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Comparing Serum AMH, InhB, Testosterone Levels and Finger Length Ratio (2D/4D) of Male Children with Specific Learning Disorder and Controls

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ABSTRACT

Introduction: It has been suggested that inhibin B (InhB), Anti-Müllerian hormone (Müllerian-inhibiting substance, AMH) levels, and 2D/4D finger length ratios are related to sex differences in neurodevelopmental disorders. The aim of this study is to investigate the role of InhB, AMH levels, and 2D/4D finger length ratios in male children with specific learning disorder (SLD).

Methods: The study included 38 male children diagnosed with SLD and 38 males of similar ages without SLD as the control group. Tests used in the evaluation were the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children-Present and Lifetime version, Specific learning disorder clinical observation battery, Wechsler Intelligence Scale for Children-Revised (WISC-R), and Conners' Parent Rating Scale-Revised: Short Form. Serum AMH, InhB, and Testosterone levels were measured using an enzyme-linked immunosorbent assay.

Results: Male children diagnosed with SLD demonstrated significantly higher levels of serum InhB compared to controls (t= 2.59 p=0.009); both groups had similar levels of serum testosterone and AMH. The 2D/4D finger ratios in the SLD group were found to be lower than those in the control group (t= 2.92 p= 0.005). Serum InhB levels were positively correlated with WISC-R verbal scores (p= 0.003).

Conclusion: Our findings suggest that serum InhB levels and the 2D/4D ratio, which is an indicator of prenatal testosterone exposure, may play a role in the male predominance of SLD.

Keywords: Anti-müllerian hormone/mullerian-inhibiting substance/AMH, inhB/inhibin B, male dominance, specific learning disorder, 2D/4D finger length ratio

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INTRODUCTION

Specific Learning Disorder (SLD) is a neurodevelopmental disorder characterized by an ongoing difficulty in the ability to learn or use one or more fundamental academic skills, including reading, writing, and mathematics (1). A number of genetic and environmental risk factors play role in the etiology of SLD (2). The prevalence of SLD has been reported to be 6-12% based on different methodological approaches (3). SLD is three times more prevalent among males, and this male predominance is more prominent in the areas of reading and writing (4,5). Rutter et al reported the results of four epidemiological studies, all of which showed a greater prevalence of reading disability in boys than girls (5).

Highlights

- **Serum Inhibin B levels are higher in male children with specific learning disorder.**
- **In male children with specific learning disorder, 2D/4D finger length ratio is lower.**
- **Inhibin B and prenatal testosterone may contribute to specific learning disorders.**

In addition, despite the growing interest in the scientific literature regarding the etiology of male dominance in autism and attention deficit hyperactivity disorder (ADHD) (6-8), there is insufficient research on the etiology of male dominance in SLD. The etiological components of male dominance in neurodevelopmental disorders have mostly been attributed to genetic factors pertaining to sex and hormonal factors associated with sex differentiation (9). Hormonal factors have been shown to play a significant role in neurodevelopment in numerous studies (10). Specifically, research examining the relationship between fetal testosterone with neurodevelopmental disorders is frequently encountered in the literature. This concept is supported by the current hypothesis that prenatal androgen exposure may be associated with the occurrence of neurodevelopmental disorders (11-14).

Since the genes involved in the etiology of SLD typically display an autosomal inheritance, the etiology of male dominance in SLD can be

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better explained by hormonal factors as opposed to genetic factors. This suggests that the male dominance observed in SLD is associated with sex-specific hormonal factors, which may influence the modulation of this pathology (15). High levels of intrauterine testosterone are linked to atypical cerebral lateralization (16). Furthermore, atypical cerebral lateralization is frequently observed in SLD, which suggests that prenatal fetal testosterone may play a role in the etiology of SLD. In summary, boys exposed to higher levels of fetal testosterone may exhibit right hemisphere dominance, which could be attributed to delays in left hemisphere development. These hemispheric differences may have a potential link to dyslexia (17). Although many studies have found it difficult to precisely determine fetal testosterone levels during early-stage development, the 2D/4D ratio, (second finger length/ fourth finger length), is accepted in the literature as a specific indicator of fetal testosterone levels throughout life (18).

