

Investigation of *CHL1* Gene Polymorphism as Potential Predictive Biomarker in Idiopathic Scoliosis in Bulgarian Patients

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Abstract

It was previously unraveled that the *CHL1* gene could be considered as a predisposition gene for adolescent idiopathic scoliosis (AIS) in Caucasian population. This is the first study of the possible correlation between the *CHL1* candidate-gene and the susceptibility to primary scoliosis in a Bulgarian population sample.

A total of 105 patients with a primary curve with unknown etiology and 210 unrelated gender-matched control subjects were included. The genotyping of *CHL1* (rs1400180 T/G) was carried out by using TaqMan real-time PCR technology. The statistical analysis was conducted by the Pearson's Chi-squared Test or the Fisher's Exact Test with a value of p less than 0.05 as statistically significant.

The results indicated that *CHL1* (rs1400180) is not associated with susceptibility to idiopathic scoliosis (IS), onset of the disease, family history or gender. In conclusion, the *CHL1* polymorphism could not be considered as a predisposing factor of IS or AIS.

Replication extended case-control studies would be necessary to explore the relationship between the *CHL1* gene and IS in distinct population groups. Predictive biomarkers for IS could be a useful means for early detection of the predisposition among relatives and for prevention of the curve progression in affected children.

Keywords: *idiopathic scoliosis, CHL1, association study, predisposition*

1. Introduction

Idiopathic scoliosis (IS) is a social-significant condition characterized by

phenotypic variability and convincing evidence of a genetic susceptibility.

According to the common disease – common variant hypothesis (CDCV hypothesis), there is an accumulation of common genetic variants creating a predisposition that is triggered under the influence of non-genetic factors. The aim of a genome wide association study (GWAS) is a primary identification of statistically significant associations between common diseases (conditions) and common genetic polymorphisms in a large cohort.

It was previously unraveled that the *CHL1* gene could be considered as a predisposition gene for adolescent idiopathic scoliosis (AIS) in Caucasian population. The first genome study conducted by Sharma et al. [1] reported a list of single nucleotide polymorphisms (SNPs) significantly associated with susceptibility to AIS in North American population. The authors found the most significant association between *CHL1* (rs1400180) and AIS in the discovery cohort of 1122 individuals ($p < 8.10^{-8}$). Then they tested replication of their most significant findings in two follow-up case-control cohorts, obtaining the most significant results when all three cohorts having a total of 3431 individuals were combined. The SNP *CHL1* (rs10510181) was found to be in a strong association with AIS (odds ratio: OR = 1.49, 95% confidence interval: CI = 1.29–1.73, $p = 2.58 \times 10^{-8}$), but this association was not confirmed in a separate GWAS [1].

The *CHL1* gene encodes a neural recognition molecule similar to the roundabout guidance receptor 3 (*Robo3*). Mutations in the *Robo3* gene cause a rare

hereditary syndrome characterized by congenital horizontal gaze palsy and severe progressive scoliosis with usually late onset [2-4]. Although mutations in *Robo3* were associated with progressive scoliosis, the authors of the first whole genome association study found no significant association between AIS and common variants of *Robo3* [1].

Two independent case-control replication studies were performed. Zhou et al. [2012] found no significant association between the deformity and genotype or allele frequencies of *CHLI* (rs10510181, rs2055314, rs331894, rs2272524 and rs2272522) in a Han Chinese population (648 cases/ 573 controls) [5]. Qiu et al. [2014] found no significant association between the clinical phenotype and genotype or allele frequencies of *CHLI* (rs10510181, rs2055314, rs331894, rs2272524 and rs2272522) in a Han Chinese population (500 cases/ 500 controls) [6].

There is no replication study on the possible correlation between the SNP *CHLI* (rs1400180 T/G) and deformity. The main goal of the current study is to investigate the reported association between the *CHLI* (rs1400180 T/G) polymorphism and the genetic predisposition to IS and AIS in Bulgarian patients. This is the first study of the possible correlation between the *CHLI* candidate-gene and the susceptibility to primary scoliosis in an Eastern European population group.

2. Materials and Methods

The study was approved by the University Ethics Committee and was performed according to the ethical principles contained in the Declaration of Helsinki. All participants were included only after their informed consent was signed.

2.1. Materials

A total of 105 Bulgarian patients with a primary curve with unknown etiology and 210 unrelated gender-matched control subjects were referred by the orthopaedic

physicians. IS diagnosis was clinical and radiographic. Secondary deformity including syndromic scoliosis was excluded. The Cobb angle was evaluated radiologically and the mean value was 54.6 ± 22.7 . The mean age at the beginning of IS was 11.2 ± 3.1 years old. Cases were separated according to the age of onset of IS in three subgroups: infantiles ($n = 4$), juveniles ($n = 23$) and adolescents ($n = 78$). Additionally, we included familial ($n = 28$) and non-familial cases ($n = 77$), male individuals ($n = 19$) as well as female individuals ($n = 86$). Primary radiological examination was not performed in the control group consisting of adult subjects with skeletal maturity (≥ 18 years old) for exclusion of IS at a later stage.

