ANALYSIS OF ASSOCIATIONS OF GENETIC MARKERS WITH THE DEVELOPMENT OF CONGENITAL SCOLIOSIS

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Objective. To study the associations of single-nucleotide polymorphisms: rs6570507 in GPR126 gene, rs1800795 in IL-6 gene, rs1800469 in TGFB1 gene, rs731236 in VDR gene, rs625039 and rs11598564 polymorphisms in LBX1 gene, and rs12946942 in SOX9 gene with congenital scoliosis.

Material and Methods. The study included 90 patients with verified congenital anomalies of the spine (single and multiple malformations of the spine, ICD-10 Code: Q76.3) and 157 clinically healthy volunteers without diagnosed spinal deformity and without family history of spinal malformations or osteoarticular system diseases. Molecular genetic testing was performed by PCR with real-time registration of a signal from the developed oligonucleotides used to determine rs6570507, rs1800795, rs1800469, rs625039, rs11598564, rs12946942, and rs731236 polymorphisms. Reference sequences were selected from the dbSNP database, and sequence design was performed on the BLAST platform. Data analysis was performed using the R free software computing environment. Data were compared using Pearson's χ^2 test, and 95 % confidence interval limits were calculated to assess the significance of OR.

Results. Statistically significant association of the G allele and GG genotype of the rs1800795 polymorphism in the interleukin-6 gene with congenital scoliosis was found in group of Russian patients ($p < 0.001$). No significant association of alleles and genotypes of polymorphic variants of rs6570507, rs1800469, rs625039, rs11598564, rs12946942, and rs731236 with congenital scoliosis was found.

Conclusion. The rs1800795 polymorphism can be considered as a promising marker for molecular genetic diagnostics of congenital scoliosis.

Key Words: congenital scoliosis, single-nucleotide polymorphisms, SNPs, association of genetic markers.

Please cite this paper as: *Klyuchnikov DYu, Filatov EYu, Tyumin IV, Tyumina OV. Analysis of associations of genetic markers with the development of congenital scoliosis. Hir. Pozvonoc. 2022;19(2):33–39. In Russian.*

DOI: http://dx.doi.org/10.14531/ss2022.2.33-39.

Congenital scoliosis is frontal or sagittal spine curvature, caused by abnormal vertebral segmentation and development [1].

Congenital scoliosis accounts for about 10% of spinal deformities [2]. The incidence of congenital spine anomalies ranges from 0.5 to 1.0 per 1,000 of liveborns [3]. The cause of congenital vertebral malformations may be disorders that occur at the stage of somitogenesis. [4].

Congenital spinal deformities are progressive in 50 % of cases, and patients with this disease need constant monitoring.

Currently, congenital anomalies of spine are detected with ultrasound screening. However, the possibilities of ultrasound in prenatal diagnostics of such anomalies are limited. Spine curvature may not be obvious at birth and diagnostics can fail. Therefore, developmental anomalies are often detected in the postnatal period much later, when an extended primary scoliotic curve, quite often with structural compensatory curves, have already been formed [5].

In addition to ultrasound diagnostics, congenital scoliosis can be detected using X-ray methods, but they create undesirable radiation exposure on patients.

In this regard, studying the genetic nature of the onset of congenital scoliosis is of great interest in order to develop early molecular genetic screening tests identifying the risks of the disease onset and progression. It is known that abnormal vertebral segmentation can be associated with more than 150 genetic disorders [6]. Data given by different authors, however, are contradictory and have not been reproduced in other studies.

The objective is to study the association of polymorphisms array with the risk of the development of congenital scoliosis in order to assess the possibilities of early molecular genetic testing of this pathology.

Despite the fact that congenital and idiopathic scolioses are clinically different, their pathogenetic mechanisms are similar [7]. Therefore, SNPs with the described association with the development of congenital and idiopathic scoliosis in different groups of patients were selected for analysis: polymorphism rs6570507 in GPR126 gene [8], rs1800795 in IL-6 gene [9], rs1800469 in TGFB1 gene [10], rs731236 in VDR gene [11], polymorphisms rs625039 and rs11598564 in LBX1 gene [12], as well as rs12946942 in SOX9 gene [13].

