

## ASSOCIATION STUDY BETWEEN IDIOPATHIC SCOLIOSIS AND *MTNR1B* AND *CHD7* GENE POLYMORPHISMS IN BULGARIAN PATIENTS

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### ABSTRACT

Idiopathic scoliosis (IS) is a primary complex three-dimensional deformity that affects the spine's balance in the frontal plane, and the sagittal and axial planes. A number of studies have examined the role of genetic factors in the development and progression of IS.

We conducted a case-control study aimed to investigate the association between 2 single nucleotide polymorphisms in 2 candidate-genes: *MTNR1B* (*rs4753426*) and *CHD7* (*rs4738824*) and the predisposition to IS in Bulgarian patients.

DNA probes were extracted from peripheral blood of 94 patients with IS and 188 unrelated healthy controls. The genetic variants were analyzed by Fast Real-Time TaqMan PCR technology. The statistical analysis was performed by Pearson's Chi-squared test with SPSS 19.0 for Windows.

This case-control study didn't reveal statistically significant association between *CHD7* and *MTNR1B* gene polymorphisms and the susceptibility to IS among Bulgarian patients. The genotype and allele frequencies of the *MTNR1B* and *CHD7* gene polymorphisms were comparable in patients and controls ( $p > 0.05$ ). A much larger population-based case control study will be needed to investigate the contribution of these polymorphic variants to the development and progression of IS.

The identification of molecular markers with diagnostic and prognostic value could be a useful means for the early detection of children at risk for the development of IS and for prognosis of the risk for a rapid deformity progression in affected children. That would permit prophylaxis and early stage treatment of the patient with the least invasive procedures.

**Key words:** idiopathic scoliosis, *MTNR1B*, *CHD7*, association study

### INTRODUCTION

Idiopathic scoliosis (IS) is a primary complex three-dimensional deformity that affects the spine's balance in the frontal plane, and the sagittal and axial planes.

Since its first documentation by Hippocrates (Hippocrates. Articulations, par. 47), the diagnosis, cause and treatment of IS have been the focus of a great deal of research and yet the aetiology remains enigmatic. A number of studies have examined the role of genetic factors in the development and progression of IS. Genetic complexity is inferred from inconsistent inheritance [1], discordance among monozygotic twins [2, 3] and highly variable results from human genetic studies [4].

With the case-control study design, genes with high biological relevance to the disease would be selected as candidates. A case-control study conducted by Gao et al. in 2007 [5] was the first one which investigated the role of the *CHD7* single nucleotide polymorphisms (SNPs) in the pathogenesis of IS and concluded that the *CHD7* gene constitutes important factor for the genetic predisposition to IS in Caucasian population. *CHD7* is a transcriptional regulator that binds to enhancer elements in the nucleoplasm (OMIM 608892). *CHD7* also functions as a positive regulator of ribosomal RNA (rRNA) biogenesis in the nucleolus [6].

No association between more than twenty genotyped SNPs in the *CHD7* gene and familial IS was found by Tang et al. [7] and Tilley et al. [8], failing to replicate the earlier findings [5]. The absence of association has indicated that common variants in *CHD7* could play a minor role in genetic predisposition to sporadic IS, at least in the Chinese population. Patten et al. [9] demonstrated a key role of *CHD7* in eye, heart, and ear development but not in the onset of skeletal deformities as observed in scoliosis.

One of the leading theories on the etiology of IS is a defect in the melatonin signaling pathway. The data regarding human melatonin levels are controversial and the melatonin deficiency as a causative factor in the etiology of scoliosis cannot be supported. These considerations led to look instead at the melatonin signal transduction pathway because a defect of melatonin signaling activity could generate effects similar to melatonin deficiency [10].