During development of male fetus and boys, the secretion profile of testicular hormones undergoes variations, with testosterone, anti-Müllerian hormone (AMH) and inhibin B (InhB) being the primary secretions for extended periods (6). AMH hormones trigger regression of the uterine precursor while inhibin B contributes to the control of spermatogenesis. Testosterone is a key regulator for the development of a normotipical male phenotype. This male dominance could be associated with the release of testosterone, AMH and Inhibin B, as their levels in boys might be linked to their developmental path. A significant part of brain development stretches into the postpartum period when testosterone levels are minimal (19). In this regard, various studies have also mentioned other hormonal factors that may play a role in brain development; AMH and InhB are particularly notable (20). AMH and inhibin B are family members of TGF-B and the crucial regulators of SMAD proteins in neural development (6). In addition, there are studies in the literature showing that AMH receptors may have an effect on the size and function of the cerebellum, which is involved in the etiology of dyslexia (21).

To our knowledge, there is currently no study that has investigated the potential role of AMH and InhB in the etiology of SLD, a neurodevelopmental disorder. Identification of biomarkers that contribute to the male dominance in SLD may possibly contribute to the understanding of the etiopathogenesis of this disorder. Indeed, a significant sex difference in the prevalence of a disorder can provide valuable insights into its underlying etiology or pathogenesis. The purpose of this study is to determine the role of free testosterone, InhB, AMH levels, and 2D/4D finger length ratios in male children with SLD by comparing them with healthy controls.

The present research addresses the following inquiries:

- Does high intrauterine testosterone play a role in male dominance of SLD?
- Is there a relationship between serum testosterone level and male dominance of SLD?
- What can be the potential role of AMH and InhB in the etiology of male dominance in SLD?

METHOD

Setting and Design

The study was conducted between January 2020 and July 2020 at the Child and Adolescent Psychiatry outpatient clinic of Istanbul University, Istanbul Faculty of Medicine.

The relationship between sex hormones, finger length ratios and specific learning disorders was determined using a case-control design. This was a cross-sectional analysis of hormone levels and finger length ratios in boys who had already developed learning disorders.

Sample

The case group in our study consisted of 38 volunteer male children aged 7–12 who applied to our clinic and were diagnosed with a specific learning disorder according to DSM-5 diagnostic criteria and the Specific Learning Disorder Clinical Observation Battery. The control group included 38 volunteer male children without SLD who applied to the hospital for routine medical checkups and were similar in terms of age and class. SLD was excluded from the control group through DSM-5 diagnostic criteria and the SLD battery.

The children included in the case and control groups were enrolled in this study after the study was fully explained to them, and verbal consent was obtained from the children, while written consent was obtained from their parents.

Our control group was selected from healthy children who did not have any chronic disease and no history of upper respiratory tract infection in the last 1 month and who came to the hospital for control visits to different departments for a one-year routine.

The study's exclusion criteria for both groups: a) having a diagnosis of any metabolic, genetic, or progressive neurological disease, b) having a diagnosis of ADHD, autism spectrum disorder (ASD), tic disorder, or an IQ score below 80, c) having impaired hearing/eyesight, d) having a history of infection within the last month, e) receiving routine medication for a chronic medical disease or psychiatric disorder, f) failure to complete examinations/evaluations or withdrawal from the study and, g) being female (To avoid the physiological effect of puberty and/or menstrual cycle-dependent variations in hormonal profile, females were excluded.)

Study Course Overview

Of the patients who applied to our clinic, a DSM-5-based diagnostic interview was conducted and 90 SLD cases were initially considered. ADHD was present in 35 cases, language disorder in 20, and intellectual disability or borderline mental capacity in 18; these patients were excluded from the study. There was cases that presented with multiple diagnoses. After excluding 52 children based on exclusion criteria, the final case group consisted of 38 children with SLD.

