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The Association between the 5-HTTLPR Polymorphism of the Serotonin Transporter Gene with the Development of Somatoform Disorder in Children

Ассоциация полиморфизма гена 5-HTTLPR серотонинового транспортера с развитием соматоформного расстройства у детей

Abstract

The purpose of the study is to reveal the association of 5-HTTLPR polymorphism, including the single nucleotide polymorphism rs25531 (A/G) of the polymorphic region of the L-allele, with the risk of development of somatoform disorder (SD) in children.
Materials and methods. There were examined 94 children with SD. The average age of children was 13.4±2.1 years. The control group consisted of 32 children. Serum serotonin was determined with a biochemical method. Genetic polymorphism was determined with PCR.
Results. In children with SD, the S/S 5-HTTLPR polymorphism is 1.5 times more common and the S/L polymorphism is 1.9 times less frequent than in children without SD. The average serotonin level in children with SD was 1.16±0.37 μmol/l, in children without SD – 1.35±0.34 μmol/l ($p<0.012$). The level of serotonin in children with SD and the presence of S/S allele was 1.15±0.39 μmol/l, and it was lower, if compared to children without SD ($p<0,014$). Serotonin levels in children with SD and S/L were 1.24±0.34 μmol/l. High-expressing 5-HTT rs25531 (A/G) polymorphism of L_A/L_A in children with SD was 2.9 times less frequent, if compared with children, who did not have SD; and the average-expressing 5-HTT polymorphism S/L_A – 1.7 times less frequent.
Conclusion. The risk of development of SD with the presence of S/S allele increases by 2.96 times (odds ratio – OR 2.96±0.42, where 95% CI, 1.29–6.78). The risk of development of SD with the presence of S/L allele decreases by 0.37 times (OR 0.37±0.43; 95% CI, 0.16–0.86). The presence of the S/S allele may be a factor of impaired serotonin metabolism and, accordingly, increase the risk of SD and comorbid depression and anxiety.
Keywords: somatoform disorder, 5-HTTLPR, polymorphism, serotonin, children.

Резюме

Цель. Установление взаимосвязи полиморфизма 5-HTTLPR (короткий S- и долгий L-аллель), включая однонуклеотидный полиморфизм rs25531 (A/G) полиморфной области L-аллеля, с риском развития соматоформного расстройства (СР) у детей.

Материалы и методы. Обследовано 94 ребенка, у которых было диагностировано СР. Средний возраст детей составлял 13,4±2,1 года. Контрольную группу составили 32 обследованных ребенка. Определение в сыворотке крови серотонина проводили биохимическим методом. Определение генетического полиморфизма проводили методом ПЦР.

Результаты. У детей с СР-полиморфизм S/S («аллель риска») 5-HTTLPR встречался в 1,5 раза чаще, а полиморфизм S/L встречается в 1,9 раза реже по сравнению с детьми без СР. Средний уровень серотонина у детей с СР составлял 1,16±0,37 мкмоль/л, у детей без СР – 1,35±0,34 мкмоль/л (95% ДИ, 0,04–0,33; p<0,012). Уровень серотонина у детей с СР и наличием S/S-аллели составлял 1,15±0,39 мкмоль/л и был ниже по сравнению с детьми без СР (95% ДИ, 0,04–0,36; p<0,014). Уровень серотонина у детей с СР и наличием S/L составлял 1,24±0,34 мкмоль/л. Высокоэкспрессирующий 5-HTT rs25531 (A/G) полиморфизм L_A/L_A у детей с СР в 2,9 раза встречался реже по сравнению с детьми, которые не болели СР, а среднеэкспрессирующий 5-HTT полиморфизм S/L_A в 1,7 раза реже.

Выводы. Риск развития СР с наличием S/S-аллели увеличивается в 2,96 раза (отношение шансов – ОШ 2,96±0,42, где 95% ДИ 1,29–6,78). Риск развития СР с наличием S/L-аллели уменьшается в 0,37±0,43 раза (ОШ 0,37±0,43, где 95% ДИ 0,16–0,86). Наличие аллели S/S может быть фактором нарушения обмена серотонина и, соответственно, увеличивать риски развития как СР, так и коморбидных с ним депрессии и тревоги.

Ключевые слова: соматоформное расстройство, 5-HTTLPR, полиморфизм, серотонин, дети.

