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Brain-Derived Neurotrophic Factor (BDNF) Genomic Variants and Salivary Progesterone Levels in Female Patients with a History of Idiopathic Scoliosis: An Historical Cross-Sectional Study

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Abstract

Purpose: Adolescent idiopathic scoliosis is a condition characterized by a three-dimensional curvature of the spine. However, in addition to the spinal curvature, it has also been reported that patients with idiopathic scoliosis can display other abnormal physiologic parameters, such as hormone imbalances, genetic variants, and micronutrient deficiencies. The present study evaluated the salivary progesterone levels, as well as the results of brain-derived neurotrophic factor (BDNF) genomic testing, from a historical cohort of patients seeking treatment at a single integrative medicine clinic. **Method:** A group of female patients with a history of adolescent idiopathic scoliosis was compared to a group of non-scoliosis female patients. **Results:** Salivary progesterone levels were 41% higher in non-scoliosis patients compared to the scoliosis group ($P < 0.05$), and while 71% of the scoliosis group was positive for the BDNF functional genomic group compared to 19% of the non-scoliosis group ($P < 0.05$). These results were unchanged regardless of menstrual status. **Conclusion:** These results suggest a potential relationship between salivary progesterone, BDNF, and adolescent idiopathic scoliosis among female patients. Prospective studies are needed for data extrapolation. The current data warrants further investigation.

Keywords

Genomics, Hormone, Progesterone, Scoliosis

1. Introduction

Adolescent idiopathic scoliosis is a condition characterized by a spinal curvature measuring 10˚ or more on radiographic study by Cobb's angle [1]. It is estimated that 0.47% - 5.2% of the US population ages 0 - 17 have this condition, increasing to 8% of the adult population [2]. Conservative treatments for adolescent idiopathic scoliosis are based upon initial presenting curve magnitude. According to the 2016 SOSORT treatment guidelines [3], curves between 10 - 15 are treated with exercises, while curves between 15 and 50 degrees should be treated with exercises and/or bracing, depending upon the patient's growth stage. Curves exceeding 50˚ are typically referred for surgical interventions such as vertebral body tethering or spinal fusion surgery [4].

While these treatment recommendations focus solely on the spinal curvature itself, they do not address some of the non-spinal comorbidities commonly associated with the spinal curvature. These may include vitamin D deficiency [5], neurotransmitter deficiencies and/or signaling abnormalities [6], low bone mineral density [7], and various hormone imbalances [8] [9] [10] [11]. These discoveries over time have lead to more robust hypothetical modeling of scoliosis etiopathogenesis [12].

Investigations into the genetic origins of scoliosis have also lead to the identification of several genes associated with scoliosis, such as CELSR2 [13], SLC39A8 [14], MAPK7 [15], and LBX1 [16]. More recently, groups of functional genomic variants have been observed more commonly in idiopathic scoliosis patients [17]. It has become a generally accepted theory that idiopathic scoliosis has a genetic [18] component and/or epigenetic [19] [20] component related to both its onset and risk of progression.

Given these previous observations in idiopathic scoliosis, the present study investigated the occurrence of specific hormone and genomic differences in scoliosis patients. Progesterone is widely known for its impact on reproductive health. However, progesterone maintains an important influence in the motor memory centers of the brain, such as the hippocampus and the thalamus via synaptic plasticity [21]. Progesterone also has an important regulatory relationship in these brain centers with brain-derived neurotrophic factor (BDNF), which is also responsible for the maintenance of the central nervous system's body schema, via the vast network of thalamocortical networks and cerebellum [22] [23]. The current study reports on the test results of patients with and without idiopathic scoliosis and the differences observed in their salivary progesterone levels and BDNF variants. Any potential connections between progesterone, BDNF SNPs, and idiopathic scoliosis have not been previously described in the literature.

2. Methods and Materials

The data in this study were collected and reported according to the STROBE format (http://www.strobe-statement.org) for cross-sectional studies. A consecutive sampling of all patient records from a single multidisciplinary medical clinic in Grand Blanc, Michigan, USA, was conducted on patients who sought

treatment there for any reason, from January 2018 through November 2020. Patient charts were included in the present study if they met the following inclusion criteria: 1) patient had a history of idiopathic scoliosis, 2) patient was female, 3) patient completed a salivary hormone test, and 4) the patient completed a genomic analysis through a commercial lab (Functional Genomic Analysis™). Using these inclusion criteria, a total of 51 patient files were selected. All patient records meeting the inclusion criteria were included in the sample group. To verify that these patients had a history of adolescent idiopathic scoliosis, the chart needed to show documentation of a scoliosis radiograph taken at the presenting clinic, or that the patient had a scoliosis radiograph performed elsewhere, with a radiology report documenting the existence of idiopathic scoliosis.