The patients were initially evaluated by a member of the research team through the Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version (K-SADS-PL) and subsequently evaluated by an experienced psychologist using the Wechsler Intelligence Scale for Children - Revised Version (WISC-R) and the Specific Learning Disorder Clinical Observation Battery. The parents were then requested to fill out the Conners' Parent Rating Scale - Revised Short Form (CPRS-RS). Five cases were diagnosed with ADHD following the K-SADS-PL evaluation, four cases yielded SLD battery results inconsistent with the SLD diagnosis, another four cases received IQ scores below 80 on the WISC-R, and four other cases could not successfully give blood; these cases were excluded from the study and the remaining 38 were included as the case group.

The control group was selected from patients who applied to the hospital for routine medical checkups and were similar in terms of age and class. Some participants were excluded from the study following the initial DSM-5-based diagnostic interview; these included seven children with ADHD, three with language disorder, one with borderline mental capacity, and four with SLD. Afterward, two participants were diagnosed with ADHD via the K-SADS-PL, the SLD battery results were consistent with SLD for others two individuals received IQ scores below 80 on the WISC-R, and two could not successfully give blood; these participants were refused from the study, and 38 healthy participants were included in the control group.

The parents of both cases and controls gave written and verbal consent, and the subjects gave verbal assent for publication. This study was approved by the Istanbul University, Istanbul of Medicine Clinical Research Ethics Committee (numbered 2020/215) and all procedures were in accordance with the Declaration of Helsinki. Also, it was supported by the Istanbul University Scientific Research Projects Commission (numbered 2020/8).

Scales Used During the Study

Kiddie Schedule for Affective Disorders and Schizophrenia for School-Age Children - Present and Lifetime Version, DSM-5 Turkish Adaptation (K-SADS-PL-DSM-5-T)

This is a semi-structured interview chart used to evaluate psychiatric signs in children and adolescents determining past and present psychopathology. The Schedule for Affective Disorders and Schizophrenia was originally developed by Chambers et al (1985) and was later adapted by Kaufman et al as a "Present and Lifetime Version" according to DSM-III and DSM-IV diagnostic criteria (22). Unal et al conducted a reliability and validity study of the latest DSM-5version adapted to the Turkish population (23).

Specific Learning Disorder Clinical Observation Battery

This battery was developed through the addition of recent sub-tests to the original SLD battery developed by Korkmazlar (1992). It consists of nine sub-tests that involve mathematics, Gesell developmental assessments, writing, clock drawing, right-left discrimination, lateralization, judgments regarding priority, and sequence tests (24). Each sub-test of the battery can be interpreed as "risk factor" or "not a risk factor". Reading speed was determined by dividing the individual's words read per minute by the expected age-wise words read per minute.

Conners' Parent Rating Scale-Revised Short Form (CPRS-RS)

Conners rating scales are used to identify ADHD symptoms as well as several other types of problematic behavior in children and adolescents. The CPRS-RS consists of 27 questions aimed at parents to evaluate their children (25). This scale consists of additional sub-scales pertaining to oppositional defiance (OD), cognitive problems/inattention (CP-I), hyperactivity (HA), and the total score (TS). The scale's Turkish standardization study was conducted by Kaner et al (25).

Wechsler Intelligence Scale for Children - Revised (WISC-R)

This scale was developed by Wechsler to measure IQ levels in children aged 6–16 years. WISC-R is a version of WISC revised in 1974 and Savasir and Sahin (1995) standardized the scale for its use in evaluating Turkish children (26).

Collection and Storage of Samples and Assessment of Serum Levels

Both the case group and the control group fasted for 12 hours and subsequently gave venous blood samples between 8:00 and 10:00 in the morning. Participants were also notified to avoid heavy exercise, eating, and drinking prior to sampling. Samples of venous blood were 5–8 ml and stored in biochemistry tubes. The biochemistry tubes were centrifuged, and the serum specimens were kept at -80°C until assayed. Routine biochemical parameters and testosterone and AMH levels were measured with the Cobas e602 autoanalyzer in the Central Laboratory of Istanbul Medical Faculty. InhB levels were measured using ELISA (Bioassay Technology Laboratory, Shanghai, China) at the Medical Biochemistry Department of Istanbul Medical Faculty. The operating range and the sensitivity of the kit were 5–1000 and 2.52 pg/mL, respectively.