Materials and Methods

The study included 90 patients aged from 10 months up to 16 years, who underwent treatment at the National Ilizarov Medical Research Centre for Traumatology and Orthopaedics in 2019–2020, with verified congenital spine anomalies (single and multiple malformations of the spine, ICD-10 code: Q76.3 congenital

scoliosis caused by bone malformation). Also, the study included 157 clinically healthy volunteers aged 19–49 years without a diagnosed spinal deformity who did not have family history of spinal developmental pathology and diseases of the osteoarticular system. All patients or their legal representatives as well as volunteers from the comparison group, gave written informed consent to collecting biomaterial and conducting research. This study was performed on the basis of the immunological typing laboratory of the Dynasty Medical Center, the study protocol was approved by the Ethics Committee of the Centre as of January 11, 2019. The research was carried out in accordance with the WMA Declaration of Helsinki – Ethical Principles for Medical Research Involving Human Subjects with amendments as of 2000.

Venous blood samples and buccal epithelium scrapings were collected as a DNA source. Real-time PCR was performed on CFX96, BioRad and iQ5, Biorad (USA) amplifiers using the designed oligonucleotide sequences to determine rs6570507, rs1800795, rs1800469, rs625039, rs11598564, rs12946942, and rs731236. Reference sequences were selected from dbSNP database [14], sequence design was performed on Basic Local Alignment Search Tool (BLAST) platform [15].

The data analysis was performed with R free software computing environment (v.3.5.1) [16], the nominal data were compared using Pearson's 2 test, and 95 % confidence interval limits were calculated to assess the significance of OR (95 % CI).

Results

Allele and genotype frequency distributions in the study (n = 90) and the control (n = 157) groups by the analyzed polymorphisms rs11598564, rs12946942, rs1800469, rs625039, rs1800795, rs6570507, and rs731236 are given in Tables 1, 2.

rs11598564. Comparison of the allele carriership of the rs11598564 polymorphism in the study and control groups showed that the odds ratio (OR) for carriership of the G allele against the A allele is equal to 1.118 (95 CI 0.769–1.624). An analysis of a recessive model GG against AA + AG gives OR for the GG genotype as a risk factor at 1.235 (95 CI 0.688–2.215).

rs12946942. When comparing the study and the control groups, OR of the T allele as a risk factor is equal to 1.086 (95 CI 0.615– 1.917), and the TT genotype against GG + GT – 1.144 (95 CI 0.188–6.979).

rs1800469. OR of the G allele against an alternative A allele in the sample analysis is equal to 1.152 (95 CI 0.770–1.724), and the homozygote genotype GG against AA + AG – 1.260 (95 CI 0.749–2.120).

rs1800795. Analyzing this polymorphism, it is found out that OR of the G risk allele against C risk allele is equal to 2.152 (95 CI 1.456–3.182). At the same time, 2 = 15.011, higher than the critical value, the significance of 2 < 0.001. OR of the genotype GG against CC + CG is equal to 2.764 (95 CI 1.602–4.770), at the same time the confidence interval limits, 2 = 13.686 and the significance level of 2 < 0.001 indicate the confidence of the GG genotype association.

rs625039. OR of the G allele as a risk factor against the A allele is equal to 1.368 (95 CI 0.76–2.464), and the GG genotype against AA + AG is equal to 1.344 (95 CI 0.704–2.564).

rs6570507. An analysis of this polymorphism shows that the odds of the G allele against an alternative A allele is 1.324 (95 CI 0.860–2.038), the GG genotype against AG + AA – 1.247 (95 CI 0.729–2.132).

rs731236. An analysis demonstrates that the odds of the A allele variant against G is 1.247 (95 CI 0.835–1.861), the AA homozygote genotype against AG + GG – 1.201 (95 CI 0.713–2.024).

The results of OR of alleles and genotypes are given in Tables 3, 4.

The determined alleles and genotypes in the analyzed polymorphic loci were more frequent in the group of patient with congenital spine pathology: the G allele (OR 1.324) and the GG genotype (OR 1.247) for rs6570507 in GPR126 gene; the G allele (OR 1.152) and the GG genotype (OR1.26) for rs1800469 in TGFB1 gene; the A allele (OR 1.247) and the AA genotype (OR 1.201) for rs731236 in VDR gene; the G allele (OR 1.368) and the GG genotype (OR1.344) for rs625039 in LBX1 gene; the G allele (OR 1.118) and the GG genotype (OR 1.235) for rs11598564 of LBX1 gene; the T allele (OR 1.086) and the TT genotype (OR 1.144) for rs12946942 in SOX9 gene. However, confident association was found only for the G allele and the GG genotype for rs1800795 in IL-6 gene. Thus, it is shown that carriership of the GG genotype can increase the risk of congenital scoliosis by 2.7 times.

