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MOLECULAR GENETIC ANALYSIS OF GENES FOR DETOXIFICATION AND DNA REPAIR IN CHILDREN WITH CONGENITAL DEFORMITIES OF THE THORACIC AND LUMBAR SPINE

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Introduction. Spine congenital curvatures, which form from anomalies in the development of vertebral bodies, comprise 3.2% of the general structure of vertebral column deformities. Several such anomalies present during adolescence lead to severe and rigid curvature of the spinal column and are often accompanied by irreversible neurological disorders. The timely detection of the progressive forms of curvature and early surgical treatment are measures that prevent against neurological deficit development and gross congenital deformities of the spine in children. However, it is extremely difficult to predict the course of congenital spinal column deformation in infants based on clinical and radiological investigations alone. Therefore, the study of congenital malformation genetic markers is an essential and immediate task.

Materials and methods. Two hundred 1.2–16-year-old children with congenital deformities of the thoracic and lumbar spine were examined using clinical and radiation diagnostic methods. Molecular genetic studies were performed by analyzing several polymorphic regions in the genes for the first and second stages of detoxification and DNA repair, which are of clinical importance as predisposing factors in several congenital malformations. Polymorphisms were determined using the polymerase chain reaction (PCR) method. The results were determined using gel electrophoresis of DNA in a polyacrylamide gel.

Results and discussion. The polymorphisms of the genes *CYP1A2*, *NAT2*, *GSTM1*, *GSTT1*, *GSTP1*, *XRCC1*, *XRCC3* and their frequency distributions among patients with congenital spine deformities (CSD) were studied. The results for each gene are presented in the digital diagrams, and their indicators are compared with the values of the control group.

Conclusion. In most patients (83%) with spinal congenital deformations, there were mutations of candidate genes in the homozygous state; however, the simultaneous carriage of several mutant alleles in patients with CSD was more than twice that in the control group. Children with multiple and combined defects in spine development noted the presence of more mutations in the genes for detoxification and DNA repair. The obtained results already assume to a certain extent the course of the spine congenital deformity in patients at an early age. However, the final evaluation and identification of molecular genetic criteria for the progressive course of spine congenital deformities in children requires further study.

Keywords: children; congenital defects of the spine; molecular genetic analysis; genes of detoxification and reparation.

МОЛЕКУЛЯРНО-ГЕНЕТИЧЕСКИЙ АНАЛИЗ ГЕНОВ ДЕТОКСИКАЦИИ И РЕПАРАЦИИ ДНК У ДЕТЕЙ С ВРОЖДЕННЫМИ ДЕФОРМАЦИЯМИ ГРУДНОГО И ПОЯСНИЧНОГО ОТДЕЛОВ ПОЗВОНОЧНИКА

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Введение. Доля врожденных искривлений позвоночника, сформированных в результате аномалий развития тел позвонков, в общей структуре деформаций позвоночного столба составляет 3,2 %. Ряд подобных аномалий в подростковом возрасте приводит к тяжелым и ригидным искривлениям позвоночного столба, нередко сопровождающимся необратимыми неврологическими нарушениями. С целью профилактики развития неврологического дефицита и предотвращения развития грубых врожденных деформаций позвоночника у детей необходимо своевременно выявлять прогрессирующие формы искривлений и предпринимать раннее хирур-

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гическое лечение. Однако, основываясь на данных только клинического и лучевого методов исследования, у детей раннего возраста крайне тяжело предсказать вариант течения врожденной деформации позвоночного столба. Исследование генетических маркеров прогрессирующего течения врожденного искривления позвоночного столба на фоне врожденных пороков тел позвонков представляет собой важную и актуальную задачу.
Материалы и методы. Обследовано 200 детей с врожденными деформациями грудного и поясничного отделов позвоночника в возрасте от 1 года 2 месяцев до 16 лет с применением методов клинической и лучевой диагностики. Молекулярно-генетические исследования проводили путем анализа ряда полиморфных участков в генах 1-й и 2-й стадий детоксикации и репарации ДНК, имеющих клиническое значение в качестве предрасполагающих факторов при ряде врожденных пороков развития. Полиморфизмы определяли методом полимеразной цепной реакции. Результаты оценивали с помощью метода гель-электрофореза ДНК в полиакриламидном геле.
Результаты и обсуждение. Были исследованы полиморфизмы генов *CYP1A2*, *NAT2*, *GSTM1*, *GSTT1*, *GSTP1*, *XRCC1*, *XRCC3* и их частотное распределение среди больных с врожденными деформациями позвоночника (ВДП). Результаты по каждому гену, представленные на цифровых диаграммах, и их показатели сравнивали со значениями в контрольной группе.
Заключение. У большинства пациентов (83 %) с ВДП имелись мутации кандидатных генов в гомозиготном состоянии, причем частота одновременного носительства нескольких мутантных аллелей у больных ВДП более чем в два раза превышает данный показатель в контрольной группе. Установлено, что у детей с множественными и комбинированными пороками развития позвоночника отмечается наличие большего количества мутаций генов детоксикации и репарации ДНК. Полученные результаты позволяют в определенной степени предполагать характер течения врожденной деформации позвоночника у пациентов раннего возраста. Однако для окончательной оценки и выявления молекулярно-генетических критериев прогрессирующего течения врожденной деформации позвоночника у детей требуются дальнейшие исследования.