Anthropometric Measurements of the Hand

Finger lengths were measured using photocopies of the hand's palmar surface (27). The 2nd finger was measured, on the palmar surface, as the distance between the tip of the index finger and the middle of the proximal crease, where the finger joins the palm. The 4th finger was measured, on the palmar surface, according to the distance between the tip of the ring finger and the middle of the proximal crease, where the finger joins the palm (28). To calculate the ratio of the 2nd finger to the 4th finger, the 2D/4D formula was used to divide the length of the 2nd finger by the length of the 4th finger. In the anthropometric measurements of the hand, a STAINLESS brand digital caliper with 0.01 mm precision was used by two separate researchers taking independent measurements, and the average of the two evaluations was considered.

Statistical Analysis

Study data were analyzed using the IBM Statistical Package for Social Sciences (SPSS) program, version 21.0, for Windows. Descriptive data were expressed as mean, standard deviation (SD), frequency distribution, and percentage. Chi-squared and Fisher's exact tests were used to compare categorical data. Depending on the characteristics of distribution, continuous variables were compared using the Mann–Whitney U test or a t-test for independent groups. When evaluating the correlation between parameters, both Pearson and Spearman correlation analyses were used in variables with and without normal distribution, respectively. A p-value below 0.05 was accepted as statistically significant. A power analysis conducted by the Biostatistics Department of the Istanbul Medical Faculty of Medicine by using a two-group independent t-test with the power of 80%, α=0.05 two-sided significance level based upon results from Pankhurst et al, resulted that at least 256 children should be included in this study (18). Unfortunately, we only recruited 76 participants, rendering a power of 70%.

RESULTS

The case and control groups were entirely male. Each group consisted of 38 participants. The mean age of the control group was 8.76±1.01, while the mean age of the case group was 9.16±1.39 (t [90]=-1.422; p=0.159). The birth weight distribution of the case group was 34.2% (n=13) below 2500 grams and 65.8% (n=25) above 2500 grams. Of the control group participants, 18.4% (n=7) were below 2500 grams at birth, and 81.6% (n=31) were above 2500 grams (χ^2=2.44; p: 0.11). When comparing the two groups' gestational ages, 2.6% of both the control group (n=1) and the case group (n=1) were premature (χ^2=0.00; p: 1). The median (25–75%) BMI (Body Mass Index) was 17.64 kg/m^2 (15.77–19.73) for the case group and 17.99 (15.92–20.46) for the control group (z=-0.30; p: 0.76). Preterm birth and low birth weight are confounding factors that increase the risk of developmental problems. Also, BMI and age are confounding factors that can have an impact on the AMH and InhB levels. There was no statistically significant difference between groups in any of these confounding factors. Evaluation with the K-SADS-PL-T showed that 42.6% (n=16) of the case group had accompanying psychopathology. Thirteen cases had at least one anxiety disorder, and four cases had a diagnosis of enuresis. As determined by the K-SADS-PL-T, 28.9% (n=11) of the control group had at least one mental disorder. Ten cases had one or more anxiety disorders, and one had enuresis. The WISC-R total IQ score was found to be lower in the case group (MV: 94.89±11.38) than in the control group (OD: 107.76±8.47) (p<0.001). Likewise, the SLD group had lower WISC-R performance scores (OD: 102.11±14.27) in comparison to the control group (OD: 109.74±11.50) (p=0.017). The case group had significantly higher levels of InhB compared to the control group (p=0.009; t=-2.59). Both groups were similar in terms of serum-free testosterone (z=-0.81; p=0.42) and AMH (z=1.25; p=0.80)

Table 1. Comparison of blood values between the patient group and the control group

	SLD (n: 38)	Control (n: 38)		
	Median (25%-75)	**Median (25%-75)**	**z**	**p**
Free Testosterone	0.11 (0.08–0.15)	0.13 (0.09±0.17)	-0.81	0.42
INHIBIN B	269.58 (206.66–370.65)	207.94 (174.14–257.39)	-2.59	0.009
	Mean ± SD	**Mean ± SD**	**t**	**p***
AMH	94.47±37.77	96.36±27.25	0.25	0.80

P: Mann-Whitney U test p-value; p*: Students t-test p-value.