■ INTRODUCTION

Somatoform disorder (SD) connects a group of psychogenic diseases characterized by pathological symptoms reminiscent of somatic disease, but in which, according to the survey, there are no morphological manifestations, although there are often nonspecific functional disorders [1, 2]. These symptoms lead to numerous referrals to the doctor, additional unnecessary research and manipulation, and in children cause family and social maladaptation, impaired educational functioning, causing considerable psychosocial distress [3].

With the release of the DSM-5 SD were renamed "Somatic Symptoms Disorder". "Somatic symptom disorder" – is characterized by somatic symptoms that are either very unpleasant or lead to significant impairment of function, as well as significant thoughts about the severity of the symptoms, high levels of anxiety about the symptoms and appropriate behavior in relation to these symptoms [4].

It has been found that psychosocial stress, especially in interpersonal relationships in the family, can be an important factor in the development of SD in children [5].

However, it is known that the development of various psychiatric disorders, more often than not, arises as a result of a combination of environmental factors and genetic factors, of which genetic polymorphism plays a special role, which consists in single nucleotide substitutions or tandem repeats of nucleotide regions.

Unlike mutations that lead to pathological changes and reduce viability, genetic polymorphism is less pronounced in the phenotype. However, genetic polymorphism is not always neutral, but more often it leads to the appearance of protein products with slightly altered physicochemical properties and, accordingly, parameters of functional activity [6].

According to scientific studies, various single-point polymorphisms of alleles of genes of transmitters or their transporters determine the strength of the organism's response to a psycho-traumatic situation. Thus, "susceptibility genes" are alleles of mutants that, immediately after birth, may not be clinically apparent but, under certain adverse conditions, lead to the development of a disease [6].

One of the key neurotransmitters that plays an important role in human emotional reactions is serotonin. Serotonin (5-HT) is involved in the regulation of important biological and mental mechanisms, including mood, and may be associated with the development of many psychiatric disorders. Studies of the influence of the gene polymorphism of the 5-HTT gene-linked promoter region (5-HTTLPR) of the serotonin transporter (SERT or 5-HTT) and serotonin receptors have demonstrated significant associations with many psychiatric disorders [7].

Thus, the involvement of the serotonin receptor polymorphism SLC6A4 rs4795541 and HTR2A rs6311 was found in the development of depression in patients with irritable bowel syndrome (IBS), and that the HTR2C rs6318 polymorphism may be associated with a predisposition to anxiety disorders [8].

Among the genes of the serotonin system, the gene of the serotonin transporter (5-HTT) has goten special attention [9].

Correlations were found between serotonin transporter gene expression and environmental factors. Polymorphism of the serotonin transporter in addition to life stressful situations may determine the onset of a depressive episode or other disorders [10].

5-HTT is a high-affinity Na^+ – dependent presynaptic transporter, located in the cell wall and responsible for the reverse absorption and transport of serotonin from the synaptic cleft, thus replenishing the serotonin stores in neurons. The gene encoding 5-HTT is located on the long arm of 17 chromosomes (17q11.1-q12) and consists of 14 exons encoding 630 amino acids [7, 11].

Polymorphism (5-HTTLPR, rs4795541) (serotonin-transporter-linked polymorphic region) is associated with insertions or deletions of 44 bp long, localized in the 5'-promoter region of the SLC6A4 gene encoding a serotonin vector, including a long L-allele consisting of 16 repeats and a short S-allele consisting of 14 repeats. Genotype combinations may include S-homozygous (S/S), S/L heterozygous, or L-homozygous (L/L). Cells homozygous for the L (L/L) allele have three or more times higher basal activity with 5-HTT mRNA production than in cells with the S allele. Thus, a polymorphism with a long L-allele leads to an increase in gene expression and correspondingly more 5 -HTT in the cell membrane, which increases the re-entry of serotonin into the presynaptic cleft with its replenishment [7, 10, 11].

Decreased transcriptional activity of the S-allele ("risk allele") may be associated with an increased amygdala response, leading to anxiety-related personality traits, major depressive episode, suicide attempts, and bipolar disorder. On the contrary, the increased transcriptional activity of the L-allele has a protective effect on the development of depression, but is also associated with complete suicide, nicotine addiction and attention deficit hyperactivity disorder (ADHD) [7, 11].

Carriers of the short allele S were also shown to have lower amygdala volume and activity than carriers of the LL genotype [12].

It was further discovered that the expression of the 5-HTTLPR gene may depend on an additional single nucleotide polymorphism (SNP) rs25531, which is to replace adenine with guanine in the sixth nucleotide of the L allele [13].