Once the first group of patient files were collected and grouped, a second data collection was performed from the files not initially selected as part of the study group. For this group, there were 3 inclusion criteria: 1) the patient completed the same salivary hormone test as the first group, 2) patient was female, and 3) the patient completed the same genomic analysis through the same commercial lab (Functional Genomic Analysis™). Based upon these inclusion criteria, 113 patient files were identified. This collection of files served as the non-scoliosis group. **Figure 1** shows a flow chart of the file selection process. Data were only collected from female patients since female reference progesterone levels are different than males. Therefore, males were excluded to improve data homogeneity. It is important to note that both groups were patients at the same clinic, being managed for various symptoms and conditions, not necessarily just scoliosis. For example, a salivary progesterone level may be ordered for headaches or menstrual symptoms regardless of a patient's history of scoliosis. None of the patients in either group were pregnant or breastfeeding at the time of salivary collection, due to their potential impacts on hormone ratios. Since oral contraception also affects salivary hormone results, none of the patients whose results were used for this study were on oral contraceptive medication at the time of collection.

The following non-identifying data was extracted from all the files in both groups: age, baseline salivary progesterone level (used when patients had completed more than one salivary hormone test), and if the patient was positive or negative for a group of brain-derived neurotrophic factor (BDNF) genomic variants. The determination of a "positive" or "negative" BDNF genomic variant is made based upon the number of specific genes that have a least 1 mutated allele. The genomic analysis that was performed evaluates a total of 19 single nucleotide polymorphisms (SNPs). **Table 1** shows a complete listing of the SNPs tested for all patients in both groups, including their reference SNP cluster ID (rs number). If 10 or more of the 19 BDNF SNPs showed a variant, then that patient was considered positive (>50%). In some patient results, some SNPs were not present. In those instances, a greater than 50% positive BDNF SNP count still resulted in that patient being classified as positive for study purposes. An advi-

sory review was conducted by Advarra Institutional Review Board (IRB) where they determined that the study design was exempt from IRB approval.

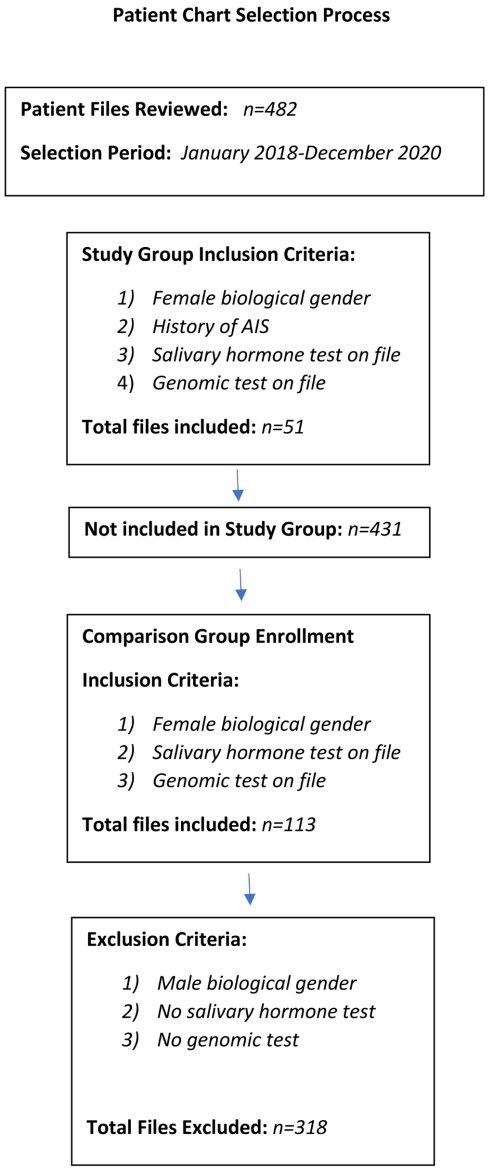

Figure 1. Attached as separate pdf document.