Table 2. Comparison of right-left 2D/4D and hand preference between the patient group and the control group

	SLD (n: 38)	Control (n: 38)		
	Mean ± SD	**Mean ± SD**	**t**	**p**
RIGHT 2D/4D	0.94±0.050	0.97±0.029	2.92	0.005
LEFT 2D/4D	0.95±0.046	0.97±0.027	1.98	0.050
	n (%)	**n (%)**	**x**	**P***
Right-Handed	24 (64.9)	33 (86.8)	4.96	0.026
Left-Handed	13 (35.1)	5 (13.2)		

p: Student's T-Test p-value; p*: Chi-Square Test p-value.

Table 3. Serum testosterone, Inhibin B, AMH levels and the right hand 2D/4D finger ratio, and their correlation to SLD severity, Conner's Parent Rating Scale - Revised Short form (CPRS-RS) total and sub-scale scores, and WISC-R IQ scores

		Testosterone	Inhibin B	AMH	Right hand 2D/4D
SLD Severity	r	0.029	0.090	0.208	0.125
	p	0.862	0.591	0.210	0.480
Reading Speed	r	0.283	-0.300	-0.329	0.070
	p	0.086	0.068	0.044	0.692
CPRS-RS-TS	r	0.228	-0.263	-0.164	0.017
	p	0.169	0.111	0.324	0.922
CPRS-RS-HA	r	0.017	-0.354	-0.57	0.099
	p	0.920	0.029	0.733	0.579
CPRS-RS-CP	r	0.123	-0.364	-0.248	0.278
	p	0.460	0.025	0.134	0.111
CPRS-RS-OD	r	-0.222	-0.337	0.068	0.086
	p	0.180	0.039	0.686	0.627
WISC-R Total	r	0.052	0.371	0.114	0.291
	p	0.757	0.022	0.495	0.095
WISC-R Verbal	r	0.137	0.489	0.048	0.462
	p	0.434	0.003	0.782	0.009
WISC-R Performance	r	0.029	0.068	0.153	0.158
	p	0.868	0.697	0.382	0.396

CPRS-RS-CP: Conners' Parent Rating Scale - Revised Short Form cognitive problems, CPRS-RS-HA: Conners' Parent Rating Scale - Revised Short Form hyperactivity, CPRS-RS-OD: Conners' Parent Rating Scale - Revised Short Form oppositional defiance, CPRS-RS-TS: Conners' Parent Rating Scale - Revised Short Form Total Score, SLD: Specific Learning Disorder, WISC-R Performance: Wechsler Intelligence Scale for Children - Revised Version Performans, WISC-R Total: Wechsler Intelligence Scale for Children - Revised Version Total, WISC-R Verbal: Wechsler Intelligence Scale for Children - Revised Version Verbal.

levels. Table 1 displays the serum testosterone, InhB, and AMH levels of the case and the control groups.

Compared to the control group, the case group exhibited lower 2D/4D finger length ratios in the right (t=2.92; p=0.005) and left (t=1.98; p=0.05) hands. Left-hand preference was statistically significantly more common in the case group in comparison to the control group. Table 2 presents the 2D/4D finger length ratios and hand preference rates for the case and control groups.

InhB levels were negatively correlated with CPRS-RS-HA (p=0.029), CPRS-RS-CP (p=0.025), and CPRS-RS-AD (p=0.039) and were positively correlated with WISC-R-Total (p=0.022) and WISC-R-Verbal (p=0.003) scores. Serum AMH levels were negatively correlated with reading speed (p=0.044). A positive correlation was determined between the 2D/4D ratio of the right hand and the WISCR-Verbal score. Table 3 shows serum testosterone, InhB, AMH levels, and the right-hand 2D/4D finger length ratio in relation to SLD severity, CPRS-RS total and sub-scale scores, and WISC-R IQ scores.

DISCUSSION

This study investigated certain factors that may have a role in the male dominance of SLD, such as serum-free testosterone level, InhB, AMH, 2D/4D finger length ratio, and hand dominance. Serum InhB levels were found to be significantly higher in male SLD cases compared to the healthy controls. In addition, the case group had significantly lower 2D/4D ratios and a significantly higher rate of left-hand preference in comparison to the control group.