The L allele in combination with the rs25531 G allele (L_G genotype) and the S_A allele with respect to mRNA expression are functionally equal. It means the replacement of adenine by guanine at the sixth nucleotide of the L allele leads to a decrease in the levels of the L allele transcription, to the level of the short allele S. Thus, only the L-allele with adenine at the sixth nucleotide (L_A genotype) is associated with relatively high transcriptional activity [14].

The combination of two polymorphisms (5-HTTLPR/rs25531) is shown as a triple locus, L_A, L_G, and S (trial polymorphism), in which the L_G allele is functionally equivalent to the S allele [15].

Heterozygous carriers of the S/L_G allelic polymorphism 5-HTTLPR/rs25531 are more sensitive to the effects of negative emotional signals than homozygotes for the L_A allele [16].

In fact, today it can be assumed that some people are more likely to develop depression and other disorders that occur after exposure to negative factors, and this tendency may be in the human genome [17].

Therefore, one of the important tasks of research on the development of SD in children with comorbid or associated with affective states is to search for polymorphisms of genes that determine susceptibility / resistance to stress.

■ PURPOSE

The aim of the study was to establish a relationship between 5-HTTLPR polymorphism (short S- and long L-allele), including the genotype of the single nucleotide polymorphism (SNP) rs25531 (A/G) polymorphic region of the L-allele, with the risk of developing somatoform disorder in children.

■ MATERIALS AND METHODS

The studies were performed on the clinical basis of the Department of Pediatrics, Obstetrics and Gynecology Faculty of Postgraduate Education, National Pyrogov Memorial Medical University, Vinnytsya (Ukraine) in the gastroenterological, cardiology, nephrological and neurological departments of the Khmelnytskyi Regional Children's Hospital, Khmelnitskyi (Ukraine).

After the informed consent of parents and children was signed, 94 patients were diagnosed with SD with an average age of 13.4±2.1 years (M±σ), of whom 39.4% (n=37) and girls – 60.6% (n=57). In the gastroenterology department of SD 36 children were diagnosed with cardiology, 15 children, nephrology in 16 children, and neurology in 27 children. In addition, 32 children with an average age of 13.2±2.0 years (M±σ) were examined, who were treated in somatic wards and did not have emotional disorders that made up the control group.

The selection of children and the diagnosis of SD were performed after a general clinical examination and according to the criteria of CD ICD-10. To identify the presence of depression and its clinical symptoms in a selected

group of children with SD, the Children's Depression Inventory – CDI (M. Kovacs, 1992) was used to assess the affective and cognitive symptoms of depression. The overall normal CDI score may range from 0 to 54, 50 being the critical value after which the depth of symptomatology increases [18].

To investigate anxiety, was used the Spielberger test questionnaire (STPI – State Trait Personal Inventory) modified by A.D. Andreeva (1988). Spielberger's questionnaire modified by A.D. Andreeva, reveals the level of cognitive activity, anxiety and anger as an existing condition and as a personality trait. The scale consists of two parts. The minimum score on each scale is 10 points, the maximum is 40 points [19].

In all 111 children diagnosed with SD, blood samples were taken to determine serum concentrations of serotonin, which were determined in the certified laboratory of Diagnostics Plus LLC, Kharkiv (Ukraine).

Serotonin was examined by a biochemical method. This transmitter is known to be found predominantly in platelets, so it is determined in whole blood after protein precipitation with chlorine lime. For purification and separation of serotonin use its property at alkaline pH to go into the organic phase, with acid – into aqueous. The analysis is completed by measuring the fluorescence of the condensation products of serotonin with ninghydrin.

Genetic studies were performed to determine the genetic polymorphism of the serotonin transporter (5-HTT), which is associated with insertions or deletions 44 bp long, localized in the 5'-promoter region of the SLC6A4 gene encoding the serotonin 5-HTTLPR (serotonin-transporter-linked polymorphic region), and includes the long (L) and short (S) alleles affecting the transcription rate of serotonin, as well as the SNP of the promoter region of the 5-HTT gene at point rs25531, which replaces A at G in the sixth repetition of the L- and S-alleles. This substitution inhibits the transcription of the L-allele encoding serotonin synthesis.

Determination of genetic polymorphism was performed by PCR at the State Institution "Institute of Gerontology. D.F.Chebotaryov NAMS of Ukraine, in the laboratory of epigenetics with the use of the AmpliPrime DNA Sorb-B reagent kit (manufacturer of NextBio, Russia).

The study was carried out in accordance with the principles of the Declaration of Helsinki. The study protocol was approved by the Local Ethics Committee for all participants.

