circulating testosterone, as well as other gonadal hormones, is bound to sex hormone-binding globulin (SHBG), while a lesser proportion is bound to albumin and a yet smaller proportion is present as free hormone (Hammar et al., 2018). The small amount that circulates as free testosterone is believed to be the metabolically active fraction (Shea et al., 2014). Some believe that testosterone potentially influences behaviors and physiology, as well as neurodevelopment (Nguyen et al., 2016b; Rice, 2015). Several investigations have demonstrated that the saliva and plasma of children with aggressive tendencies contains higher testosterone levels than those of children without aggressive tendencies (Pajer et al., 2006; Scerbo and Kolko, 1994). However, other investigations have not found a significant relationship between testosterone levels and children's disruptive behavior or ADHD (Dorn et al., 2009; Wang et al., 2017b,c). Evidence of the connection between testosterone levels and the pathogenesis of ADHD remains scarce and inconsistent.

SHBG is a protein that is produced by the liver and affects the bioavailability of sex hormones (Hammar et al., 2018). SHBG levels are highest at age five years and decline following the prepubertal stage of development (Jurewicz and Hanke, 2011). Recent evidence has shown that SHBG is not just synthesized peripherally, but also originates from the hypothalamus and pituitary in the brain (Goldstajn et al., 2016). Since SHBG is taken up into brain cells, whether SHBG affects only the delivery of steroids into neurons or also affects neurophysiology is undetermined (Caldwell et al., 2007). Moreover, SHBG is one of the mediators between endocrine mechanisms and chronic inflammation (Liao et al., 2012). Recent findings suggest that SHBG has potential pathophysiological involvement in various diseases and may serve as a useful clinical marker for these diseases, including metabolic syndrome, polycystic ovary syndrome and cancer (Goldstajn et al., 2016). However, whether SHBG independently influences brain function or neurodevelopment and is further associated with the susceptibility to ADHD, remains unclear.

To gain insight into the roles of DHEA-S, testosterone, and SHBG in the pathophysiology of ADHD, a cross-sectional study was carried out to determine whether the levels of these hormones differ between ADHD patients and healthy controls of each gender. The relationships among behavioral symptoms, neuropsychological functions, and the levels of aforementioned androgen hormones are examined.

2. Material and methods

2.1. Study participants

The Institutional Review Board at Chang Gung Hospital in Taiwan approved the research protocol. Patients with ADHD who were treated in the outpatient Department of Child Psychiatry at Chang Gung Children's Hospital in Taiwan were recruited for this cross-sectional study and obtained informed consent was obtained in writing from all participants or their guardians. The inclusion criteria were as follows; (a) clinical diagnosis of ADHD by a senior child psychiatrist based on the DSM-IV-TR and structured interviews using the Chinese version of