There is growing interest in the literature regarding the high levels of testosterone seen in male children with neurodevelopmental disorders (12,29). However, the literature contains only one study that investigated testosterone levels in male children with SLD (30). The study assessed testosterone levels in saliva and found them to be significantly in the case group higher than in the control group (30). However, the study used the 1986 diagnostic criteria of the Alabama State Department of Education in its SLD diagnoses and did not exclude ADHD comorbidity. Several studies have shown that children with ADHD have higher testosterone levels compared to controls (11). Therefore, the fact that ADHD cases were not excluded may have influenced the study results pertaining to SLD. In contrast to the study by Kirkpatrick and colleagues, our study showed similar serum testosterone levels in prepubescent male children and controls. Our study results may have differed from those of Kirkpatrick et al due to the methodological differences between the two studies.

In our study, although male SLD patients and controls showed a cross-sectional similarity between levels of prepubescent serum testosterone; the 2D/4D finger length ratio, which is an indicator of prenatal testosterone exposure, was significantly lower in SLD cases compared to controls. A lower 2D/4D finger length ratio is indicative of higher testosterone exposure during the prenatal period (31). This ratio was lower in both the right and left hands of SLD cases in comparison to controls. The literature has found that the finger ratio of the right hand is more valuable in assessing prenatal testosterone exposure (32). In our study, this ratio was similarly more pronounced in the right hand. To the best of the researchers' knowledge, the literature contains limited studies investigating 2D/4D ratios in SLD patients. In their study, Boets et al reported that cases with SLD had lower 2D/4D finger length ratios compared to controls; however, this difference was not statistically significant (33). In the aforementioned study, the dyslexia group contained only 12 cases. Therefore, the insufficient number of participants may have contributed to the difference not being statistically significant. Left hand dominance is also an indicator of atypical cerebral lateralization. Many studies have reported higher frequencies of left-hand dominance in children with SLD (17). Similar to this previous research, our study found a significantly higher rate of left hand preference among the SLD group. There is evidence that atypical cerebral lateralization may also be linked to prenatal testosterone exposure (16). Our research findings support the hypothesis that prenatal testosterone exposure may play a role in the etiology of SLD.

Although our research revealed differences between 2D/4D ratios, serum testosterone levels were found to be similar, which implies that testosterone sensitivity may be more influential than the level of serum testosterone in the etiology of SLD. The literature has reported that androgen sensitivity had a greater effect on finger length ratio compared to androgen concentration (34). Since the finger length ratio stays consistent from birth and early infancy to adulthood, and because it could not be linked to serum sex hormone levels, androgen sensitivity may have a greater impact on this ratio (34).

The continuous development of the brain during the prepubertal period, when testosterone levels are low, indicates that sex hormones other than testosterone may play a role in male dominance (19). Morgan et al have also expressed that InhB and AMH may be key hormones associated with sex difference (20). While we could not pinpoint any studies evaluating the levels of InhB and AMH in children with SLD, other works suggest a significant relationship between the social interaction and communication and language domains and levels of InhB in individuals with autism (18). Our study shows that InhB levels differ significantly in patients with SLD. SLD most commonly causes problems in relation to verbal skills and may have a mutual genetic and etiological basis as language disorders; (35,36) accordingly, this suggests that InhB may play a role in the verbal skill deficit of patients with SLD. The significant correlation between InhB levels and WISC-R verbal IQ scores is a prominent finding in our study, which dictates that InhB could be related to verbal deficits.

AMH is another hormone examined in studies investigating male dominance in neurodevelopmental disorders. Gökcen et al reported that their control group had AMH levels similar to children with ADHD, but symptoms pertaining to attention deficit and behavioral problems varied with AMH levels (7). In the aforementioned study of Pankhurst and McLennan, AMH levels did not vary between the ASD group and the control group (6). The modern literature does not present a single study that assesses the levels of AMH in SLD patients. Similar to other neurodevelopmental disorders, such as ASD and ADHD, the AMH levels in our case group did not differ significantly from the control group. Although belonging to the same TGFβ family, the observed variations between InhB and AMH may be connected to the different SMAD pathways used. The TGFβ superfamily has a regulatory effect in almost all stages of brain development, making SMAD pathways plausible determinants of pathogenesis in neurodevelopmental disorders. Most neurons contain AMH receptors, which contributes to sex differences in the brain (20). InhB is a negative modulator of the signal in these pathways, and the receptors it modulates are widely expressed in the brain (37). The male dominance in SLD is especially evident in reading and writing. There are studies in the literature showing that boys outperform girls in mathematics, while girls show superiority in language skills. The lack of male dominance in the subtype of SLD with arithmetic (math) disorder may be attributed to the natural advantage of males in math skills compared to literacy skills (38).

