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ASSOCIATION OF ALLELIC VARIANTS OF COMBINED GENETIC RISK OF VITAMIN D DEFICIENCY WITH SEVERE IDIOPATHIC SCOLIOSIS IN RUSSIAN PATIENTS

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Abstract. ***Introduction.*** Idiopathic scoliosis (IS) is a common spinal disorder that affects 2–4% of people worldwide. There is a large research array investigating the relationship between blood vitamin D levels and IS. ***The purpose of the work*** — to study the association between polymorphic variants in the *CYP2R1* and *GC* genes and severe idiopathic scoliosis (SIS) in Russian patients. ***Materials and methods.*** Patients with IS and controls were selected based on the results of preliminary clinical studies. Blood samples were genotyped using allele-specific polymerase chain reaction (PCR). ***Results.*** The distribution of CYP2R1 rs2060793 alleles and the distribution of CYP2R1 rs10766197 genotypes and alleles were statistically different ($p < 0.05$) between patients and controls. No statistically significant differences were found between TIS and the control group for single nucleotide variations in CYP2R1 rs10741657 and GC rs4588, rs842999, rs2282679. Stratification of the combined genetic risk (GSR) score for vitamin D deficiency into three subgroups was performed by dividing the score according to the sum of risk alleles. A statistically significant difference ($p < 0.05$) was found between the groups. Paradoxically, low GSR was detected in patients with TIS more often than in the controls (OR=2.28). The lowest GSR value (0 alleles) was found only in the TIS group, but not in the control group: 0/92 (0%) and 8/91 (9%), respectively. ***Conclusions.*** The results obtained in this study are not sufficient to make any clinical decision on the significance of testing vitamin D metabolites in patients with IS or the therapeutic use of vitamin D preparations. Further studies are needed to determine the influence of genetic factors of vitamin D metabolism on the progression of idiopathic scoliosis, prognosis of its course and choice of treatment tactics.

Keywords: idiopathic scoliosis, vitamin D deficiency, CYP2R1, GC, mutation

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АССОЦИАЦИЯ АЛЛЕЛЬНЫХ ВАРИАНТОВ КОМБИНИРОВАННОГО ГЕНЕТИЧЕСКОГО РИСКА ДЕФИЦИТА ВИТАМИНА D С ТЯЖЕЛЫМ ИДИОПАТИЧЕСКИМ СКОЛИОЗОМ У РОССИЙСКИХ ПАЦИЕНТОВ

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Резюме. ***Введение.*** *И*диопатический сколиоз (ИС) — распространенное заболевание позвоночника, которое встречается у 2–4% людей во всем мире. Имеется большой массив исследований по определению связи между концентрацией витамина D в крови и ИС. ***Цель исследования*** — изучение ассоциации между полиморфными вариантами в генах *CYP2R1* и *GC* и тяжелым идиопатическим сколиозом (ТИС) у российских пациентов. ***Материалы и методы.*** Подбор пациентов с ИС, а также лиц контрольной группы проводился по результатам предварительных клинических исследований. Генотипирование образцов крови проводилось методом аллель-специфической полимеразной цепной реакции (ПЦР). ***Результаты.*** Распределение аллелей CYP2R1 rs2060793 и генотипов и аллелей CYP2R1 rs10766197 статистически различалось ($p < 0,05$) между пациентами и контрольной группой. Не было обнаружено статистически значимых различий между ТИС и контрольной группой для однонуклеотидных вариаций CYP2R1 rs10741657 и GC rs4588, rs842999, rs2282679. Стратификация шкалы комбинированного генетического риска (КГР) дефицита витамина D на три подгруппы была проведена делением шкалы в соответствии с суммой аллелей риска. Было выявлено статистически значимое различие ($p < 0,05$) между группами. Парадоксально, но низкий КГР был выявлен у пациентов с ТИС чаще, чем в контроле (OR=2,28). Самое низкое значение КГР (0 аллелей) было обнаружено только в группе ТИС, но не в контрольной группе: 0/92 (0%) и 8/91 (9%) соответственно. ***Выводы.*** Полученные в данном исследовании результаты не являются достаточными для принятия какого-либо клинического решения о значимости тестирования метаболитов витамина D у пациентов с ИС или терапевтического применения препаратов витамина D. Необходимы дальнейшие исследования, чтобы определить влияние генетических факторов метаболизма витамина D на прогрессирование идиопатического сколиоза, прогнозирование его течения и выбор тактики лечения.