Table 1. BDNF genes assessed (SNP Cluster IDs).

rs6265	rs11030119	rs11785351
rs3750934	rs1491850	rs143442829
rs191472263	rs142303821	rs10835210
rs2030324	rs1967554	rs962369
rs56820186	rs41282916	rs79187269
rs6484320	rs988748	rs78531552
rs10767664		

The salivary progesterone levels were ordered through Labrix (http://www.doctorsdata.com). Salivary progesterone was chosen for a variety of reasons. Salivary progesterone collection is noninvasive, and hence easier to obtain from adolescent patients. It also appears to maintain a consistent correlation with serum progesterone values [24]. In a large percentage of healthy subjects, salivary progesterone levels will reach ovulatory levels, even in premenarchial females [25]. This may make salivary progesterone a more sensitive marker in premenarchial females for comparative purposes. Salivary BDNF seems to follow the natural estradiol rhythm throughout a normal menstrual cycle, and does not seem to correlate to the progesterone peak during the luteal phase [26].

Salivary progesterone levels were compared among the following groups: the scoliosis group and non-scoliosis group. These groups were subdivided according to menstrual status: scoliosis non-menarchial group (pre or post combined), scoliosis menarchial group, non-scoliosis, non-menarchial group, and non-scoliosis menarchial group. The rate of BDNF positivity was compared between the scoliosis group and non-scoliosis groups. The onset of menarche, as well as menopause, are associated with significant changes in hormone production. Therefore, stratification was conducted based upon menstrual status. Paired t-tests were used to compare the average progesterone levels among the stratified subgroups. This was performed to minimize confounding factors, such as cycling status.

3. Results

The average age of the scoliosis patient group was 26 years (range 11 - 62), compared to 44 years (range 16 - 67) for the non-scoliosis group. For the scoliosis group, the average salivary progesterone level was 73.57 pg/mL ± 61.42, and 36 out of 51 patients (71%) had 10 or more BDNF gene variants. They had an average of 12 of 19 variant genotypes ± 7. The salivary progesterone for the non-scoliosis group was 103.47 pg/mL ± 52.54, and 19% (22 out of 113) had 10 or more BDNF gene variants. These values are shown in **Figure 2**. The non-scoliosis patient group had an average of 7 of the 19 BDNF variant genotypes ± 6. A summary of these data are shown in **Tables 2-4**.

In the scoliosis patient group, 39 of the 51 patients were cycling, 12 were not. Among the 39 cycling scoliosis patients, their average progesterone was 76.13 pg/mL ± 36.34. In the non-cycling scoliosis patients, their average progesterone was 66.33 pg/mL ± 47.24.

The non-scoliosis patient group had 50 women who were cycling while 63 were not. The non-scoliosis cycling patients had an average progesterone 105.14 pg/mL ± 53.99. The 63 non-scoliosis, non-cycling patients had average progesterone levels of 100.58 pg/mL ± 64.68. The progesterone levels among each group are illustrated in **Figure 3**.

Paired t-tests were then used to determine if any of the observed differences were statistically significant. **Table 5** shows a summary of the t-test comparisons and the resultant P values. Intragroup comparisons in both the scoliosis and

non-scoliosis patient groups showed that differences in menstrual cycling status and BDNF variants were not statistically significant. These were performed to identify these as confounding variables had the calculations shown a statistically significant difference.

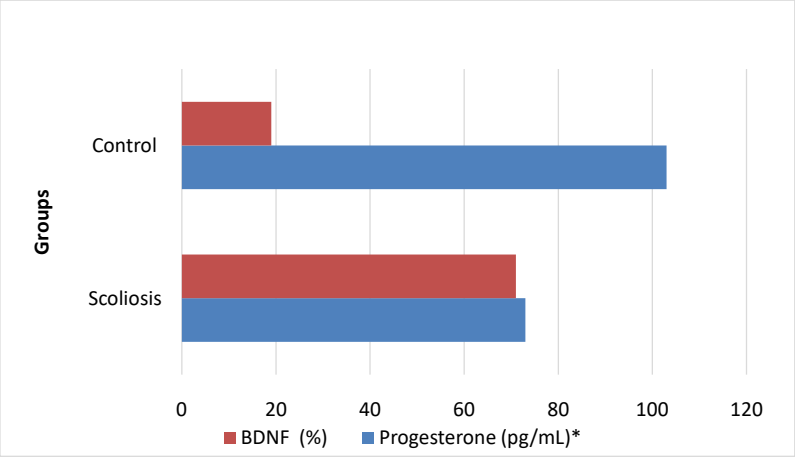

Figure 2. Progesterone levels and the percent of patients with >50% BDNF gene variants, *P < 0.05.