Statistical analysis was performed using Statistica 8.0.360 software package, MedCalc.7.4.4.1. Quantitative features are given as M±σ (arithmetic mean±rms deviation).

The statistical processing of the material used the absolute risk (AR) definition of the exponential group (ie the risk of developing the disease under the influence of the risk factor in this group) and the non-exponential group or Experimental Event Rate (EER) and Control Event Rate (CER). The attributable risk (AtR) was determined to determine the extent to which a significant authority contributes a given risk factor to the incidence rate. In addition, relative risk (RR) and odds ratio (OR) were determined. The potential damage index was calculated – the required number of individuals who are under the influence of a provoking factor for the development of a single disease.

The likelihood of differences was assessed using a two-tailed Student's t-test and building a 95% confidence interval (CI) for the mean difference,

as well as using the Fisher's exact, X^2 test with Yates' correction and the Pearson's coefficient. The values at $p<0.05$ were considered significant.

■ RESULTS

In our research SD in children was mainly manifested as gastrointestinal symptoms (functional dyspepsia, nausea, vomiting, loss of appetite, diarrhoea, constipation) in 27 (28.7%) children, irritable bowel syndrome (IBS) in 8 (8.5%), various pain syndromes (fibromyalgia, joint pain, back pain, chest pain) in 6 (6.4%), thermoregulation disorder in 6 (6.4%), cardiorespiratory symptoms (cardiac, lump in one's throat, coughing, shortness of breath or sensation of suffocation, heart palpitations, heart flutter and hyperventilation syndromes) in 30 (32.9%), neurogenic dysuric disorders (pain during urination, pain in or around the genital area) in 16 children (17.1%).

It should be noted that in all patients with SD there were significant anxiety disorders that could be a factor in the tendency to develop SD and a key cause of their occurrence. Thus, according to the questionnaire, among 94 children, moderate anxiety was detected in 30 (31.9%) children and severe personal anxiety in 64 (68.1%) children.

In general, in the study of the entire cohort of children with SD, 48 (51.0%) of children showed depression. Also, according to the questionnaire, among 48 children with existing depression, 14 (29.2%) were able to identify mild depressive disorders, moderate depression was found in 27 (56.2%) children, and severe depression in 7 (14.6%) children.

Among the children we tested for SD, the homozygous 5-HTTLPR (L/L) variant was found in 5 (5.4%) individuals, four of whom (80%) had excessive anxiety, but no depressive disorders were observed in any child. Homozygous (S/S) variant of this gene was detected in 68 (72.3%) children, among them 9 (13.2%) showed depression, 27 (39.7%) had excessive anxiety, 21 (30.9%) were noted as signs of depression and manifestations of excessive anxiety (fig. 1).

Among the 21 children (22.3%), a heterozygous variant of this gene (S/L) was identified. Among these children, 6 people (28.6%) had depression, 1 (4.7%) had severe anxiety, and 5 children (23.8%) had both depression and anxiety.

As the diagram shows, the presence of the L allele is probably protective for the development of depression.

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In a survey of 32 non-SD children, S/S polymorphism was detected in 15 children, accounting for 46.8%, in 14 children (43.7%) S/L, and in 3 (9.5%) L/L. Fig. 2 shows the percentage of children with different variants of the allele.

As can be seen from the above, in children with SD allelic S/S polymorphism in the gene encoding transporter synthesis is found 1.5 times more frequent than in children without SD. The absolute risk (AR) of the exponential group (ie, the rate of development of SD under the action of

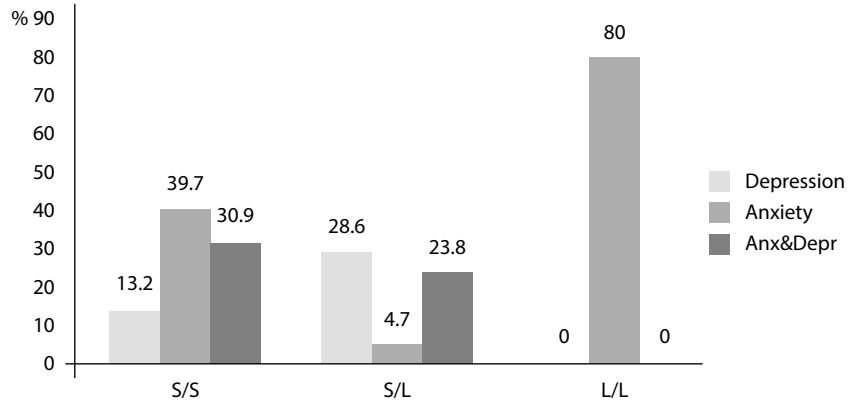

Fig. 1. Structure of anxiety-depressive disorders in children with CD depending on the allelic polymorphism 5-HTTLPR