Ключевые слова: идиопатический сколиоз, дефицит витамина D, *CYP2R1*, *GC*, мутация

INTRODUCTION

Idiopathic scoliosis (IS) is a common spinal disorder that occurs in 2–4% of people worldwide, manifesting usually in childhood [22]. The etiology and pathogenesis of IS still remain unclear [5, 23, 27, 31].

Currently, there is an active search for biomarkers to assess severity and progression of the disease, including those at the genetic level [1, 3]. Many studies have been conducted to investigate the relationship between vitamin D concentration and the clinical course of IS. At the same time, a number of studies have shown a genetic predisposition to vitamin D deficiency. However, a direct relationship between IS and the genetic risk of hypovitaminosis D has not been studied [2, 4, 6, 8]. Relatively low plasma levels of vitamin D metabolites in patients with IS have been reported in many trials [16, 21, 32]. In addition, vitamin D deficiency is a possible factor affecting a clinical course of IS. Vitamin D deficiency is common in patients with adolescent IS, and although it is similar to healthy adolescents, a correlation has been found between preoperative back pain scores and the severity of vitamin D deficiency [14]. This observation fits with another research showing that a Cobb angle was negatively correlated with serum vitamin D levels [16]. Moreover, a prospective, randomized, case-control trial showed clinical and radiological

Table 1

SNV of *CYP2R1* and *GC*, associated with vitamin D blood concentrations

Таблица 1

Однонуклеотидные вариации (SNV) в генах *CYP2R1* и *GC*, влияющие на концентрацию в крови метаболитов витамина D

Ген, SNV / Gene, SNV	Регион, популяция / Region, population	Источник данных / Data source
CYP2R1 rs10500804	Арабы / Arabs Датчане / Danes Корейцы / Koreans	[15] [25] [27]
CYP2R1 rs10741657	Датчане / Danes Северная Америка, Европеоиды / North America, Caucasoids Иордания / Jordan Смешанная (обзор) / Mixed (review)	[17–19] [23] [20] [24, 26]
CYP2R1 rs10766197	Европеоиды / Caucasoids Датчане / Danes Смешанная (обзор) / Mixed (review)	[16] [17–19] [26]
CYP2R1 rs11023374	Аляска, американские индейцы / Alaska, Americam Indians	[22]
CYP2R1 rs12794714	Арабы / Arabs	[15]
CYP2R1 rs2060793	Смешанная (обзор) / Mixed (review)	[26]
GC rs1155563	Смешанная (обзор) / Mixed (review)	[26]
GC rs17467825	Арабы / Arabs	[15]
GC rs2282679	Южная Азия, арабы / Southern Asia, Arabs Северная Америка, Европеоиды / Northen America, Caucasoids Смешанная (обзор) / Mixed (review)	[15] [23] [26]
GC rs2298850	Южная Азия, арабы / Southern Asia, Arabs	[15]
GC rs3755967	Южная Азия, арабы / Southern Asia, Arabs	[15]
GC rs4588	Датчане / Danes Иордания / Jordan Смешанная (обзор) / Mixed (review)	[17–19, 25] [20] [21, 26]
GC rs7041	Южная Азия, арабы / Southern Asia, Arabs Иордания / Jordan Датчане / Danes Смешанная (обзор) / Mixed (review)	[15] [20] [25] [21, 26]
GC rs842999	Датчане / Danes	[17–19]

improvement in patients with IS using melatonin, calcium and vitamin D supplementation [15]. These examples support the role of low levels of vitamin D metabolites in IS progression. However, there is still a lack of clarity regarding the role of vitamin D in the etiopathogenesis and clinical course of IS [13, 21].