Moreau et al. [11] reported that melatonin signaling is impaired in osteoblasts of patients with adolescent idiopathic scoliosis (AIS). In 2007 Qiu et al. [12] found association between polymorphisms rs4753426 and rs741837 of the promoter of *MTNR1B* gene and the occurrence of AIS, but not with the curve severity and suggested that *MTNR1B* is an AIS predisposition gene in Chinese population. This findings were not confirmed in Japanese population [13] and Caucasian population [14]. No significant differences in the frequencies of the *BMP4*, *Leptin*, *IL6*, *MMP3* and *MTNR1B* polymorphisms were reported between the cases and controls in Hungarian patients. However, particular *IL-6*, *MMP3* and *MTNR1B* combinations appeared significant risk factors for the development of IS [15].

In Bulgaria, in the period of 2012-2015 Nikolova et al. investigated the association between 20 SNPs in 19 candidate-genes and the susceptibility to IS and curve severity and confirmed the association of *ESR1* with the etiology and progression of IS in Bulgarian patients [16].

We conducted a case-control study aimed to investigate the association between 2 single nucleotide polymorphisms in 2 candidate-genes: *MTNR1B* (*rs4753426*) and *CHD7* (*rs4738824*) and the predisposition to IS in Bulgarian patients.

## MATERIAL AND METHODS

DNA probes were extracted from peripheral blood of 94 patients with IS and 188 unrelated healthy controls. All participants in the study were informed about its purpose and were included only after the subjects/families signed their informed consent.

*Patients.* The IS diagnosis was confirmed clinically and radiologically. The curves were measured by the Cobb method. The mean value of Cobb angles was  $54.6 \pm 23.2$ . The mean age at the beginning of the disease was  $11.2 \pm 3.1$  years. Male ( $n=16$ ) and female ( $n=78$ ) patients were included.

*Controls.* The control group including healthy subjects without clinical signs of IS was recruited from a pool of unrelated gender-matched volunteers. The controls were selected among adult patients with skeletal maturity with negative family history of IS. Radiological examination was not performed in the control group.

*Genotyping.* Genomic DNA was extracted from the peripheral blood leucocytes using magnetic bead technology (chemagic DNA Blood Kit special, Chemagen) on automated high throughput nucleic acid isolation platform (chemagic Magnetic Separation Module I, Chemagen). The genetic variants were analyzed by Fast Real-Time TaqMan PCR technology. The genotyping of the selected SNPs of the *CHD7* and *MTNR1B* genes was carried out by using TaqMan SNP Genotyping Assay (Life Technologies) and 7900 Real-time PCR (Life Technologies). The results were analysed with Sequence Detection Software (Life Technologies).

*Statistical analysis.* The statistical analysis was performed by Pearson's Chi-squared test with SPSS 19.0 for Windows. A value of  $p < 0.05$  was considered to be statistically significant for comparison between data sets. Odds ratios (OR) of major versus minor homozygote genotypes and alleles were calculated with 95% confidence interval (95% CI).