S/S allele in this group) in patients is 82±4% (95% CI, 74.2–89.8%), whereas the AR of the non-exponential group (ie, the frequency of development SD under the action of S/L and L/L alleles in this group) is 60±5% (95% CI, 50.2–69.8%). Thus, absolute rates of incidence of SD in the groups that are and are not under the influence of a risk factor (in this case the S/S allele) are obtained. An attributive risk (AtR) is determined to determine how important the authority contributes to this risk factor in increasing the incidence of SD. AtR is 22±8.2% (95% CI, 6–38%), ie the presence of the S/S allele increases the average incidence of SD by 22±8.2%. By determining the relative risk (RR), it is possible to determine how many times the incidence of SD in the presence of the S/S allele is increased. However, RR is 1,36±0,13 (95% CI, 1.04–1.76), ie the presence of the S/S allele leads to an increase in the incidence of SD by 1,36±0,13 times. Calculating the odds ratio (OR), it was found that in children with S/S, the OR allele is 2.96±0.42 (95% CI, 1.29–6.78), ie, the risk of developing SD with S/S allele increases 2.96 times. Calculating the potential damage index (PDI), which in this case is 4,5, it can be noted that in the presence of S/S allele in each 4,5 exposed patient (with S/S allele), SD will develop. Fisher's exact test for these samples is p=0.02 and can be a reliable indication that the presence of this polymorphism is a predictor of SD development. The value of X^2 is 6,887, with a Yates correction of 5,800, respectively, the dependence of the incidence of SD on the presence of the S/S allele is statistically significant ($p<0.009$ and $p<0.017$, respectively). When assessing the strength of the link between the risk factor (S/S allele) and the development of SD, the Pearson coefficient is 0.322, indicating an average link strength.

In our researc, it was found that S/L polymorphism occurs 1,9 times less in children with SD compared with children without SD. The absolute risk of the exponential group (ie, the incidence of SD under the action of the S/L allele in this group) in patients is 60±4.8% (95% CI, 50.6–69.4%), whereas the AR of the non-exponential group (ie the incidence of SD under the action of S/S and L/L alleles in this group) is 80±4.0% (95% CI, 72.2–87.8%). Thus,

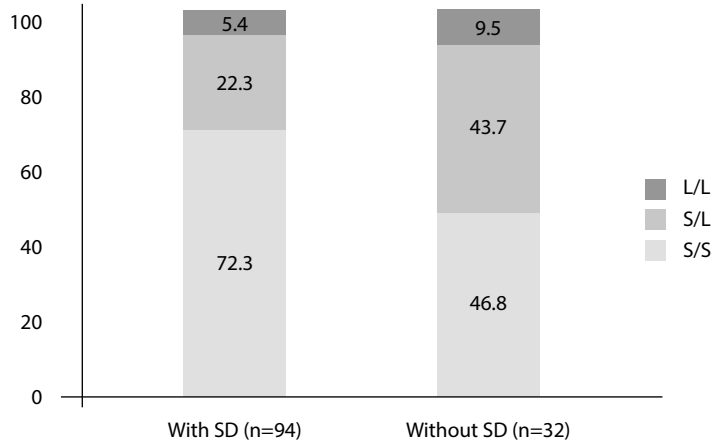

Fig. 2. Frequency of 5-HTTLPR allelic polymorphism in children

the absolute incidence of SD in the groups that are and are not at risk (in this case the S/L allele) is obtained. Attributive risk is determined by determining the extent to which a significant authority contributes a given risk factor to increasing or decreasing the incidence of SD. The AtR is –20±8.6% (95% CI, –36.9 – –3.1%), ie the presence of the S/L allele reduces the average incidence of SD by 20±8.6%. By determining the relative risk, it is possible to determine how many times the incidence or reduction of SD in the presence of the S/L allele is increased. In this case, the RR is 0.75±0.14 (95% CI, 0.56–0.99), ie, the presence of the S/L allele reduces the incidence of SD by 0.75±0.14 times. Calculating the odds ratio, it was found that in children with S/L, the OR allele is 0.37±0.43 (95% CI, 0.16–0.86), ie the risk of developing SD with S/L The L allele decreases by 0.37 times. Fisher's exact criterion for these samples is p=0.024 and can be a reliable indication that the presence of this polymorphism is a predictor of reducing the risk of developing SD. In this case, the value of X^2 is 5,455, with a Yates correction of 4,400, respectively, the dependence of the rate of decrease in the development of SD on the presence of the S/L allele is statistically significant (p<0.02 and p<0.036, respectively). When assessing the strength of the link between the risk factor (S/L allele) and the decrease in the development of SD, the Pearson coefficient is 0.288, indicating a moderate link strength.