Vitamin D deficiency or insufficiency may be caused by many factors, such as race, ethnicity, geographic and environmental factors, diet and lifestyle, and individual genetic predisposition. Numerous studies have shown that common genetic variants in the *CYP2R1* (microsomal 25-hydroxylase of vitamin D) and *GC* (vitamin D-binding protein — DBP) genes are associated with lower circulating 25-hydroxyvitamin D concentrations [7, 9–12, 18–20, 24–26, 28, 29]. Notably, the effect of *CYP2R1* and *GC* polymorphic alleles on serum 25-hydroxyvitamin D concentrations was observed in different geographic regions and populations (Table 1), although population genetic features reflect the different significance of each individual allelic variant for the development of hypovitaminosis D.

A combined effect of "unfavorable" *CYP2R1* and *GC* alleles on blood 25-hydroxyvitamin D concentrations was analyzed by J. Nissen et al. [24, 25] in the Danish population. The authors proposed the combined genetic risk scale (GRS — genetic risk score) as the sum of "unfavorable" alleles.

The genetic risk score (range 0–8) was calculated as the sum of G alleles rs10741657, A alleles rs10766197, A alleles rs4588 and C/A alleles rs842999 in each individual [25, 26] and a positive correlation was shown between the GRS and mean plasma 25-hydroxyvitamin D concentration. Thus, the impact of vitamin D deficiency regarding the progression of IS remains unclear. The study of 25-hydroxyvitamin D concentration in patients with IS is a "single snapshot", while individual genetic characteristics form a lifelong pattern.

AIM

The aim of the study was to examine associations between polymorphic genetic variants of *CYP2R1* and *GC* and severe idiopathic scoliosis (SIS) in Russian patients. Six single nucleotide variations were included: rs2060793, rs10766197, rs10741657 of the *CYP2R1* gene and rs4588, rs842999, rs2282679 of the *GC* gene. Associations of each genetic polymorphic variants with SIS and distribution of CGR in patients with TIS and healthy controls were analyzed.

MATERIALS AND METHODS

Patients and Control Group

91 patients with severe idiopathic scoliosis (TIS) and 92 healthy individuals (the control group) were included. The research was approved by the local ethical committee of the institute. All patients had a progressive form of SIS with stage III or IV according to SRS criteria [17]. Patients diagnosed with secondary form of scoliosis were not included. Members of the control group were selected after physical examination. Detailed information about both groups is presented in Table 2.

Genotyping

Genomic DNA was isolated from blood using a DNA isolation kit (Syntol, Moscow, Russia).Allele-specific real-time polymerase chain reaction with SYBR Green I detection was used for genotyping. PCR of each DNA sample was

Table 2

Clinical characteristics of Patients with severe idiopathic scoliosis (SIS) and healthy controls

Таблица 2

Клинические характеристики группы пациентов с тяжелым идиопатическим сколиозом (ТИС) и здоровых лиц контрольной группы

Испытуемые / Subjects	Численность / Number	Возраст (средний возраст) / Age (middle age)	Степень сколиоза / Scoliosis degree	
			III степень	IV степень
Пациенты ТИС / Patients SIS	91 (100%)	6–20 (17,4)	14	77
Женщины / Female	70 (77%)	6–20 (17,6)	11	59
Мужчины / Male	21 (23%)	15–19 (16,9)	3	18
Контрольная группа / Healthy controls	92 (100%)	18–56 (31,6)	–	–
Женщины / Female	82 (89%)	19–49 (28,4)	–	–
Мужчины / Male	10 (11%)	19–56 (35,7)	–	–