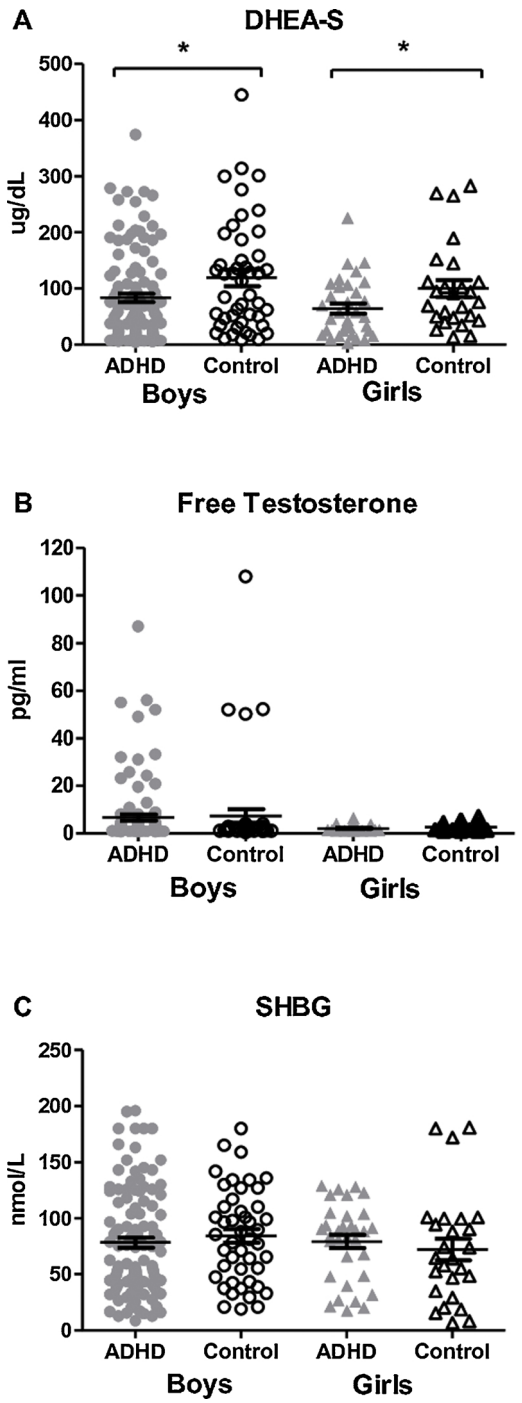

Fig. 1. Serum levels of dehydroepiandrosterone-sulfate (DHEA-S) **(A)**, free testosterone **(B),** and sex hormone binding globulin (SHBG) **(C)** in boys and girls with ADHD and healthy controls of the same sex * $p < 0.05$.

the Schedule for Affective Disorders and Schizophrenia for School-Age Children, epidemiologic version (K-SADS-E) (Kaufman et al., 1997); (b) between the ages of 6 and 16 years old; and (c) a drug-naïve patient or a patient with a current diagnosis but who had not taken ADHD medication for at least six months. Any patient with a history of comorbid pervasive developmental disorder, intellectual disability, major depressive disorder, bipolar disorder, psychosis, epilepsy, or brain injury, was excluded.

Children without ADHD or other psychiatric disorders (such as intellectual disabilities, autism spectrum disorder, bipolar disorders, major depressive disorders, psychotic disorders, substance dependence, epilepsy, or severe head trauma) were recruited from the same catchment area as healthy controls. Overall, a total of 113 boys and 35 girls with ADHD, 46 healthy control boys, and 26 healthy control girls, were recruited for this study.

2.2. Laboratory testing of androgen hormones levels

Blood samples were collected in the morning after participants had fasted overnight. DHEA-S serum levels were measured using ARCHITECT DHEA(S) Reagent Kit (8K2720) (sensitivity range: 0.2–961.1 μg/dL); free testosterone levels were measured using the IBL International Free Testosterone ELISA (DB52181) (sensitivity range: 0.25–100 pg/mL); and SHBG levels were measured using the Human Sex Hormone-binding Globulin ELISA Kit from Signalway Antibody LLC (EK1708) (sensitivity range: 7.8–500 nmol/l).

2.3. Clinical measurements

A senior psychiatrist interviewed ADHD patients and healthy control subjects using the K-SADS-E diagnostic tool. An experienced child psychologist performed neuropsychological assessments using the Wechsler Intelligence Scale for Children–Fourth Edition (WISC-IV) and the Conners' Continuous Performance Test (CPT). The Swanson, Nolan, and Pelham Version IV Scale (SNAP-IV) parent form and SNAP-IV teacher form were completed by the parents and a teacher of each patient, respectively.

The WISC-IV is an individually administered and norm-referenced test that was developed to evaluate the intelligence of children from 6 to 16 years old (Baron, 2005). The WISC-IV comprises ten core and five supplemental subtests. The core subtests generate values of the following four factor indexes; Verbal Comprehension Index (VCI), Perceptual Reasoning Index (PRI), Working Memory Index (WMI), and Processing Speed Index (PSI). The Full Scale Intelligence Quotient (FSIQ) is obtained using ten core subtests. Both the factor indices and the FSIQ have a population mean of 100 and a standard deviation of 15 (Baron, 2005).