Ключевые слова: дети; врожденные деформации позвоночника; молекулярно-генетический анализ; гены детоксикации и репарации.

Introduction

Congenital curvature of the spine caused by impaired development of the vertebral bodies comprises 3.2% of all spinal column deformities. Approximately 50% of all congenital malformations of the spine have a progressive pattern [1]. Such variants in the course during adolescence lead to severe and rigid spinal curvatures that are frequently accompanied by irreversible neurological disorders. In order to prevent neurological deficit and prevent the development of gross congenital spinal deformities in children, timely detection of the progressive forms of curvature and early surgical treatment up to the age of three years is necessary [1–3]. However, based on the data only from clinical and radiological studies, it is extremely difficult to predict the course of congenital spinal deformity in young children.

There is an urgent need to study the genetic prerequisites for the occurrence of congenital malformations. Understanding the biological nature of this phenomenon would enable us to conduct targeted prevention and develop diagnostic activities that enable the detection of spinal deformities, characterized by a progressive pattern, along with abnormal development of vertebral bodies, during the first years of a child's life. In turn, this would facilitate early surgical intervention aimed at radical correction of congenital spinal column curvature with minimal fixation of the spinal motion segments.

Congenital malformations, similar to any multifactorial pathology, are associated with both, exposure to adverse teratogenic environmental factors during pregnancy (hypoxia, several medicines, alcohol consumption, hyperthermia, insulin-dependent diabetes mellitus, and gestational diabetes) [4–9] and genetic factors (chromosome aberrations, gene polymorphisms due to hereditary predisposition, *de novo* mutations, and epigenetic changes) [10–13]. Each of these factors, individually or in combination, can impair embryogenesis and abnormal development of the vertebrae. Recent studies have analyzed molecular genetic markers accompanying congenital spinal deformities (CSD) [14–16]. These trials have considered possible factors for the etiology and pathogenesis of CSD in sufficient detail. Various studies have indicated a close relationship between congenital deformities of the spinal column and mutations in the TBX6 gene [17–19]. The main aim of these studies is the development of a set of diagnostic measures to assess the rate of progression of congenital curvature in children with spinal deformities, using data from clinical and radiological studies and the creation of a diagnostic panel based on molecular, genetic, and biochemical criteria.

This study aimed to perform the molecular genetic analysis of the genes of stages 1 and 2 of detoxification and excision repair of DNA in children with congenital deformities of the spine of the thoracic and lumbar localization.

Materials and methods

We observed 200 children with congenital deformities of the thoracic and lumbar spine, aged 1 year 2 months to 16 years whose diagnosis was confirmed using standard methods of clinical and radiological diagnostics. In the structure of congenital curvatures of the spinal column, various anomalies of vertebral development were revealed, such as abnormal formation (lateral and posterolateral semi-vertebrae as well as posterior and lateral wedge-shaped vertebrae), impaired fusion (asymmetrical butterfly-shaped vertebrae), impaired vertebral segmentation (blocking of the lateral surfaces and the anterior surfaces of the vertebral bodies), and synostosis of the ribs. Total 32% of the children had isolated malformations of the thoracic or lumbar spine; in the remaining 68% of the patients, multiple and combined malformations of the thoracic and/or lumbar spine were noted. All patients had a clinically pronounced scoliotic and/or kyphotic deformity of the thoracic and/or lumbar spine, manifested by asymmetry of the shoulder girdle, waist triangles, and pelvic distortion. The magnitude of the scoliotic deformity component ranged from 30°–72°, while that of the kyphotic deformity was 26°–52°. Most children exhibited progression of the congenital curvature in the process of growth and development.