2.2. Methods

Total DNA extraction from peripheral blood was performed by using magnetic bead technology on a chemagic Magnetic Separation Module I (Chemagen, Baesweiler, Germany). The genotyping of *CHLI* (rs1400180 T/G) was carried out by using TaqMan SNP Genotyping Assay with catalogue No C_8831533_10 (Life Technologies, NY, USA) in a 7900HT Fast Real-time PCR System (Life Technologies, NY, USA). The results were analysed with Sequence Detection Software (Life Technologies, NY, USA). The statistical analysis was conducted by the Pearson's Chi-squared Test or the Fisher's Exact Test with a value of p less than 0.05 as statistically significant. Odds ratios (OR) with 95% confidence interval (CI) were also calculated (IBM SPSS 19.0, NY, USA).

3. Results and Discussion

This is the first case-control study between the *CHLI* gene and IS in Bulgarian population. We separated the cases in subgroups according to age, gender and family history and then investigated the associations in the general sample and in the different subgroups under genotypic (co-dominant, dominant and recessive) and allelic model.

Genotypes were in Hardy-Weinberg equilibrium. The overall frequencies of the genotypes (TT vs. TG vs. GG, $p = 0.77$) and alleles (T vs. G, $p = 1$) of *CHLI* (rs1400180) in the patients with IS were comparable with the controls. On the basis of these results *CHLI* (rs1400180) could not be considered as a predisposing factor of IS in Bulgarian patients.

An allelic discrimination plot depicting genotyping results for the patients and controls is presented at Figure 1.

The population frequency of AIS, a spinal deformity with onset during the adolescent period is between 2 and 3%, making it the most common IS type [7]. Sharma et al. [2011] found a positive association between susceptibility to deformity and *CHLI* (rs1400180 T/G) but their study included only patients with AIS [1]. There is a need for studies of infantile and juvenile IS. It is possible the participation of different genetic variants in the etiopathogenesis of early and late onset scoliosis [8].

In our study we selected cases among patients with infantile, juvenile and AIS and then we investigated the associations in the general sample and in the different subgroups as well. In the subgroup of adolescents as well as in the subgroup of juveniles the observed frequencies of the genotypes and alleles of *CHLI* polymorphism were comparable between cases and controls ($p > 0.05$). Consequently, *CHLI* (rs1400180) could not be associated with the early or late onset IS.

The results for the frequency of the familial IS are controversial. Early genetic studies of IS have reported about 19% recurrence risk of mild scoliosis (Cobb angle of 10^0 to 20^0) and 11.5% recurrence rate of moderate and severe scoliosis (Cobb angle $> 20^0$) among younger siblings [9-11]. Wise et al. [2008] found that about 16% of the siblings were affected and the estimated sibling risk ratios were comparable to those for other multifactorial disorders such as diabetes

mellitus type 1 and rheumatoid arthritis [12].

Familial (26.7%) and non-familial (73.3%) IS cases were collected. The obtained results were analogous for familial and non-familial cases ($p > 0.05$). Hence, the genotypes and alleles of the *CHLI* (rs1400180) could not be associated with the familial history of IS.

The lateral curvature of the spine was more prevalent in the female patients. There are a number of studies that report higher incidence of IS in females [13-17]. Logically, some genetic factors could also influence on the higher incidence of IS in females than males. Nevertheless, a statistically significant association between the genetic variant of *CHLI* and the deformity was not found for the female patients as well as for the male patients ($p > 0.05$). In conclusion, the genotypes and alleles of *CHLI* (rs1400180) could not be associated with the gender of the IS patients.

The estimated ORs (95% CI) in the general sample and in the separate subgroups are presented at Table 1.

The results indicated that *CHLI* (rs1400180) is not associated with susceptibility to IS, onset of the disease, family history or gender. In conclusion, the *CHLI* polymorphism could not be considered as a predisposing factor of IS or AIS.

These results don't exclude a potential role of the same polymorphic markers in other population groups or impact of other polymorphisms of *CHLI* on the etiology and pathogenesis of IS in Caucasian population. Replication extended case-control studies would be necessary to explore the relationship between the *CHLI* gene and IS in distinct population groups. Predictive biomarkers for IS could be a useful means for early detection of the predisposition among relatives and for prevention of the curve progression in affected children.