performed simultaneously in two wells, each containing one of the allele-specific primers, as well as equal amounts of the second (common) primer and genomic DNA. The PCR mixture contained 400 nM of each allele-specific and common oligonucleotide primer, 0.2 units of SynTaq thermally activated Taq polymerase (Sytol Moscow, Russia) in the appropriate real-time PCR buffer with fluorescein (FAM) and SYBR Green I, 3 mM $MgCl_2$, 1 μM of each dNTP, and 1 μL of DNA solution (8–15 ng). After initial heating at 95° for 5 min, 35 cycles of 10 s each for a denaturation step at 95° and 30 s for a conjugated annealing-synthesis step at 60° were repeated, during which fluorescence data were collected. To confirm the specificity of AS-PCR, melting curve analysis of PCR products between 65° and 95° with a step temperature increase of +0.5° was used. The delta-Ct method was used to recognize homozygous and heterozygous DNA samples. The list of PCR oligonucleotide primers is given in Table 3.

Statistical analysis

An online medical statistics calculator (https://medstatistic.ru/calculators.html) was used for statistical analysis. The χ^2 test was used for intergroup comparison of genotype and allele distributions. The χ^2 test was calculated for contiguity tables of 5×5 or less. The odds ratio (OR) was calculated to assess the effect of statistically different genotypes and alleles in the comparison groups on susceptibility to SIS.

RESULTS

Detailed results of genotype and allele distribution of CYP2R1 and GC in SIS patients and healthy controls are presented in Table 4 and Table 5.

The χ^2 test was used to compare genotype and allele occurrence in SIS group and controls. The G allele rs2060793 of CYP2R1 was predominant in the control group: 129 (69%) versus 105 (58%) in the SIS group, the difference was statistically significant ($p < 0.05$) The difference in genotype and allele distribution of rs10766197 CYP2R1 and alleles was statistically significant ($p < 0.05$) between the SIS group and the control group: 19 (21%) and 29 (31%), respectively. The A allele of rs10766197 CYP2R1 was detected in 105 (56%) cases in the SIS group and 79 (43%) cases in the control group,

Table 3

List of olygonucleotide PCR-primers

Таблица 3

Список олигонуклеотидных праймеров для ПЦР

Ген, SNV / Gene, SNV	Название праймера / Primer name	5'–3' последовательность / 5'–3' sequence
CYP2R1 rs10741657	CYP2R1-657sA	GGGAGATACTTTAGCAGGCA
	CYP2R1-657sG	GGGAGATACTTTAGCAGGCG
	CYP2R1-657aB	TGTCAGCCCTGGAAGACTCA
CYP2R1 rs10766197	CYP2R1-197aG	GGTCCTTTCTGTATCTTGGCAC
	CYP2R1-197aA	GGTCCTTTCTGTATCTTGGCAA
	CYP2R1-197sB	ACCGAATACACAGCAGGCTA
CYP2R1 rs2060793	CYP2R1-793sA	GCCCACCTGGATAATCCCA
	CYP2R1-793sG	GCCCACCTGGATAATCCCG
	CYP2R1-793aB	TTTCCCCCATATCTGCCTCA
GC rs2282679	GC-679aT	CTCTGTCTCTTAATTATCTCACAA
	GC-679aG	CTCTGTCTCTTAATTATCTCACAC
	GS-679sB	TCACAGCCTCAGTTCCTATG
GC rs4588	GC-588sG	ACCAGCTTTGCCAGTTCCG
	GC-588sT	ACCAGCTTTGCCAGTTCCT
	GC-588aB	TAGAATTTTCTTGAGACAGGCA
GC rs842999	GC-999aG	CTTACACTTATACAAGAGCAGC
	GC-999aA	CTTACACTTATACAAGAGCAGT
	GC-999aC	CTTACACTTATACAAGAGCAGG
	GC-999sB	TTTAAGACTTGATGAGCCCTGT