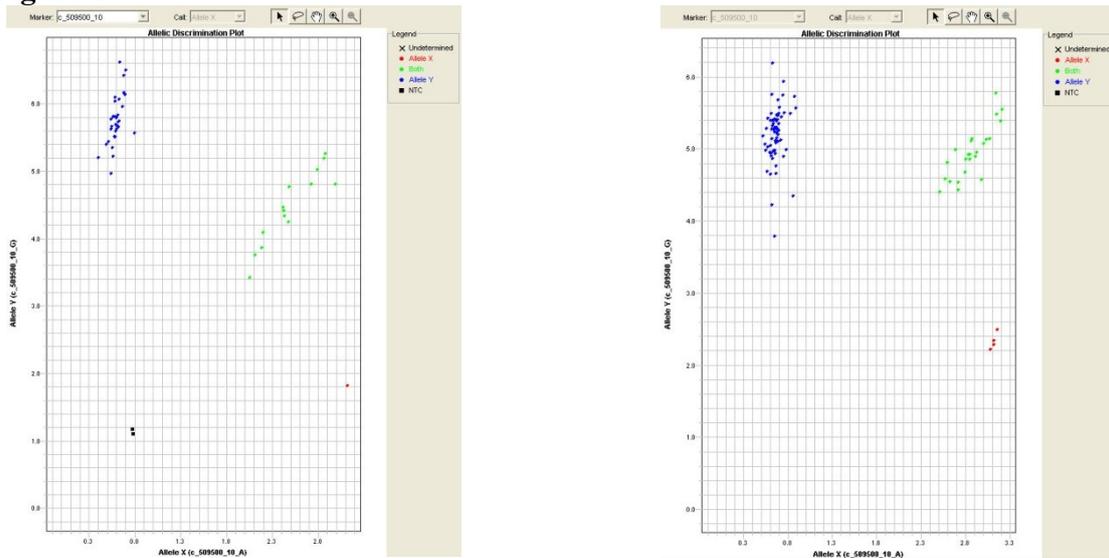
## RESULTS AND DISCUSSION

Predisposition for IS, like other examples of complex traits, does not have a specific assigned risk of heritability, but inheritance is based on multiple factors, potentially both genetic and environmental [17].

This study examined the association between IS and two SNPs: the *CHD7* gene polymorphism (rs4738824 G/A) and the *MTNR1B* gene polymorphism (rs4753426 T/C). Genotypes were in Hardy-Weinberg equilibrium.

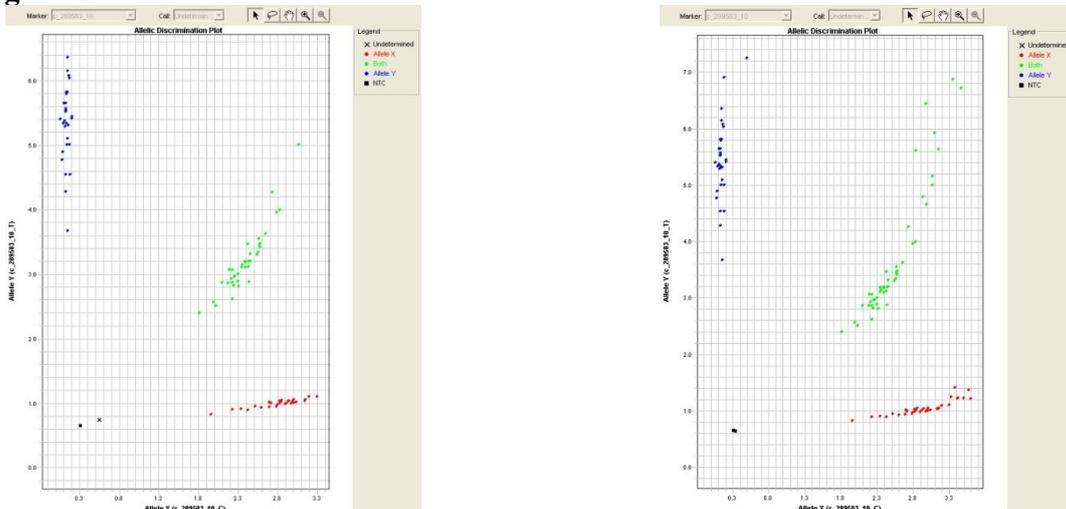
The overall frequencies of the AA and the GG genotype of the *CHD7* polymorphism in the patients with IS were comparable with the controls (GG, 61.7 vs. 63.8% and AA, 2.1 vs. 5.3%,  $p=0.35$ ). In conclusion, a specific genotype was not associated with a higher risk of scoliosis (AA vs. GG,  $p=0.35$ ; OR: 2.4; 95% CI: 0.51–11.39) and the presence of a given allele (A vs. G,  $p=0.92$ ; OR: 1.03, 95% CI: 0.52-2.05) could not be considered as a susceptibility factor to IS. The genotype distribution in cases and controls is presented at Figure 1.

Figure 1.