Discussion

Among the studied polymorphisms, a confident association was found for the GG genotype of the rs1800795 polymorphism. This polymorphism was identified on the short arm of the 7th chromosome (chr7:22727026 (GRCh38.p13) in the promoter region of IL-6 gene. The presence of the G allele in the genotype is associated with the enhanced expression of IL-6 gene [17, 18]. It is known that vertebral bodies are formed from somites as a result of budding from presomitic mesoderm, mediated by expression of the FGF, Wnt genes and genes of the Notch signaling pathway [19], while downregulation of Notch signaling pathway is observed at an elevated level of IL-6 [20]. The evidence suggests that a signal pathway disorder, which decreases Notch pathway signaling and regulatory failure of this process, are quite important in the pathogenesis of congenital spine deformities [21].

In view of the association with scoliosis, rs1800795 has been analyzed in several studies with contradictory results. It is detected that carriers of the GG genotype have a higher risk of developing adolescent idiopathic scoliosis among the representatives of the European race [9] which was not detected in the Asian population [22]. The literature also points out that such polymorphism is reliably associated with the severity of the disease [23]. The data obtained as a result of our

Table 1
Allele frequencies

Polymorphism	Allele	Study group (n = 90)		Control group (n = 157)	
		frequency	number	frequency	number
rs11598564	A	0.46	79	0.49	150
	G	0.54	93	0.51	158
rs12946942	G	0.88	158	0.89	273
	T	0.12	22	0.11	35
rs1800469	A	0.28	51	0.31	97
	G	0.72	129	0.69	213
rs1800795	C	0.31	55	0.49	143
	G	0.69	125	0.51	151
rs625039	A	0.10	18	0.13	41
	G	0.90	158	0.87	263
rs6570507	A	0.23	42	0.29	81
	G	0.77	138	0.71	201
rs731236	A	0.72	129	0.67	209
	G	0.28	51	0.33	103

Table 2
Genotype frequencies

Polymorphism	Genotype	Study group (n = 90)		Control group (n = 157)	
		frequency	number	frequency	number
rs11598564	AA	0.221	19	0.234	36
	AG	0.477	41	0.506	78
	GG	0.302	26	0.260	40
rs12946942	GG	0.778	70	0.792	122
	GT	0.200	18	0.188	29
	TT	0.022	2	0.020	3
rs1800469	AA	0.089	8	0.090	14
	AG	0.389	35	0.445	69
	GG	0.522	47	0.465	72
rs1800795	CC	0.144	13	0.265	39
	CG	0.322	29	0.442	65
	GG	0.533	48	0.292	43
rs625039	AA	0.011	1	0.026	4
	AG	0.182	16	0.217	33
	GG	0.807	71	0.757	115
rs6570507	AA	0.067	6	0.121	17
	AG	0.333	30	0.333	47
	GG	0.600	54	0.546	77
rs731236	AA	0.489	44	0.449	70
	AG	0.456	41	0.422	69
	GG	0.056	5	0.109	17

research confirm the described studies in a group of the Russian patients and allow us to attribute the GG genotype of the rs1800795 polymorphism to the risk factors for developing congenital scoliosis.

The results on the frequencies of alleles and genotypes of rs1800469 differ from the results obtained on a sample of the patients from Moscow region, where the OR for the G allele is 1.73 and the GG genotype is 4.82 in comparison with the results obtained in the experiment – 1.152 and 1.260, respectively [10]. The results on the frequencies of the GG genotype of the rs731236 polymorphism in VDR gene differ from those described in the other group of Russian patients [11].

Some differences were also noted in the frequencies of the rs1800795 and rs731236 alleles in the control group and the frequencies given in the databases of the ALFA, gnomAD-Genomes, 1000 Genomes projects and described for Caucasoids. Allele frequencies in the control group are presented in Table 5.

On the one hand, a statistically unconfirmed association may be due to the small sample of the study and relative heterogeneity of the patients. On the other hand, the data of some studies are not reproduced in other populations due to the difference of ethnic origin that is confirmed by experimental data on the difference in the allele frequencies of the rs1800795 and rs731236 polymorphisms between the control group (inhabitants of the Middle Volga region) and the data in the European populations. The difference in the frequencies of alleles and genotypes in populations speaks in favor of forming relevant comparison groups based on population features.

Congenital scoliosis can be a polygenic disease, and polymorphisms can have different severity or affect only in combination. Introduction of risks calculation for development of congenital or idiopathic scoliosis using genetic markers requires additional research, particularly, of intergenic interactions and non-genetic environmental factors. The risk of congenital spinal disorders is also associated with pregnancy course, diabetes and maternal smoking [27, 28].