Table 4

Distribution of genotypes and alleles of CYP2R1 in the groups of Patients with severe idiopathic scoliosis (SIS) and Healthy Controls (HC)

Таблица 4

Распределение генотипов и аллелей CYP2R1 в группах пациентов с тяжелым идиопатическим сколиозом (ТИС) и в контрольной группе

Ген, SNV / Gene, SNV		ТИС, (%) / SIS (%)	Контроль, (%) / Controls (%)	χ^2, p
CYP2R1 rs2060793				
Генотипы / Genotypes	GG	32 (35%)	46 (49%)	χ^2=4,9825 p=0,082806
	AG	41 (45%)	37 (40%)	
	AA	18 (20%)	10 (11%)	
	Всего / Total	91 (100%)	93 (100%)	
Аллели / Alleles	G	105 (58%)	129 (69%)	χ^2=5,4038 p=0,020093*
	A	77 (42%)	57 (31%)	
	Всего / Total	182 (100%)	186 (100%)	
Носители аллелей / Allele carries	G	73 (55%)	83 (64%)	χ^2=1,9844 p=0,158931
	A	59 (45%)	47 (36%)	
	Всего / Total	132 (100%)	130 (100%)	
CYP2R1 rs10741657				
Генотипы / Genotypes	GG	38 (42%)	45 (49%)	χ^2=1,4059 p=0,495127
	TG	35 (38%)	34 (37%)	
	TT	18 (20%)	13 (14%)	
	Всего / Total	91 (100%)	92 (100%)	
Аллели / Alleles	G	111 (61%)	124 (67%)	χ^2=1,6319 p=0,201436.
	T	71 (39%)	60 (33%)	
	Всего / Total	182 (100%)	184 (100%)	
Носители аллелей / Allele carries	G	73 (58%)	79 (62%)	χ^2=0,5968 p=0,439785
	T	53 (42%)	47 (38%)	
	Всего / Total	126 (100%)	126 (100%)	
CYP2R1 rs10766197				
Генотипы / Genotypes	GG	31 (34%)	17 (18%)	χ^2= 6,5548 p=0,037726*
	AG	41 (45%)	47 (51%)	
	AA	19 (21%)	29 (31%)	
	Всего	91 (100%)	93 (100%)	
Аллели / Alleles	G	103 (57%)	81 (44%)	χ^2=6,2616 p=0,012338*
	A	79 (43%)	105 (56%)	
	Всего / Total	182 (100%)	186 (100%)	
Носители аллелей / Allele carries	G	72 (55%)	64 (46%)	χ^2=2,1195 p=0,145436
	A	60 (45%)	76 (54%)	
	Всего / Total	132 (100%)	140 (100%)	

* The result is reliable at p<0.05. / Результат достоверен при p <0,05.

Table 5

Distribution of genotypes and alleles of GC in the groups of Patients with severe idiopathic scoliosis (SIS) and Healthy Controls (HC)

Таблица 5

Распределение генотипов и аллелей GC в группах пациентов с тяжелым идиопатическим сколиозом (ТИС) и в контрольной группе