The CPT is a 14 min computerized test that primarily evaluates attention and impulse control (Conners, 2004). Participants have to respond to the stimuli on a computer screen by pressing the space bar in response to every displayed letter, except the letter "X." The test includes a variety of assessment parameters, including Omissions, Commissions, Hit Reaction Time (RT), Hit Reaction Time Standard Error (Hit RT SE), Variability of Standard Error, and Detectability (D'). The Confidence Index (percentile) combines all data from the CPT data as a score out of 100 that represents the probability of a significant attention problem (Conners, 2004).

The SNAP-IV is a 26-item questionnaire that is used to evaluate ADHD symptoms and severity that needs to be completed by parents or teachers (Bussing et al., 2008). The 26 items comprise 18 associated with ADHD symptoms (nine for inattention and nine for hyperactivity/impulsivity) and eight associated with oppositional defiant disorder (ODD) symptoms, as defined by the DSM-IV. Each item is scored from 0 to 3 on a Likert scale. In this study, only the inattention and hyperactivity/impulsivity scores are analyzed.

The pubertal stages of the subjects were identified using the Tanner scale (Marshall and Tanner, 1969, 1970). The Tanner scale (Tanner stages) is a scale of physical development that is based on the morphology of sexual features.

2.4. Statistical analysis

Data were analyzed using the statistical software package SPSS version 21.0 (SPSS Inc., Chicago, IL, USA) and variables were presented as either mean (standard deviation) or frequency. Two-tailed p-values of < 0.05 were considered to indicate statistical significance.

One-way analysis of variance (ANOVA) was used to compare

Table 2
Factor loading scores of variables and their related principal components identified from nutritional status and dietary indexes.[a,b]

Variables	Behavior Symptoms	CPT Inattention	Intelligence Quotient	CPT Impulsivity
WISC-IV				
Full Scale Intelligence Quotient	−0.136	−0.076	**0.978**	−0.062
Verbal Comprehension Index	0.044	0.144	**0.773**	−0.048
Perceptual Reasoning Index	−0.158	−0.134	**0.795**	−0.054
Working Memory Index	−0.113	−0.098	**0.772**	−0.179
Processing Speed Index	−0.175	−0.158	**0.638**	0.105
Conners' CPT				
Confidence Index	0.259	**0.879**	−0.033	0.016
Omission	0.129	**0.824**	−0.073	0.054
Commission	0.056	−0.022	−0.128	**0.926**
Hit Reaction Time	−0.117	**0.721**	−0.056	−0.436
Hit RT SE	0.141	**0.928**	−0.105	0.081
Variability	0.121	**0.859**	−0.069	0.218
Detectability	0.068	0.168	−0.054	**0.917**
SNAP-IV				
SNAP-IV parent form (I)	**0.729**	0.193	−0.277	0.021
SNAP-IV parent form (H)	**0.817**	0.228	−0.136	0.018
SNAP-IV parent form (O)	**0.735**	0.213	−0.074	0.023
SNAP-IV teacher form (I)	**0.781**	0.031	−0.242	0.035
SNAP-IV teacher form (H)	**0.853**	−0.015	−0.005	0.072
SNAP-IV teacher form (O)	**0.830**	0.008	0.040	0.054

[a]Rotation method was Varimax with Kaiser Normalization. [b] Variables (boldface) with factor loading score more than 0.50 are regarded as main contributors to components and served as further analysis. SNAP-IV, the Swanson, Nolan, and Pelham–Version IV Scale for ADHD; WISC-IV, the Wechsler Intelligence Scale for Children–Fourth Edition; CPT, Conners' Continuous Performance Test; Hit RT SE, Hit Reaction Time Standard Error; I, inattention scores; H, hyperactivity/impulsivity scores; O, oppositional scores.

Table 3
Correlations between serum levels of androgen hormones and participants' characteristics, separated by gender.