The aim of the study was also to identify the dependence of the variant allelic polymorphism and serum serotonin levels on the development of SD.

Children with SD showed a decrease in serum serotonin levels compared with children without SD. The mean serotonin level in children with SD was 1.16±0.37 μmol/l, in children without SD the serotonin level reached 1.35±0.34 μmol/l (95% CI, 0.04–0.33, p<0.012).

The level of serotonin in patients with SD with S/S allele was 1.15±0.39 μmol/l and also exceeded its level compared with children without SD (95% CI, 0.04–0.36, p<0.014). Serotonin levels in children with SD and presence of S/L were 1.24±0.34 μmol/l, and although not significantly higher than children on SD and presence of S/S allele (p>0.05) (fig. 3).

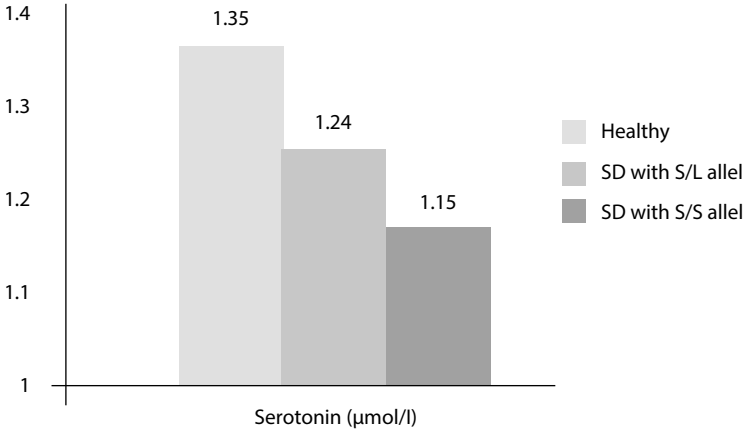

Fig. 3. Serotonin levels depending on genetic polymorphism (5-HTTLPR) in children

A single nucleotide polymorphism (SNP) of the 5-HTTLPR promoter region of the 5-HTT gene at rs25531 (A/G) encoding serotonin synthesis, which is replacing A by G in the sixth repetition of the L and S alleles, was determined separately in children. This substitution inhibits the transcription of the L-allele.

The combination of the two polymorphisms (5-HTTLPR and rs25531) is considered as a triallelic locus including three significant alleles of L_A, L_G, and S_A [14].

No significant polymorphisms have been established to date in the development of SD in children.

In our research of common polymorphism (5-HTTLPR / rs25531), children with and without SD were represented in table.

It should be noted that high-expressing 5-HTT polymorphism of L_A/L_A in children on SD was 2.9 times less frequent compared with children who did not suffer from SD, and the average-expressing 5-HTT polymorphism of S/L_A was 1.7 times less that may also indicate a possible projective effect on the development of SD in children of these alleles, which needs further confirmation with a larger sample of patients.

Children with SD were also twice more likely to be found, along with the low-expressing 5-HTT allele S/S, the low-expressing 5-HTT allele S/L_G, and the low-expressing 5-HTT allele L_G/L_G, which was not determined in healthy children.

Combined 5-HTTLPR / rs25531 polymorphism in children with SD and without SD

	Genotype															
	L_A/L_A		**L_A/L_G**		**L_G/L_G**		**S/L_A**		**S/L_G**		**S_A/S_G**		**S_A/S_A**		**Total**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Children with SD	3	3.2	1	1.0	1	1.0	20	21.3	1	1.0	6	6.4	62	66.0	94	100
Children without SD	3	9.4	0	0	0	0	12	37.5	2	6.3	1	3.1	14	43.7	32	100

■ DISCUSSION

According to the research, a separate role of serotonin and its 5-HTT transporter in the development of such disorders has been identified. The relationship between 5-HTT associated with the 5-HTTLPR polymorphic site (in particular, carriers of short S-alleles) and adverse stressful events that occurred in childhood were associated with a greater tendency for stressors and risk of developing mental disorders such as depression, anxiety and aggression [20]. Also, the presence of a short S-allele of the same polymorphism may be a prognostic factor for impulsivity in ADHD and adolescent alcohol addiction [21]. With respect to SD, it may be noted that a meta-analysis of 25 studies, including 3443 patients with IBS, showed that mutation in the 5-HTTLPR polymorphic site was associated with development with constipation (IBS-C) but not IBS-D and IBS-M [22].