Ген, SNV / Gene, SNV		ТИС, (%) / SIS (%)	Контроль, (%) / Controls (%)	χ^2, p
GC rs4588				
Генотипы / Genotypes	GG	51 (56%)	44	χ^2=2,0387 p=0,360838
	TG	33 (36%)	37	
	TT	7 (8%)	12	
	Всего / Total	91 (100%)	93	
Аллели / Alleles	G	135 (74%)	125 (67%)	χ^2=2,1562 p=0,141995
	T	47 (26%)	61 (33%)	
	Всего / Total	182 (100%)	186 (100%)	
Носители аллелей / Allele carries	G	84 (68%)	81 (62%)	χ^2=0,8234 p=0,364192
	T	40 (32%)	49 (38%)	
	Всего / Total	124 (100%)	130 (100%)	
GC rs2282679				
Генотипы / Genotypes	GG	7 (8%)	13 (14%)	χ^2=2,4248 p=0,297487
	TG	33 (36%)	36 (39%)	
	TT	51 (56%)	44 (47%)	
	Всего / Total	91 (100%)	93 (100%)	
Аллели / Alleles	G	47 (26%)	62 (33%)	χ^2=2,4882 p=0,114702
	T	135 (74%)	124 (67%)	
	Всего / Total	182 (100%)	186 (100%)	
Носители аллелей / Allele carries	G	40 (32%)	49 (38%)	χ^2=0,9092 p=0,340323
	T	84 (68%)	80 (62%)	
	Всего / Total	124 (100%)	129 (100%)	
GC rs842999				
Генотипы / Genotypes	CC	41 (45%)	33 (36%)	χ^2=1,7241 p=0,78633
	GC	30 (34%)	37 (40%)	
	GG	14 (15%)	16 (17%)	
	TC	4 (4%)	4 (4%)	
	TG	2 (2%)	2 (2%)	
	TT	0	1 (1%)	
	Всего / Total	91 (100%)	93 (100%)	
Аллели / Alleles	C	116 (64%)	107 (58%)	χ^2=1,5293 p=0,465495
	G	60 (33%)	71 (38%)	
	T	6 (3%)	8 (4%)	
	Всего / Total	182 (100%)	186 (100%)	
Носители аллелей / Allele carries	C	75 (59%)	74 (55%)	χ^2=0,5783 p=0,748897
	G	46 (36%)	55 (40%)	
	T	6 (5%)	7 (5%)	
	Всего / Total	127 (100%)	136 (100%)	

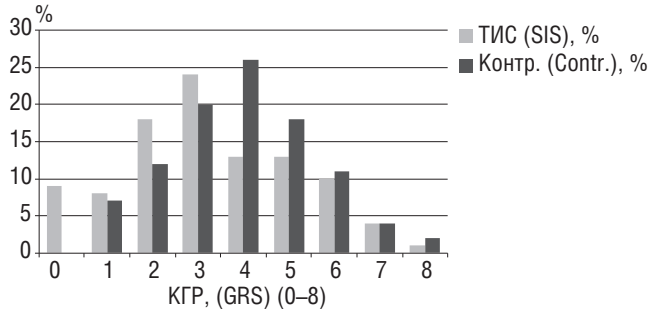

Fig. 1. Distribution the values of the combined genetic risk score (GRS, 0–8 points) for vitamin D deficiency in groups of patients with severe idiopathic scoliosis (SIS) and in the control group

Рис. 1. Распределение значений шкалы комбинированного генетического риска (КГР, 0–8 баллов) дефицита витамина D в группах пациентов с тяжелым идиопатическим сколиозом (ТИС) и в контрольной группе

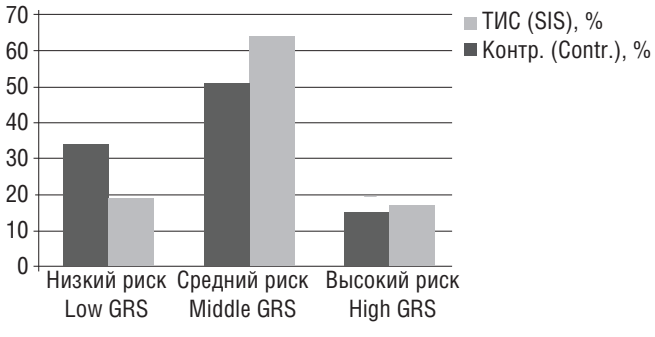

Fig. 2. Distribution of patients with severe idiopathic scoliosis (SIS) and control group individuals into subgroups of risk

Рис. 2. Распределение пациентов с тяжелым идиопатическим сколиозом (ТИС) и лиц контрольной группы на подгруппы риска

the difference was statistically significant ($p < 0.05$). No statistically significant differences were found between SIS group and control group for CYP2R1 rs10741657 and GC rs4588, rs842999, rs2282679 single nucleotide variations.