Conclusion

A frequency association of the G allele and the GG genotype of the rs1800795 polymorphism with congenital scoliosis was revealed on the example of a group

Table 3
Allele odds ratio

Polymorphism	Allele	OR (95 CI)	χ^2	p
rs11598564	A	0.895 (0.616–1.301)	0.340	0.560
	G	1.118 (0.769–1.624)		
rs12946942	G	0.921 (0.522–1.625)	0.081	0.776
	T	1.086 (0.615–1.917)		
rs1800469	A	0.868 (0.580–1.300)	0.472	0.492
	G	1.152 (0.770–1.724)		
rs1800795	C	0.465 (0.314–0.687)	15.011	0.0001
	G	2.152 (1.456–3.182)		
rs625039	A	0.731 (0.406–1.316)	1.099	0.295
	G	1.368 (0.760–2.464)		
rs6570507	A	0.755 (0.491–1.162)	1.634	0.202
	G	1.324 (0.860–2.038)		
rs731236	A	1.247 (0.835–1.861)	1.162	0.281
	G	0.802 (0.537–1.198)		

Table 4
Genotype odds ratio

Polymorphism	Model	OR (95 CI)	χ^2	p
rs11598564	GG	1.235 (0.688–2.215)	0.502	0.479
	AA + AG			
rs12946942	TT	1.144 (0.188–6.979)	0.021	0.885
	GT + GG			
rs1800469	GG	1.260 (0.749–2.120)	0.759	0.384
	AG + AA			
rs1800795	GG	2.764 (1.602–4.770)	13.686	0.0002
	CG + CC			
rs625039	GG	1.344 (0.704–2.564)	0.807	0.370
	AG + AA			
rs6570507	GG	1.247 (0.729–2.132)	0.650	0.421
	AG + AA			
rs731236	AA	1.201 (0.713–2.024)	0.475	0.491
	AG + GG			

of the Russian patients (n = 90). The obtained results show that rs1800795 can be considered as one of the promising candidates for diagnostic markers of congenital scoliosis development, and require validation.

Molecular genetic screening tests in combination with other methods for early diagnostics of congenital scoliosis development will allow early risks diagnosis and the patients stratification. It will give an opportunity to assign timely and effective treatment and will enhance the patients' quality of life.

Phenotypic heterogeneity within the groups with congenital spinal deformities and a limited number of studies on small samples remain the main difficulties in determining the genetic basis for the development of congenital and idiopathic scoliosis. Therefore, it is important and expedient to develop not only a system for registering patients with congenital spinal deformities, but also biobanking the genetic material of patients for testing and validating research results.

The authors are grateful to Natalya Sergeevna Osinovskaya, PhD in Biology, Senior Researcher at the Genomics Laboratory with the Bioresource Collection Group of the Scientific and Research Institute of Obstetrics, Gynecology and Reproductology named after D.O. Ott, for the support in preparing the manuscript for publication.

The study was carried out with the financial support of the Fund for the Promotion of Small Forms of Enterprises in the Scientific and Technical Field within the framework of the 3741ГС1/63150 project.

The authors declare that they have no conflict of interest.

Table 5

Occurrence frequency of polymorphism alleles rs6570507, rs1800795, rs1800469, rs625039, rs11598564, rs12946942, rs731236 [24–26]

Polymorphism	ALFA: European	gnomAD-Genomes, European	1000 Genome, European	Experimental data (comparison group)
rs6570507	G = 0.71	G = 0.71	G = 0.68	G = 0.71
	A = 0.29	A = 0.29	A = 0.32	A = 0.29
rs1800795	C = 0.44	C = 0.46	C = 0.42	C = 0.49
	G = 0.56	G = 0.54	G = 0.58	G = 0.51
rs1800469	A = 0.32	A = 0.30	A = 0.31	A = 0.31
	G = 0.68	G = 0.70	G = 0.69	G = 0.69
rs625039	G = 0.86	G = 0.88	G = 0.86	G = 0.87
	A = 0.14	A = 0.12	A = 0.14	A = 0.13
rs11598564	G = 0.52	G = 0.53	G = 0.54	G = 0.51
	A = 0.48	A = 0.47	A = 0.46	A = 0.49
rs12946942	G = 0.93	G = 0.92	G = 0.91	G = 0.89
	T = 0.07	T = 0.08	T = 0.09	T = 0.11
rs731236	A = 0.60	A = 0.63	A = 0.60	A = 0.67
	G = 0.40	G = 0.37	G = 0.40	G = 0.33

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Received 19.04.2022
Review completed 06.06.2022
Passed for printing 08.06.2022

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