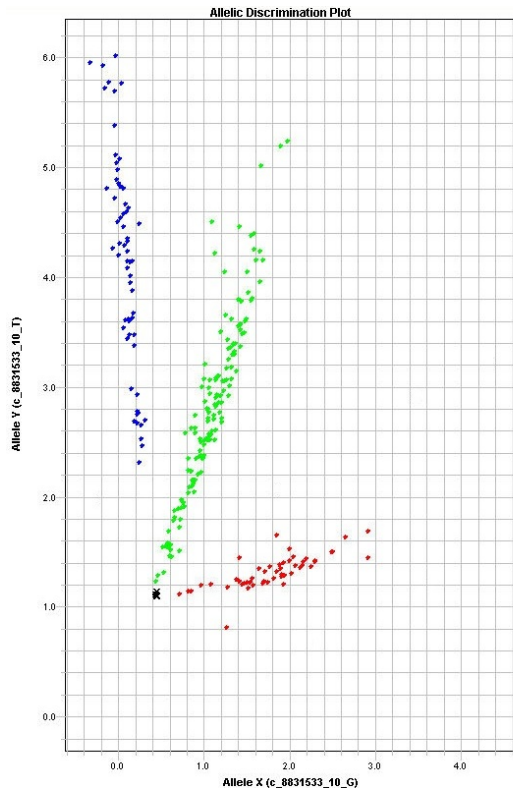


FIGURE 1 - An allelic discrimination plot depicting results from genotyping of *CHLI* (rs1400180 T/G) for patients and controls. Blue color indicates the TT homozygotes that were labeled with fluorescent FAM, red color indicates the GG homozygotes that were labeled with fluorescent VIC, and green color indicates the TG heterozygotes that were labeled with both fluorescent VIC and FAM. ■, negative control; X, undetermined.

Table 1: Odds ratios of genotypes and alleles of *CHLI* (rs1400180 T/G) polymorphism in different subgroups with idiopathic scoliosis (IS)

Subgroup	Genotype, Allele	p	OR [95% CI]
General (n ₁ =105, n ₂ =210)	TT vs. GG	1	1.00 [0.5-1.99]
	TT vs. TG + GG	0.65	1.13 [0.66-1.94]
	TT + TG vs. GG	0.69	0.89 [0.5-1.58]
	T vs. G	1	1.01 [0.72-1.41]
AIS (n ₁ =78, n ₂ =210)	TT vs. GG	0.92	1.03 [0.47-2.23]
	TT vs. TG + GG	0.81	1.08 [0.59-1.96]
	TT + TG vs. GG	0.92	0.97 [0.51-1.85]
	T vs. G	0.92	1.02 [0.7-1.47]
JIS (n ₁ =23, n ₂ =210)	TT vs. GG	0.54	1.65 [0.46-5.85]
	TT vs. TG + GG	0.31	1.66 [0.67-4.15]
	TT + TG vs. GG	1	1.19 [0.38-3.68]
	T vs. G	0.44	1.30 [0.7-2.42]

Familial group (n ₁ =28, n ₂ =210)	TT vs. GG	0.51	0.55 [0.15-2.07]
	TT vs. TG + GG	0.34	0.52 [0.17-1.57]
	TT + TG vs. GG	1	0.92 [0.35-2.40]
	T vs. G	0.48	0.8 [0.45-1.39]
Non-familial group (n ₁ =77, n ₂ =210)	TT vs. GG	0.69	1.16 [0.55-2.45]
	TT vs. TG + GG	0.24	1.41 [0.79-2.51]
	TT + TG vs. GG	0.7	0.88 [0.47-1.67]
	T vs. G	0.61	1.10 [0.76-1.6]
Males (n ₁ =19, n ₂ =38)	TT vs. GG	1	1.23 [0.26-5.85]
	TT vs. TG + GG	0.54	1.63 [0.5-5.31]
	TT + TG vs. GG	1	0.85 [0.21-3.35]
	T vs. G	0.84	1.17 [0.53-2.58]
Females (n ₁ =86, n ₂ =172)	TT vs. GG	0.89	0.94 [0.44-2.03]
	TT vs. TG + GG	0.92	1.03 [0.56-1.89]
	TT + TG vs. GG	0.74	0.90 [0.48-1.69]
	T vs. G	0.89	0.98 [0.68-1.41]

4. Conclusions

The case-control study revealed no statistically significant association between the *CHLI* (rs1400180 T/G) polymorphism and susceptibility to IS in Bulgarian patients.

No genotype or allele of the *CHLI* (rs1400180 T/G) polymorphism was found to be correlated to the age of onset, gender or family history.

Larger case-control studies would be necessary to explore the impact of this *CHLI* genetic variant on the etiopathogenesis of IS in Caucasian population.

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