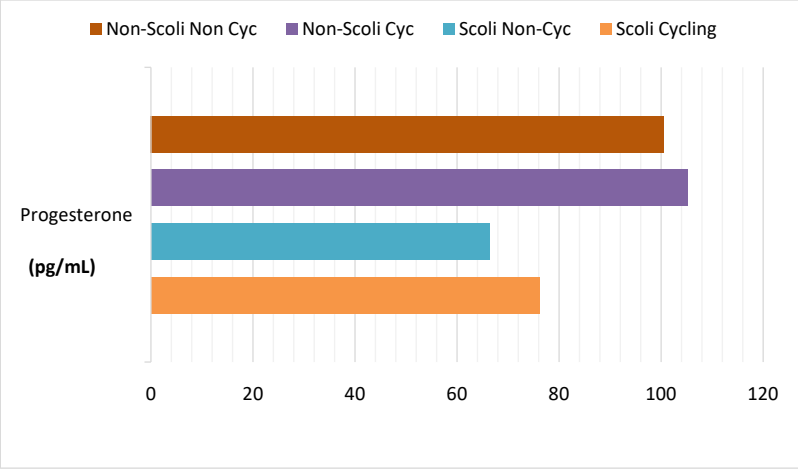

Figure 3. Progesterone levels based on history of scoliosis and cycling status.

Table 2. Progesterone and BDNF variants for entire cohort.

Group	# Patients	Progesterone	# Cycling	Avg # BDNF variants
Scoliosis	51	73.57 pg/mL	39	12
Non-Scoliosis	113	103.47 pg/mL	50	7

Table 3. Progesterone and BDNF variants for menstruating patients.

Cycling Patients	# Patients	Progesterone	Avg # BDNF variants
Scoliosis	39	76.13 pg/mL	13
Non-Scoliosis	50	105.14 pg/mL	5

Table 4. Progesterone and BDNF variants for pre-menarche and post-menopausal patients.

Non-Cycling Patients	# Patients	Progesterone	Avg # BDNF variants
Scoliosis	12	66.33 pg/mL	10
Non-Scoliosis	63	100.58 pg/mL	8

Table 5. P values among intra-group comparisons.

IntraGroup Comparison*	Scoliosis Group	Non-Scoliosis Group
BDNF Yes vs. BDNF No	0.762	0.798
Cycling Yes vs. Cycling No	0.518	0.686

*Paired t-tests, 95% CI, normal distribution.

A post-hoc power analysis was conducted for the progesterone levels using an online calculator (http://www.clincalc.com). For progesterone, the current sample size for both groups resulted in 86.2% power to show a 29 pg/mL difference at a 0.05 confidence interval. Post-hoc power analysis for the BDNF variants, with a dichotomous endpoint, showed that with the given sample patient populations, the difference in the number of BDNF genotypes between the scoliosis and non-scoliosis groups resulted in 99.3% power at 0.05 confidence interval.

4. Discussion

The evaluation of 2 groups of consecutively selected patient charts showed statistically significant differences in the salivary progesterone levels among female patients with a past medical history of adolescent idiopathic scoliosis, when compared to a group of patients without a known history of adolescent idiopathic scoliosis. The scoliosis patient group was also more likely to display a higher number of genomic variants involving brain-derived neurotrophic factor (BDNF). These values were independent of menstrual cycling status. The present study is the first to identify an inverse relationship between salivary progesterone levels and the number of BDNF variants in scoliosis patients versus non-scoliosis patients. Further studies should consider the role of BDNF in idiopathic scoliosis.