Variables	Behavior Symptoms		CPT Inattention		Intelligence Quotient		CPT Impulsivity	
	Male	Female	Male	Female	Male	Female	Male	Female
DHEA-S	−0.663*	−0.186	0.416	−0.012	0.396	−0.145	−0.678*	−0.946*
Free testosterone	−0.282	0.060	0.257	−0.564	−0.095	−1.975	0.296	0.842
SHBG	−0.871*	−0.022	0.056	−0.364	−0.208	−1.254	−0.153	0.183

Note: Data are expressed as correlation coefficient in the General Linear Model, controlling for age; DHEA-S: dehydroepiandrosterone-sulphate; SHBG, Sex hormone-binding globulin. *$p < 0.05$.

continuous variables among the boys and girls with ADHD and the healthy control boys and girls. DHEA-S, free testosterone, and SHBG levels all exhibited significant positive skewness. Arithmetic log transformations were used to obtain approximate normal distributions of testosterone levels.

To determine the interrelations among a large number of clinical variables, principal component analysis (PCA) was used to identify latent components from variables that were significantly associated with ADHD. Varimax rotation was used and orthogonal components with an eigenvalue of greater than 1.0 were chosen. Loading scores were used to weigh the selected variables associated with each component to generate a factor score, and variables with loading scores of over 0.50 were identified as the major contributors to the components.

The General Linear Model (GLM) was also employed to evaluate the effects of DHEA-S, free testosterone, and SHBG levels on each factor that was identified using PCA. To identify potential sex differences in the relationships between hormones and clinical variables, the GLM model was applied separately to the boys and girls, controlling for the confounding effect of age. The four factors that were identified from the aforementioned PCA were set as dependent variables and androgen levels and age were set as independent variables.

3. Results

The study sample included 113 boys with ADHD (mean age: 9.1 years), 35 girls with ADHD (mean age: 8.6 years), 46 healthy control boys (mean age: 9.3 years), and 26 healthy control girls (mean age: 9.2 years). No significant differences with respect to age, height, or weight were found among the four groups (Table 1). The healthy controls outperformed the ADHD groups with respect to all indexes of the WISC-IV. The ADHD groups demonstrated underperformed the control groups with respect to all indices of CPT, with the exception of the HitRT. In all of the dimensions of clinical ADHD symptoms (parent-rated and teacher-rated inattention scores, hyperactivity/impulsivity scores, and oppositional scores of the SNAP-IV), the ADHD groups exhibited greater severity than the control groups.

Fig. 1 displays the serum levels of DHEA-S, free testosterone, and SHBG of the four participant groups. Boys with ADHD had lower DHEA-S levels than the healthy control boys (ADHD: 83.2 ± 80.0 ug/dL vs. Controls: 118.8 ± 99.2 ug/dL, $p = 0.016$). Likewise, girls with ADHD had lower DHEA-S levels (ADHD: 63.9 ± 52.0 ug/dL vs. Controls: 99.8 ± 76.4 ug/dL, $p = 0.024$) than the healthy control girls. However, no significant variation with regard to free testosterone or SHBG was observed among the groups. A sensitivity test (excluding the 19 participants whose Tanner stages were II or above) was conducted and the results are displayed in **Supplementary** Fig. 1. Patients with ADHD, both boys ($p = 0.023$) and girls ($p = 0.021$), had lower DHEA-S levels than the healthy control boys and girls. No significant difference with respect to free testosterone or SHBG level was observed between ADHD patients and the controls.

The correlations between DHEA-S level and SHBG level (r = -0.533, $p < 0.001$) and between free testosterone level and SHBG level (r = -0.352, $p < 0.001$) were both statistically significant. Controlled for age and sex, the serum levels of DHEA-S, free testosterone, and SHBG

were not affected by ADHD subtype (ADHD inattentive type vs. hyperactive/impulsive or combined type), comorbidity of oppositional defiant disorder (ODD), conduct disorder or tic disorders, or the season of blood sample collection (**Supplementary** Table 1).

Table 2 provides the weights of the four symptoms factors that yielded eigenvalues of greater than 1.00 and were thus retained for PCA. The resulting four factors were labeled according to their component. Factor 1 primarily comprised SNAP-IV scores and was labeled "Behavior Symptoms". Factor 2 contained the omission-related scores in the CPT and was labeled "CPT Inattention". The main component of Factor 3 was the sub-score of the WISC-IV and was labeled "Intelligence Quotient". Factor 4 consisted of commission-related CPT performance and was labeled "CPT Impulsivity".