In our observation it was found that the presence of the S/S genotype moderately increases the likelihood of developing SD in children by 22.0±8.2% (95% CI, for AtR, 6–38%) and increases the risk of its occurrence in 2.96±0.42 times (95% CI, for OR, 1.29–6.78) and thus carriers of this genotype may be at risk for the development of SD and need special attention in the process of development and upbringing and influence of environmental factors and emotional stress to reduce the risk of developing and preventing mental disorders in adulthood.

With respect to the S/L polymorphism variant, it can be suspected that the risk of emotional distress may be lower compared to the S/S genotype due to the presence of a long allele L. Thus, in the Colombian study, the S/L genotype was associated with the development of depressive disorders in patients of all ages, however, the S/S genotype was more significantly associated with the development of such disorders in young adults compared to the S/L genotype [23].

On the contrary, we have received data confirming our assumptions that the presence of the S/L genotype does not increase the risk of developing SD in children, but on the contrary may reduce it by 20.0±8.6% (95% CI, for AtR, –36.9 to –3.1%) or 0.37 times (95% CI, for OR, 0.16–0.86) and thus may have a projective effect on the possibility of development of SD in children.

It is likely that the presence of a long allele L may reduce the psychopathological response to stress and have a projective effect on the effects of stress transmitted during SD in children.

The 5-HTTLPR polymorphic site associated with the 5-HTT gene plays an important role in the modulation of mood and behavior by regulating 5-HTT expression and thus controlling serotonin concentration at synapses. The homozygous short allele (S/S) in 5-HTTLPR leads to decreased expression of 5-HTT in combination with a more pronounced psychopathological response to stress compared to the homozygous long allele (L/L) and heterozygous S/L allele [24].

A study of SPC patients with Irritable bowel syndrome showed that its subtype with diarrhea (IBS-C) is associated with elevated serotonin levels, and its subtype with constipation (IBS-D) is associated with a decrease in serum serotonin levels. Because tryptophan is a precursor of serotonin (5-HT) but is predominantly catabolized via the kynurenine pathway, it has been suggested that this pathway may also be involved in the pathogenesis

of IBS due to deviation of tryptophan from the 5-HT pathway leading to 5-HT deficiency [25].

Such disorders may play a role in the development of SD in children. It is possible that serotonin levels can be influenced by many mechanisms, starting with the conversion of tryptophan and its various metabolic pathways and genetic factors.

Possible effects on serotonin concentrations and transcription of 5-HTT of different polymorphic genotypes may also determine the response and effectiveness of the use of selective serotonin reuptake inhibitors (SSRIs), the main mechanism of action of which is 5-HTT suppression. .

One study showed the effect of 5-HTTLPR polymorphism on the efficacy and tolerability of selective serotonin reuptake inhibitors (SSRI). It has been found that the use of SSRI class antidepressants in carriers of the S/S genotype is ineffective and may be poorly tolerated, so antidepressants with other mechanisms of action should be used in such cases [26].

The data we hold indicate that the presence of the S/S allele may be a factor in impaired serotonin metabolism (95% CI, 0.04–0.36, $p<0.014$) and, accordingly, increase the risk of developing both SD and comorbid depression and anxiety (fig. 3).

We also did not find a separate relationship between allelic polymorphism and the incidence of comorbid depression or anxiety in a group of children with SD. Thus, depression in children with SD was found in 41 children, of whom 30 (73.2%) had the S/S allele and 11 (26.8%) the S/L allele. While no depression was observed in 53 children, the S/S allele was present in 38 (71.7%), the S/L allele in 10 (18.8%) and 5 (9.5%) L/L allele.

These results require further research with the study and comparison of different variants of somatoform disorders and in a larger sample of patients, taking into account age, gender and concomitant pathologies, which may determine or exclude a certain role of serotonin in the development of a particular somatoform disorder and associated emotional disorders.

Expression of the 5-HTTLPR gene may depend on an additional single nucleotide polymorphism (SNP) rs25531, which is to replace adenine with guanine in the sixth nucleotide of the L allele [13].

The combination of the two polymorphisms (5-HTTLPR and rs25531) is considered as a triallelic locus including three significant alleles of L_A, L_G, and S_A [14].