It has been known for several decades that scoliosis can continue to progress throughout adulthood after skeletal maturity [27]. Marty-Poumarat *et al.* [28] showed that adult scoliosis progression is linear and is estimated to occur in 68% of curves measuring ≥30˚ at the time of skeletal maturity, regardless of curve pattern [4] [29]. Although it is unknown how progesterone might impact scoliosis onset or progression, progesterone maintains an important influence in the motor memory centers of the brain, such as the hippocampus and the thalamus via synaptic plasticity [21]. Central pattern generator differences have been observed in scoliosis patients [12]. Progesterone's involvement in central pattern generation would be via the central nervous system upregulation of brain-derived

neurotrophic factor (BDNF) receptors [22] [23]. It is possible that the observed differences may play some role in the continued progression of idiopathic scoliosis across the lifespan.

There are some limitations involved in this study, based on the study design, that warrant discussion. As with any retrospective design, selection bias is a potential factor. However, as described in the paper, all patient charts within a historical time frame were selected, and both groups of charts comprised all of the charts, as long as the inclusion criteria were fulfilled. However, male patients were excluded from the study. Therefore, the results of the study may not be applicable to male patients with a history of idiopathic scoliosis. Information bias was also addressed in the study in multiple ways. First, by evaluating the impact of confounding factors upon the progesterone levels and BDNF genomic variants. Menstrual cycling status was statistically insignificant between the patient groups, it was unlikely to have an impact on progesterone levels. **Table 5** shows the intra-group P values for the groups, based upon their cycling status. Their menstrual cycling status could be used as an independent variable in efforts to reduce selection bias. Second, we assessed the statistical difference of 1 out of 19 BDNF SNPs using the online calculator to assess a dichotomous endpoint, given that this was the minimum difference between classifying a patient as BDNF positive or negative. Since the outcome measures were obtained from independent laboratory testing not performed by the author, and not collected from self-rated surveys or questionnaires, self-selection bias and outcome-based information bias could also be minimized.

One additional limitation is that a certain unknown portion of the non-scoliosis patient group may, in fact, have a previously undiagnosed idiopathic scoliosis. Using the 5% - 8% estimate of teen and adult prevalence of idiopathic scoliosis [2], it is possible that 5 - 9 patients in the non-scoliosis group would be correctly added to the scoliosis group. However, if their progesterone levels were consistent with the scoliosis group, meaning they would be closer to the scoliosis group average versus the non-scoliosis group average, it may have further decreased the resultant P value, thus increasing its statistical significance had they been placed into the corrected group (the scoliosis patient group).

5. Conclusion

The results of the present study suggest that progesterone is significantly lower in female patients with a history of adolescent idiopathic scoliosis (AIS) when compared to female patients with a negative history of AIS. Scoliosis patients were also more likely to have a higher number of genomic variants involving brain-derived neurotrophic factor (BDNF). Menstrual cycle status did not impact the differences between groups. The study design prevents any extrapolation on causality, nor are these results necessarily applicable to male scoliosis patients. Future investigations are warranted in male patients, as well as comparing salivary and serum progesterone levels in similar groups.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Grivas, T.B., Vasiliadis, E., Chatzizrgiropoylos, T., Polyzois, V.D. and Gatos, K. (2003) The Effect of a Modified Boston Brace with Antirotatory Blades on the Progression of Curves in Idiopathic Scoliosis: Aetiologic Implications. *Pediatric Rehabilitation*, **6**, 237-242. https://doi.org/10.1080/13638490310001636808

[2] Konieczny, M.R., Senyurt, H. and Krauspe, R. (2013) Epidemiology of Adolescent Idiopathic Scoliosis. *Journal of Children's Orthopaedics*, **7**, 3-9. https://doi.org/10.1007/s11832-012-0457-4

[3] Negrini, S., Donzelli, S., Aulisa, A.G., *et al.* (2018) 2016 SOSORT Guidelines: Orthopaedic and Rehabilitation Treatment of Idiopathic Scoliosis during Growth. *Scoliosis and Spinal Disorders*, **13**, Article No. 3. https://doi.org/10.1186/s13013-017-0145-8

[4] Weinstein, S.L., Dolan, L.A., Spratt, K.F., Peterson, K.K., Spoonamore, M.J. and Ponseti, I.V. (2003) Health and Function of Patients with Untreated Idiopathic Scoliosis: A 50-Year Natural History Study. *Journal of the American Medical Association*, **289**, 559-567. https://doi.org/10.1001/jama.289.5.559