The frequencies of the CC and the TT genotype of the *MTR1B* polymorphism in the IS patients were also comparable with the control subjects (TT, 20.2 vs. 29.8% and CC, 29.8 vs. 29.3%,  $p=0.19$ ). In conclusion, a specific genotype was not associated with a higher risk of scoliosis (TT vs. CC,  $p=0.25$ ; OR: 1.5; 95% CI: 0.75–2.99) and the presence of a given allele (T vs. C,  $p=0.47$ ; OR: 1.23, 95% CI: 0.7-2.14) could not be considered as a susceptibility factor to IS. The genotype distribution in cases and controls is presented at Figure 2.

Figure 2.



The genotype and allele frequencies of cases and controls are summarised in Table 1.

**TABLE 1. Genotype and allele frequency distributions in patients and controls.**

Gene, Polymorphism	Genotype, Allele	Cases N, %	Controls N, %	p-value
<i>CHD7</i> (rs4738824 G/A)	GG	58 (61.7)	120 (63.8)	0.35
	AG	34 (36.2)	58 (30.9)	
	AA	2 (2.1)	10 (5.3)	
	G	150 (79.8)	298 (79.3)	0.92
A	38 (20.2)	78 (20.7)		
<i>MTNR1B</i> (rs4753426 T/C)	TT	19 (20.2)	56 (29.8)	0.19
	CT	47 (50.0)	77 (41.0)	
	CC	28 (29.8)	55 (29.3)	
	T	85 (45.2)	189 (50.3)	0.25
	C	103 (54.8)	187 (49.7)	

In the subgroup of surgical cases (n=75) where Cobb angle >40° the genotype and allele frequencies of the *CHD7* and *MTNR1B* polymorphisms were comparable between cases and controls (AA vs. GG, p=0.19; OR: 4; 95% CI: 0.5-32.1; A vs. G, p=0.72; OR: 1.13; 95% CI: 0.56-2.28 and TT vs. CC, p=0.26; OR: 1.53; 95% CI: 0.73-3.18; T vs. C, p=0.43; OR: 1.25; 95% CI: 0.72-2.18). Odds ratios of genotypes and alleles are summarised in Table 2.

**TABLE 2. Odds ratios of genotypes and alleles in the general and in the surgical subgroup.**

Subgroup	Genotype, Allele	p-value	OR [95% CI]
General	GG vs. AA	0.35	2.4 [0.51–11.39]
	G vs. A	0.92	1.03 [0.52-2.05]
	TT vs. CC	0.25	1.5 [0.75–2.99]
	T vs. C	0.47	1.23 [0.7-2.14]
Cobb angle >40° (n=75)	GG vs. AA	0.19	4 [0.5-32.1]
	G vs. A	0.72	1.13 [0.56-2.28]
	TT vs. CC	0.26	1.53 [0.73-3.18]
	T vs. C	0.43	1.25 [0.72-2.18]

This case-control study didn't reveal statistically significant association between *CHD7* and *MTNR1B* gene polymorphisms and the susceptibility to IS among Bulgarian patients. The genotype and allele frequencies of the *MTNR1B* and *CHD7* gene polymorphisms were comparable in patients and controls (p>0.05). On the basis of these results the examined polymorphic variants of *CHD7* and *MTNR1B* genes could not be considered as genetic variants with predisposing effect or a risk factor for the progression of the curve. In this way previously reported negative single associations were confirmed [7, 8, 13-15]. A much larger population-based case control study will be needed to investigate the contribution of these polymorphic variants to the development and progression of IS.

In addition, these results don't exclude synergistic effect of the polymorphisms of the *CHD7* and *MTNR1B* genes in the etiology and pathogenesis of IS. Morocz et al. [15] reported positive associations between *BMP4-MTNR1B*, *Lep-MTNR1B* and *MMP3-MTNR1B* genotype combinations and AIS. Their findings suggest that the effect of two or more predisposing genetic variants can be synergistic. In addition, there are probably other potential predisposing and modifying genetic variants of *CHD7* and *MTNR1B* associated with the disease.

The identification of molecular markers with diagnostic and prognostic value could be a useful means for the early detection of children at risk for the development of IS and for prognosis of the risk for a rapid deformity progression in affected children. That would permit prophylaxis and early stage treatment of the patient with the least invasive procedures.

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