This single-nucleotide polymorphism (SNP) uses only 5-HTT. Combining the G-allele (rs25531) with the L-allele (5-HTTLPR) requires the addition of 5-HTT transcription to the level of the S-allele in order to suppress its G-allele into the L-allele. Thus, you use genotypes and using their transcriptional functionality, individual numbers exist in the genotype, you can classify the following rank: high transcriptional activity 5-HTT (genotype L_A/L_A), average transcriptional activity 5-HTT (genotype S_A/L_A, L_A/L_G) and 5-HTT (genotype S_A/S_A, S_A/S_G, S_A/L_G) is used with low transcriptional activity [27].

According to the results of the use of genotypes, it was observed that the nominal rate of hypertension in the human population is found to be 1% [13]. African-American compared to European subjects with low high low frequencies found motifs S allele (0.25 vs 0.43) and genotype S/S (0.06 vs 0.19) for the 5-HTTLPR polymorphism, and more height terrain of the G allele at rs25531 polymorphism (0.21 versus 0.075) [28].

Research has shown that combined or trial polymorphism (5-HTTLPR / rs25531) can actually be developed by major depressive sweeps using carrier infants of S or L_G allelites [29].

Thus, in children with SD, the value of the effect of combined polymorphism (5HTTLPR, rs25531) S_A/S_G and S_A/S_A where adenine replacement by guanine does not affect the expression of 5-HTT and are related to low-expression 5-HTT alleles can be fully interpolated allele S/S. Also, the triallelic polymorphism of S/L_A and S/L_G, in patients with SD in 95.2% of cases revealed the expression of the 5-HTT allele S/L_A, and therefore the data in the total obtained by the allele S/L, can also be interpolated to polymorphism S/L_A. The very low incidence of trial polymorphism L_A/L_A, L_A/L_G, L_G/L_G did not allow us to assess their effect on the risk of developing SD in children, since this polymorphism was only found in 5 children, of whom 60% showed high-expressing 5-HTT allele, and 20% medium-expression and low-expression 5-HTT allele.

Obtained data can be important in understanding the development of mental disorders, the impact of various factors, both external and biological factors on their occurrence. However, they should be interpreted with caution, as confirmation of the data obtained requires further more in-depth studies involving more patients and research methods. Also, SD may manifest themselves in different organ systems, which in future observations requires the formation of separate groups of patients suffering from a particular form of SD, as well as factors that influence their development to identify key mechanisms for their occurrence, including genetic polymorphisms and impaired neurotransmitter exchange.

■ CONCLUSIONS

In children with SD, triallal low-expression 5-HTT polymorphism S/S (S_A/S_G and S_A/S_A) in the gene encoding the synthesis of the serotonin 5-HTTLPR transpotter occurs 1.5 times more frequently, whereas the average-expressing 5-HTT polymorphism S/L_A and high-expression 5-HTT polymorphism of L_A/L_A occur respectively 1.9 and 2.9 times less than in children without SD. Other trial polymorphisms occur in isolated cases.

The presence of the S/S allele, or the triallelic S_A/S_G and S_A/S_A polymorphism increases the average incidence of SD by 22±8.2%. The risk of developing SD with the presence of S/S allele increases 2.96-fold (OR 2.96±0.42 where 95% CI, 1.29–6.78) and indicates that the presence of this polymorphism is a predictor of SD development in children (p=0.02), and the incidence of SD has a statistically significant dependence on the presence of the S/S allele, as evidenced by the value of X^2 (p<0.009), with a Yates correction (p<0.017). The link strength between the presence of the S/S allele and the development of SD has an average link strength (r=0.322).

The presence of an S/L allele or a tri-allelic S/L_A polymorphism reduces the average chance of SD disease by 20±8.6%. The risk of developing SD with the presence of S/L allele decreases by 0.37±0.43 times (95% CI, 0.16–0.86) and indicates that the presence of this polymorphism is a predictor of decreased risk of SD in children (p=0.024), and the incidence rate of SD cases has a statistically significant dependence on the presence of the S/L allele, as evidenced by the value of X^2 (p<0.02), with a Yeats correction

($p<0.036$). The strength of association between the presence (S/L) of the allele and the decrease in the development of SD has a moderate binding force ($r=0.288$).

The presence of the S/S allele or its trial polymorphism may be a factor in impaired serotonin metabolism and therefore increase the risk of both SD and comorbid depression and anxiety.

Presumably, the presence of the allelic variant L/L or its trialelic L_A/L_A polymorphism has a protective effect on the development of SD in children, but needs further, with a whimsical study.

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