[5] Ng, S.Y., Bettany-Saltikov, J., Cheung, I.Y.K. and Chan, K.K.Y. (2018) The Role of Vitamin D in the Pathogenesis of Adolescent Idiopathic Scoliosis. *Asian Spine J*, **12**, 1127-1145. https://doi.org/10.31616/asj.2018.12.6.1127

[6] Fadzan, M. and Bettany-Saltikov, J. (2017) Etiological Theories of Adolescent Idiopathic Scoliosis: Past and Present. *The Open Orthopaedics Journal*, **11**, 1466-1489. https://doi.org/10.2174/1874325001711011466

[7] Pourabbas Tahvildari, B., Erfani, M.A., Nouraei, H. and Sadeghian, M. (2014) Evaluation of Bone Mineral Status in Adolescent Idiopathic Scoliosis. *Clinics in Orthopedic Surgery*, **6**, 180-184. https://doi.org/10.4055/cios.2014.6.2.180

[8] Kikanloo, S.R., Tarpada, S.P. and Cho, W. (2019) Etiology of Adolescent Idiopathic Scoliosis: A Literature Review. *Asian Spine Journal*, **13**, 519-526. https://doi.org/10.31616/asj.2018.0096

[9] Silva, R.T.E., Fernandes, R.J.R., Ono, A.H.A., Marcon, R.M., Cristante, A.F. and De Barros Filho, T.E.P. (2017) Role of Different Hormones in the Pathogenesis and Severity of Adolescent Idiopathic Scoliosis. *Acta Ortopedica Brasileira*, **25**, 15-17. https://doi.org/10.1590/1413-785220172501168600

[10] Kulis, A., Goździalska, A., Drąg, J., Jaśkiewicz, J., Knapik-Czajka, M., Lipik, E. and Zarzycki, D. (2015) Participation of Sex Hormones in Multifactorial Pathogenesis of Adolescent Idiopathic Scoliosis. *International Orthopaedics*, **39**, 1227-1236. https://doi.org/10.1007/s00264-015-2742-6

[11] Wang, Q., Wang, C., Hu, W., Hu, F., Liu, W. and Zhang, X. (2020) Disordered Leptin and Ghrelin Bioactivity in Adolescent Idiopathic Scoliosis (AIS): A Systematic Review and Meta-Analysis. *Journal of Orthopaedic Surgery and Research*, **15**, Article No. 502. https://doi.org/10.1186/s13018-020-01988-w

[12] Burwell, R.G., Aujla, R.K., Grevitt, M.P., *et al.* (2009) Pathogenesis of Adolescent Idiopathic Scoliosis in Girls—A Double Neuro-Osseous Theory Involving Disharmony between Two Nervous Systems, Somatic and Autonomic Expressed in the Spine and Trunk: Possible Dependency on Sympathetic Nervous System and Hor-

mones with Implications for Medical Therapy. *Scoliosis*, **4**, Article No. 24. https://doi.org/10.1186/1748-7161-4-24

[13] Einarsdottir, E., Grauers, A., Wang, J., Jiao, H., Escher, S.A., Danielsson, A., Simony, A., Andersen, M., Christensen, S.B., Åkesson, K., Kou, I., Khanshour, A.M., Ohlin, A., Wise, C., Ikegawa, S., Kere, J. and Gerdhem, P. (2017) CELSR2 Is a Candidate Susceptibility Gene in Idiopathic Scoliosis. *PLoS ONE*, **12**, e0189591. https://doi.org/10.1371/journal.pone.0189591

[14] Haller, G., McCall, K., Jenkitkasemwong, S., Sadler, B., Antunes, L., Nikolov, M., *et al.* (2018) A Missense Variant in SLC39A8 Is Associated with Severe Idiopathic Scoliosis. *Nature Communications*, **9**, 4171. https://doi.org/10.1038/s41467-018-06705-0

[15] Gao, W., Chen, C., Zhou, T., Yang, S., Gao, B., Zhou, H., *et al.* (2017) Rare Coding Variants in MAPK7 Predispose to Adolescent Idiopathic Scoliosis. *Human Mutation*, **38**, 1500-1510. https://doi.org/10.1002/humu.23296

[16] Grauers, A., Wang, J., Einarsdottir, E., Simony, A., Danielsson, A., Åkesson, K., *et al.* (2015) Candidate Gene Analysis and Exome Sequencing Confirm LBX1 as a Susceptibility Gene for Idiopathic Scoliosis. *The Spine Journal*, **15**, 2239-2246. https://doi.org/10.1016/j.spinee.2015.05.013

[17] Morningstar, M.W., Stitzel, C.J. and Strauchman, M. (2019) Functional Genomic Variant Patterns in Caucasian Patients Diagnosed with Idiopathic Scoliosis: A Controlled, Observational Study. *Medical Research Archives*, **7**, 9 p.