Total 38% of children were expected to have concomitant congenital anomalies of other organs and systems, such as esophageal atresia, tracheoesophageal fistula, renal aplasia, anus atresia, congenital complete cleft of the upper lip, congenital malformation of the tracheobronchial tree, pulmonary hypoplasia, and congenital heart disease, most likely because of chromosomal aberrations in the group of linkage with other genes. The control group comprised 96 healthy children aged 2–16 years who had no spinal pathology.

Molecular genetic studies were performed by analyzing several polymorphic sites in the genes of stages 1 and 2 of detoxification and DNA repair that are clinically significant as predisposing factors in various congenital malformations [20, 21].

The polymorphisms of genes *CYP1A2*, *NAT2*, *GSTM1*, *GSTT1*, *GSTP1*, *XRCC1*, and *XRCC3* (Table 1) were studied; their frequency distribution among patients with CSD was also examined.

The definition of polymorphisms was performed using the method of polymerase chain reaction (PCR). DNA required for analyses was isolated from whole blood using diagnostic kits manufactured by "Interlabservice" and "DNA-technology" as per the manufacturer's instructions. PCR studies were performed using a Bio-Rad T100 instrument. Mixtures for PCR and amplification modes were developed independently. To determine nucleotide substitutions, the method of restriction fragment length polymorphism (RFLP) was used. The results of PCR and RFLP, in terms of the detection of polymorphisms, were evaluated using the method of gel electrophoresis of DNA with polyacrylamide gel.

Statistical analyses were performed using the Statistics 6.0 software. The significance of differences between the observation groups was assessed using the non-parametric paired student *t*-test with a two-tailed distribution and the determination of the statistical confidence indicator. Indicator differences were considered significant at a level of $p < 0.05$.

Table 1

Allelic variants of the genes under study

Gene	Polymorphism	Genotypes
CYP1A2	164 A → c	A/A, A/c, c/c
GSTM1	+/0	+/00
GSTT1	+/0	+/00
GSTP1	Ile105Val	A/A, A/G, G/G
GSTP1	Ala(C)114Val(T)	c/c, c/T, T/T
NAT2	C481T (KpnI)	*5
NAT2	G590A (TaqI)	*6
XRCC1	Arg399Gln	G/G, G/A, A/A
XRCC3	Thr241Met	c/c, c/T, T/T

Results and discussion

During the study period, the frequency of occurrence of polymorphism in the structure of the genes studied in patients with CSD was analyzed.

We also performed a comparative analysis of the severity of their polymorphisms with those in healthy children. The research results are presented in Fig. 1–4.

CYP1A2 is a member of the cytochrome superfamily p450 and is localized in the endoplasmic reticulum; its expression is induced by certain polycyclic aromatic hydrocarbons (PAHs). The endogenous enzyme substrate is able to metabolize some PAHs to carcinogenic intermediates. Changes in the *CYP1A2* activity in humans may be attributable to various environmental exposures, genetic differences, and intergenic interactions.

Research has shown that [22] the presence of the C-allele is characterized by a slower xenobiotic metabolism. In our study, 56.5% of the CSD patients had the allele of the poor metabolizer, while in the control group this indicator amounted to 48.1%.

The genes *GSTM1* and *GSTT1* belong to the group of genes for the second phase of detoxification of xenobiotics; their products convert xenobiotics and carcinogens into non-toxic water-soluble products, thus preventing DNA destruction.

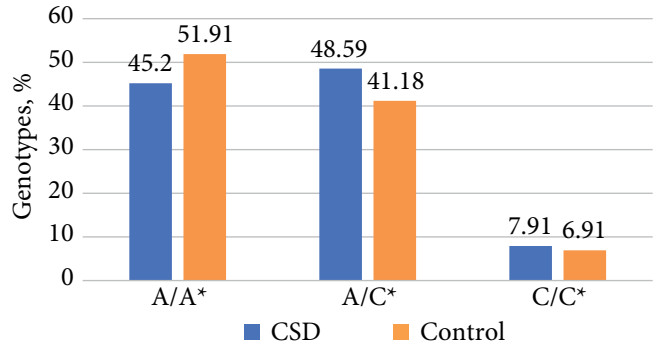

Fig. 1. The frequency of occurrence of genotypes for the *CYP1A2* 164A/c gene, % (* $p < 0.05$ compared to the control): CSD — congenital spinal deformities