Table 3 summarizes the relationships between androgen levels, neuropsychological functions, and ADHD clinical symptoms separately for boys (N = 159) and girls (n = 61). Among boys, the confounding effect of age was controlled for and DHEA-S level was found to be negatively correlated with Behavior Symptoms (B = -0.663, p = 0.013) and CPT Impulsivity (B = -0.678, p = 0.017). SHBG level was negatively correlated with Behavior Symptoms (B = -0.871, p = 0.028). Among girls, DHEA-S level was negatively correlated with CPT Impulsivity (B = -0.946, p = 0.006). The level of free testosterone exhibited no significant relationship with either ADHD clinical symptoms or neuropsychological functions in either gender.

4. Discussion

The findings herein of this investigation provide new insights into the effect of neuroendocrine on susceptibility to ADHD. First, sex hormones may have sex-specific neuropsychiatric effects. This current study examined whether levels of androgen-related hormones differ between ADHD patients and healthy controls of the same sex. Second, this study is the first case-control study of the link between SHBG and ADHD. Third, the relationship between androgen hormone levels and the full range of characteristics of ADHD with respect to behavioral symptoms as observed by parents and teachers, neuropsychological test (CPT) performance, and cognitive function (WISC-IV).

The primary finding of this study is that patients with ADHD had lower DHEA-S serum levels than healthy control subjects - a phenomenon consistently observed in both boys and girls. DHEA-S levels were negatively correlated with Behavior Symptoms and CPT Impulsivity. Previous investigations have indicated that DHEA/DHEA-S levels are negatively correlated with ADHD symptom severity (Golubchik et al., 2007; Isik et al., 2018; Strous et al., 2001; Wang et al., 2011b). The protective effects of DHEA/DHEA-S on patients' psychopathology may involve regulating $GABA_A$ and NMDA activity (Golubchik et al., 2007; Rahmani et al., 2013) or modulating the complex process of cortical maturation during middle childhood (Nguyen et al., 2016a, 2013). DHEA-S also has neuroprotective effects and can facilitate neurite growth such as by increasing the number of neurons and the length of neuritis (Maninger et al., 2009), improving neuronal survival (Brewer et al., 2001), and stimulating catecholamine synthesis and secretion (Ziegler et al., 2008). Animal models have also demonstrated that *STS* gene deficiency, which is related to androgen synthesis and metabolism, may cause ADHD-like behavioral features of neurodevelopmental disorders (Trent and Davies, 2012; Trent et al., 2013). Together with the findings herein, these findings support the claim that circulating DHEA-S levels are associated with ADHD susceptibility.

We previously demonstrated that peripheral DHEA levels (desulfated form of DHEA-S) are lower in ADHD children than in healthy controls (Wang et al., 2011b). DHEA exhibits neuroprotective and antiglucocorticoid activity and modifies immune reactions. DHEA can cross the blood-brain-barrier and is actively carried out of the central nervous system (CNS) (Starka et al., 2015). However, both DHEA and DHEA-S exhibit biological activities that may have critical roles in promoting neurodevelopment (Golubchik et al., 2007). The level of DHEA/DHEA-S increases throughout prepubertal to pubertal stages to an extent that depends on sex (Kim et al., 2016), and DHEA has sex-specific effects on glucose metabolism in the CNS (Vieira-Marques et al., 2017). Our findings reveal that DHEA-S, and not only DHEA, may be a protective factor of ADHD, regardless of a patient's gender.

Free testosterone levels did not differ significantly between the ADHD and control groups, and testosterone was not significantly involved in the behavioral symptoms or neuropsychological effects of ADHD. Some researchers have identified a significant positive correlation between testosterone levels and a tendency toward disruptive behavior disorders (Pajer et al., 2006; Scerbo and Kolko, 1994). Testicular hormones are generally considered to provide resiliency against chronic stress in males (Wainwright et al., 2011). However, one investigation did not find a close relationship between testosterone and disruptive behavior disorders (Dorn et al., 2009). Our previous studies also revealed no connection between testosterone level and ADHD pathogenesis (Wang et al., 2017b,c). Testosterone may be implicated in dominant behavior and changes in muscular physiology but it does not necessarily contribute to ADHD or external behavioral problems (Nguyen et al., 2016b; Rice, 2015).