This study involved some limitations and strenghts. The limitations of the current study include its single-centre nature, solely hospital-based population, cross-sectional design, small sample size, and potential selection bias due to the absence of randomisation. Since this study was designed as a cross-sectional study, it is not possible to determine a causal relationship between sex-specific hormones and SLD. Additionally our relatively small sample size due to the large number of exclusion criteria and the scarcity of isolated SLD types in the clinical population prevented us from making comparisons. Some of our findings may appear somewhat ambitious since there was no previous literature on this topic related to SLD, and they were compared with a study on autism. The strenghts of the present study were that the clinical diagnoses of SLD were supported with the K-SADS, which is a semi-structured interview method accepted as a gold standard test for the diagnosis of psychiatric disorders in children, and with the SLD battery, which was made reliable and valid according to Turkish norms; in addition, the parents of participants provided informative support during the study. Although the fact that neurodevelopmental disorders, including ADHD, autism, intellectual disability, and tic disorders were excluded using DSM 5 diagnostic criteria to provide accurate results specific to SLD, internalizing disorders were not excluded in subjects. However, the prevalence of internalizing disorders were similar. Furthermore, this study has a relatively small sample size as stated above. The limited sample size may decrease the power of the study and its findings. However, the sample size of studies investigating male dominance in other neurodevelopmental disorders was also small. Difficulty in recruiting patients due to the pandemic and lack of previous studies in this field make this limitation understandable.

As a results, the findings of this study indicate that exposure to InhB and /or prenatal testosterone is associated with male dominance in SLD. To the best of the researchers' knowledge, this is the first study to assess testosterone, InhB, and AMH in SLD cases, which are the factors suspected in the etiology of SLD. These results suggested that one of the mechanisms in the etiology of SLD may be InhB and /or prenatal testosterone. Such results may also be helpful to better elucidate the etiology of SLD. Additionally, one of the important findings from this study is a significant correlation between InhB levels and WISCR verbal IQ scores. These data point out that InhB may be associated with verbal deficits. However, since the data we have obtained is not strong enough to explain the sex difference, it may be recommended to investigate genetic and shared environmental factors that may affect male dominance in SLD in future studies. More research will be beneficial to examine the links between SLD and male dominance more closely. Moreover, the markers we investigated are related to communication areas, verbal deficits, and neurodevelopmental disorders. There is no study in the literature on language disorders in which these markers are examined. It will be beneficial to carry out future studies in this area of language disorders.

Explanation: Preliminary results of this research was presented at 33rd ECNP Congress Virtual (12–15 September 2020 - Virtual) as an e-poster "Serum testosterone, inhibin b, anti-mullerian hormone, dehydroepiandrosterone-sulfate levels and 2 d: 4 d digit ratio in boys with specific learning disorder". This paper had not been published elsewhere previously.

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Ethics Committee Approval: This study was approved by the Istanbul University, Istanbul Faculty of Medicine Clinical Research Ethics Committee (numbered 2020/215).

Informed Consent: The children included in the case and control groups were enrolled in this study after the study was fully explained to them, and verbal consent was obtained from the children, while written consent was obtained from their parents.

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Author Contributions: Concept- TBK, AK; Design- TBK, AK, NS; Supervision- TBK, NS, AK, PV; Resource- TBK, NS, PV; Materials- PV, TBK, GSU; Data Collection and/or Processing- TBK, GSU, PV; Analysis and/or Interpretation- AK, NS; Literature Search- TBK, GSU, PV; Writing- TBK, NS, AK; Critical Reviews- NS, AK.

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