GSTM1 gene deletion results in the absence of a corresponding enzyme; thus, sensitivity to the effects of mutagens and carcinogens is increased. The joint carriage of the 105Ile variant of the *GSTP1* gene and the deletion of the *GSTM1* gene increases the level of immunoglobulin E and histamine under the influence of xenobiotics and allergens. *GSTM1* gene deletion increases the risk of several diseases, including various types of pathologies of pregnancy, leading to impaired

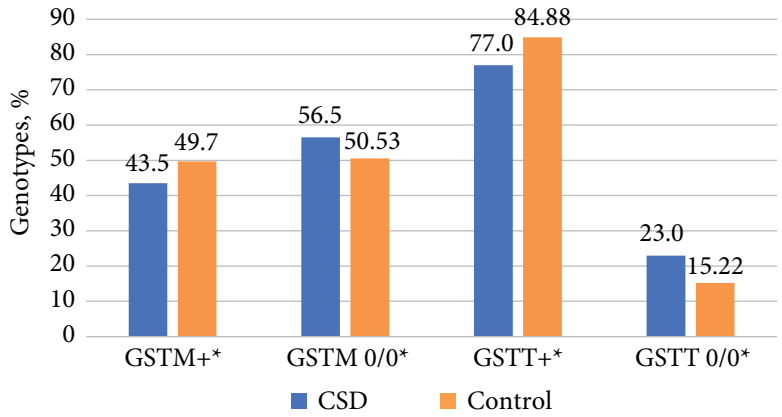

Fig. 2. The frequency of occurrence of genotypes for genes *GSTM1* and *GSTT1*, % (* $p < 0.05$ compared to the control): CSD — congenital deformities of the spine

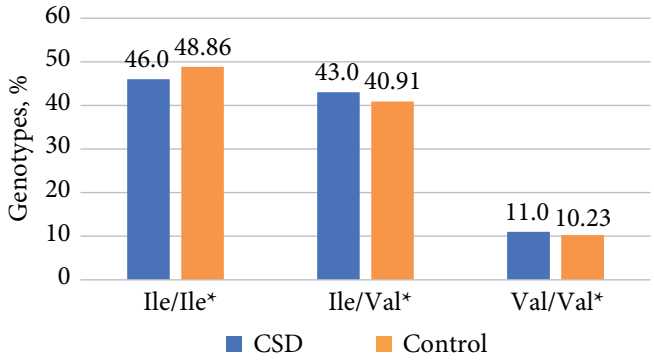

Fig. 3. The frequency of occurrence of genotypes for the *GSTP1* gene (Ile105Val), % (* $p > 0.05$ compared to the control): CSD — congenital spinal deformities

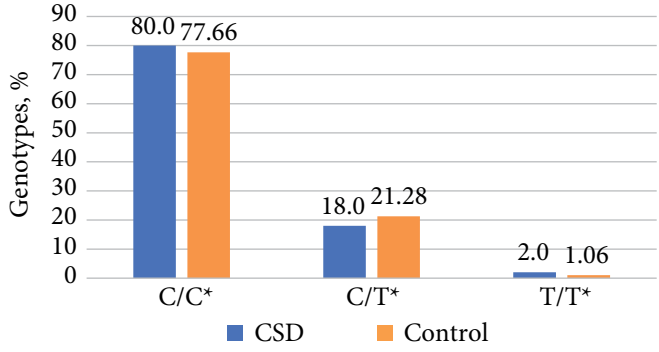

Fig. 4. The frequency of occurrence of genotypes for the *GSTP1* (c114T) gene, % (* $p > 0.05$ compared to the control): CSD — congenital spinal deformities

embryogenesis and congenital malformations of the spine.

When the *GSTT1* gene is deleted, the enzyme is also not formed; thus, the body's ability to metabolize xenobiotics decreases. This effect is especially significantly with simultaneous deletion of the *GSTM1* and *GSTT1* genes.