[18] Grauers, A., Einarsdottir, E. and Gerdhem, P. (2016) Genetics and Pathogenesis of Idiopathic Scoliosis. *Scoliosis and Spinal Disorders*, **11**, Article No. 45. https://doi.org/10.1186/s13013-016-0105-8

[19] Peng, Y., Wang, S.R., Qiu, G.X., Zhang, J.G. and Zhuang, Q.Y. (2020) Research Progress on the Etiology and Pathogenesis of Adolescent Idiopathic Scoliosis. *Chinese Medical Journal*, **133**, 483-493. https://doi.org/10.1097/CM9.0000000000000652

[20] Zaydman, A.M., Strokova, E.L., Pahomova, N.Y., Gusev, A.F., Mikhaylovskiy, M.V., Shevchenko, A.I., *et al.* (2021) Etiopathogenesis of Adolescent Idiopathic Scoliosis: Review of the Literature and New Epigenetic Hypothesis on Altered Neural Crest Cells Migration in Early Embryogenesis as the Key Event. *Medical Hypotheses*, **151**, Article ID: 110585. https://doi.org/10.1016/j.mehy.2021.110585

[21] Baudry, M., Bi, X. and Aguirre, C. (2013) Progesterone-Estrogen Interactions in Synaptic Plasticity and Neuroprotection. *Neuroscience*, **239**, 280-294. https://doi.org/10.1016/j.neuroscience.2012.10.051

[22] Parkinson, A., Condon, L. and Jackson, S.R. (2010) Parietal Cortex Coding of Limb Posture: In Search of the Body-Schema. *Neuropsychologia*, **48**, 3228-3234. https://doi.org/10.1016/j.neuropsychologia.2010.06.039

[23] Saywell, N. and Taylor, D. (2008) The Role of the Cerebellum in Procedural Learning—Are There Implications for Physiotherapists' Clinical Practice? *Physiotherapy Theory and Practice*, **24**, 321-328. https://doi.org/10.1080/09593980701884832

[24] Bolaji, I.I. (1994) Sero-Salivary Progesterone Correlation. *International Journal of Gynecology & Obstetrics*, **45**, 125-131. https://doi.org/10.1016/0020-7292(94)90119-8

[25] Gray, S.H., Ebe, L.K., Feldman, H.A., Emans, S.J., Osganian, S.K., Gordon, C.M. and Laufer, M.R. (2010) Salivary Progesterone Levels before Menarche: A Prospective Study of Adolescent Girls. *The Journal of Clinical Endocrinology & Metabolism*, **95**, 3507-3511. https://doi.org/10.1210/jc.2010-0125

[26] Matsuki, C., To, M., Kondo, Y., Sugiyama, H., Yamamoto, Y., Shimizu, T., *et al.* (2014) Associations between Brain-Derived Neurotrophic Factor and Estradiol in Women's Saliva. *Neuroendocrinology Letters*, **35**, 236-241.

[27] Collis, D.K. and Ponseti, I.V. (1969) Long-Term Follow-Up of Patients with Idiopathic Scoliosis Not Treated Surgically. *The Journal of Bone and Joint Surgery*, **51**, 425-445. https://doi.org/10.2106/00004623-196951030-00001

[28] Marty-Poumarat, C., Scattin, L., Marpeau, M., Garreau de Loubresse, C. and Aegerter, P. (2007) Natural History of Progressive Adult Scoliosis. *Spine*, **32**, 1227-1234. https://doi.org/10.1097/01.brs.0000263328.89135.a6

[29] Weinstein, S.L. (1999) Natural History. *Spine*, **24**, 2592-2600. https://doi.org/10.1097/00007632-199912150-00006