The genetic risk score (GRS) of susceptibility to vitamin D deficiency (range 0–8) was calculated as the sum of G alleles rs10741657, A alleles rs10766197, A alleles rs4588 and C/A alleles rs842999, in accordance with J. Nissen et al. [21]. Detailed information about distribution of GRS in SIS patients and healthy individuals is presented in the graph (Fig. 1).

Stratification of the GRS into three subgroups was carried out by dividing it by the sum of risk alleles: low GRS — 0 to 2 alleles, medium GRS — 3 to 5 alleles, high GRS — 6 to 8 alleles. The χ^2 test and odds ratio (OR) calculation were used to compare subgroups of low GRS and medium+high GRS for the SIS group and the control group. A statistically significant difference ($p < 0.05$) was found between the groups; low GRS values were more frequent in SIS patients compared to controls (OR=2.28). The lowest GRS value (0 vitamin D deficiency risk alleles) was found exclusively in SIS group alone: 8/91 (9%) vs. 0/92 (0%) (Fig. 2).

DISCUSSION

The significance of vitamin D in IS progression remains uncertain [13]. The aim of our study was to identify a possible direct association between idiopathic scoliosis and common *CYP2R1* and *GC* polymorphic alleles, provided that: 1) relatively low blood levels of vitamin D metabolites are associated with the occurrence and progression of IS; 2) an association between certain alleles in the *CYP2R1* and *GC* genes with susceptibility to hypovitaminosis D has been shown; and 3) CGR calculated as the sum of the number of alleles of the *CYP2R1* and *GC* genes correlates with mean blood levels of 25-hydroxyvitamin D.

Contrary to our expectations, the results show an association of SIS with low GRS of vitamin D deficiency compared to medium and high CRS (OR=2.28). Notably, the lowest GRS value (0 vitamin D deficiency risk alleles) was exclusively found in the SIS group but not in the control group (0% vs. 9%, Figure 1). In addition, the -A risk allele and AA *CYP2R1* rs10766197 genotype were more common in controls.

In order to determine the association between IS and allelic variants of *CYP2R1* and *GC* genes, we used a case-control study design comparing groups at “extreme risk” of predisposition to the disease [30]. Such approach is based on the comparison, on the one hand, of a group with severe clinical manifestations and, on the other hand, of a control group with no risk of IS development. In our opinion, such a research design is justified for testing promising genetic variants before conducting larger trials. However, the study results obtained with this approach of comparing extremes cannot be directly extrapolated to any clinical forms of idiopathic scoliosis.

CONCLUSIONS

The results obtained are not sufficient to make any clinical decision regarding testing vitamin D metabolites in patients with IS or the therapeutic use of vitamin D supplements. Further studies are required to investigate the influence of genetic factors of vitamin D metabolism on the progression of idiopathic scoliosis, to predict its course and, in the long term, to determine rational treatment tactics.

ADDITIONAL INFORMATION

Author contribution. Thereby, all authors made a substantial contribution to the conception of the study, acquisition, analysis, interpretation of data for the work, drafting and revising the article, final approval of the version to be published and agree to be accountable for all aspects of the study.

Competing interests. The authors declare that they have no competing interests.

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Consent for publication. Written consent was obtained from the patient for publication of relevant medical information within the manuscript.

ДОПОЛНИТЕЛЬНАЯ ИНФОРМАЦИЯ

Вклад авторов. Все авторы внесли существенный вклад в разработку концепции, проведение исследования и подготовку статьи, прочли и одобрили финальную версию перед публикацией.

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

Источник финансирования. Авторы заявляют об отсутствии внешнего финансирования при проведении исследования.

Информированное согласие на публикацию. Авторы получили письменное согласие пациентов на публикацию медицинских данных.

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