In the group of patients that was examined, 79.5% had deletions of the *GSTM1* or *GSTT1* genes, and 13.5% showed deletion of both genes. This may be an important factor in CSD etiology. In the control group, the deletion of one or another gene of this group was 65.77%, and that of both genes was 9.47% ($p < 0.05$).

There are two polymorphisms in the *GSTP1* gene, 105Val and 114Val, wherein an enzyme with reduced activity is produced. Thus, the body's susceptibility to the effects of mutagens and carcinogens is increased. This disadvantage is aggravated further with simultaneous carriage of these polymorphisms with deletion of the *GSTM1* gene.

Our study revealed no significant differences in the distribution of *GSTPI* genotypes in patients with CSD and in the control group. However, 8.5% of the CSD patients had a joint carrier of homozygotes on the minor allele with the zero allele of the *GSTM1* gene that was not registered in the control group. This may also be a predisposing factor for CSD.

The *NAT2* gene refers to the second-phase genes of the detoxification system and encodes the enzyme N-acetyltransferase 2 that is responsible for N-acetylation and o-acetylation reaction of heterocyclic amines that possess mutagenic and carcinogenic activity. Carrying "slow" alleles of the *NAT2* gene reduces enzyme activity, increasing the body's sensitivity to this group of mutagens and carcinogens.

In the study of the *NAT2* gene, an increase by 35.46% (9.13% versus 6.74%) in the frequency of the homozygous genotype of the slow allele acetylizer *6/6 (G590A) was revealed in CSD patients compared with that in the control group.

The *XRCC1* gene is a DNA repair gene that effectively restores single-strand breaks formed by ionizing radiation and alkylating agents. Arg399Gln polymorphism is located in a functionally important region of the gene. The replacement of arginine with glycine changes the conformation of the protein product and reduces its reparative activity.

The *XRCC3* gene also belongs to the category of DNA repair genes and contributes to the maintenance of chromosome stability and DNA damage repair.

The study of the alleles of the Arg399Gln polymorphism of the *XRCC1* gene showed no significant differences from that in the control group; nevertheless, the percentage of the mutant allele in the homozygous state was higher than that in the CSD patients.

With respect to the *XRCC3* gene, the difference in the distribution of the allelic variants of the Thr241Met polymorphism between the CSD patients and the control group was more significant (Table 2).

It is also noteworthy that the carriers of the mutant allele among the CSD patients accounted for 46.36%, while that in the control group accounted for only 21.87%.

All the CSD patients who were examined had at least one mutant allele of detoxification and reparation genes in the heterozygous state. However, from our viewpoint, the indicator of homozygous carriage of mutant alleles of the studied genes is more important. In CSD patients, this indicator was 83% (62.2% in the control group).

The combination of several mutant alleles in the homozygous state is even more significant. Such an indicator was revealed in 53% of the CSD patients, significantly different from that in the controls (22.5%).

To create a clear algorithm for the overall assessment of molecular genetic disorders (suscep-

Table 2

Distribution of genotypes by the *XRCC3* gene in patients with CSD and those in the control group

Gene	Genotype	CSD, %	Control group, %
XRCC3	C399C C399T T399T	53.64* 33.66* 12.7*	78.13 13.54 8.33

Note: * $p < 0.05$ compared with control.

tibility to CSD and prediction of their course) when compared with clinical data and their interpretation, further application of modern bioinformatics methods is required.

Conclusion

This study enabled us to obtain a large array of data with various combinations of mutant alleles of the genes of detoxification and DNA repair in children with congenital deformities of the thoracic and lumbar spine. We performed a comparative analysis of the frequency of the identified combinations of gene polymorphisms studied in patients and controls, comparing them with the results of the clinical picture and radiation diagnostics. Most patients (83%) with congenital spinal deformities had mutations of the candidate genes in the homozygous state, and the carriage of several mutant alleles in CSD patients exceeded this figure in the controls by more than 2 times.

Based on the present results, it was possible to reveal a part of the adverse genetic burden that contributes to the onset and progression of this severe pathology. We determined that in children with multiple and combined malformations of the spine, there is a higher number of mutations of the genes for detoxification and DNA repair. The results enable us to suggest, to a certain extent, the nature of the course of congenital deformity of the spine in young patients. However, further research is necessary for the final evaluation and identification of the molecular and genetic criteria of the progressive pattern of congenital spinal deformity in children.

Additional information

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Ethical review. Patients (their representatives) agreed to the processing and publication of personal data.

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