To the best of our knowledge, this investigation is the first to examine the association between SHBG and ADHD. SHBG level was negatively correlated herein with both DHEA-S level and free testosterone level. In humans, the key physiological function of SHBG is the prevention of the accumulation of excessive amounts of endogenous or exogenous androgen (Laurent et al., 2016). Based on the "free hormone hypothesis", the concentrations of free forms of androgen and estrogen are thought to be determined by SHBG (Bhasin et al., 2011). Furthermore, SHBG levels were observed herein to be negatively correlated with Behavior Symptoms of ADHD, even when the confounding effect of age was controlled for. New findings suggest that SHBG is not just a peripherally synthesized protein but it also originates from the hypothalamus and the pituitary in the brain (Goldstajn et al., 2016). SHBG was also found not only to regulate gonadal hormones but also to influence children's ADHD-related behaviors. Although the underlying mechanism warrants further investigation, we hypothesize that SHBG plays a role in neurodevelopment and may be a useful assessment marker.

With age and sex controlled for, the serum levels of DHEA-S, free testosterone, and SHBG were not related to ADHD subtype, comorbidity of oppositional defiant disorder or tic disorders, or the season of blood sample collection (**Supplementary** Table 1). Previous studies have indicated that neurosteroids have a role in the pathophysiology of disruptive behavior disorders and ADHD comorbidity (Dorn et al., 2009; Isik et al., 2018; Molina-Carballo et al., 2014). However, consistent with our earlier investigation (Wang et al., 2017b, 2011b), no such correlation among ADHD subtype, comorbidity and androgen hormone level was observed. Notably, ADHD hyperactive-impulsive/combined types or comorbidities of disruptive behavior disorders are male-dominant, and the risk of comorbidity increases as patients grow older (Taurines et al., 2010). Therefore, whether androgen hormone levels are related to heterogeneous characteristics of ADHD patients requires clarification. Moreover, Taiwan is located in the subtropical region, and seasonal variation may be too small to affect hormone levels.

This study has certain limitations. First, its cross-sectional nature and the single measurement of androgen hormone levels do not suffice to establish a definite causal relationship. For example, this cross-sectional survey would not have been able to discover ay effect of androgen hormones on brain development in a critical period in early childhood. Second, androgen hormone levels in serum samples did not necessarily reflect action in the brain. Third, some potentially confounding variables, such as socio-economic status and other environmental variables, were not evaluated, and these ought to be controlled in future studies. Fourth, the age range of the study sample was wide (6–16 years) and some of the participants may have been in the pubertal stage. While an attempt was made to eliminate the confounding

effect of age by setting it as a covariate in the GLM and performing an additional sensitivity analysis, age may still have confounded the results. Finally, the numbers of boy and girl cases were unequal, reducing the statistical power of the results, and thereby reducing the likelihood of finding a potential difference in androgen hormone levels between ADHD and controls - particularly females.

5. Conclusions

We suggest that DHEA-S is a potential biomarker of ADHD, and may be consistently involved in the pathogenesis of ADHD in both boys and girls. Moreover, SHBG may affect ADHD behavioral characteristics in boys. However, free testosterone level was not clearly related to susceptibility to ADHD. Further studies using basic scientific methods must be carried out to clarify the relationships between androgen hormones and ADHD clinical presentations.

Contributors

LJW participated in interpreting data, reviewing references, and drafting the manuscript. MCC and YSL participated in data collection and patient recruitment. WJC and MJL are co-corresponding author and contribute equally to this manuscript. WJC and MJL participated in protocol development and revised the manuscript. All authors read and approved the final manuscript and contributed to the drafting and revising of the paper.

Conflict of interest

All authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.psyneuen.2019.01.025.

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