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Association Study between CHL1 Molecular Marker and Progression of Idiopathic Scoliosis

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Abstract

Several genetic factors associated with a higher risk for rapid curve progression have been proposed. The objective of the present case-control study is to examine the association between the *CHL1* (rs1400180) polymorphism and the progression of IS in Bulgarian population sample.

A total of 105 ambulatory and hospitalised patients with IS and 210 unrelated gender-matched control subjects were selected by the orthopaedic physicians. The genotyping was carried out by using TaqMan SNP Genotyping Assay. The results were analyzed 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.

In conclusion, the *CHL1* gene polymorphism rs1400180 could not be associated with curve severity, curve pattern, bracing and finally with IS progression. Therefore, the examined polymorphic variant could not be considered as a genetic modifying factor for IS.

The identification of molecular markers could be a useful means for a more accurate prognosis of the risk for a rapid progression of the curve. That would permit early stage treatment of the patient with less invasive procedures.

Keywords: *idiopathic scoliosis, CHL1, association, progression*

1. Introduction

The contribution of genetic factors in the etiopathogenesis of idiopathic scoliosis (IS) has been well supported [1]. First, a number of predisposition genes associated with susceptibility to development of IS have been reported [2-12]. In addition, several genetic factors associated with a higher risk for rapid curve progression have been proposed [10-18].

The cell adhesion molecule L1-like (*CHL1*) gene could be regarded as a predisposing candidate-gene in adolescent idiopathic scoliosis (AIS) in Caucasian population. In 2011 Sharma et al. [2011] conducted the first whole genome screening for single nucleotide polymorphisms (SNPs) significantly associated with susceptibility to AIS. The results from the transmission disequilibrium test on a cohort of 419 trios and two replication case–control studies identified some polymorphic variants of the *CHL1* gene associated with AIS in North American population [19]. The candidate-gene is a member of the L1 gene family encoding neural cell adhesion molecules. The *CHL1* gene product is homologous with the roundabout guidance receptor 3 (Robo3). Single base modifications as missense or nonsense mutations as well as frameshift and splice site mutations in the *Robo3* gene cause a rare syndrome [OMIM, #607313] with autosomal recessive inheritance that is characterized by congenital external ophthalmoplegia and progressive childhood-onset scoliosis [20-23]. The neuroanatomical and neurophysiological studies found maldevelopment of some dorsomedial brain-stem structures [21] and disturbances in the axon pathway crossing the midline in the hindbrain [22, 24]. Although mutations in the *Robo3* gene were associated with severe scoliosis, the common polymorphisms of the *Robo3* gene were not associated with late-onset AIS [19].

Other significant associations in the first genome wide association study were

between SNPs in the *DSCAM* and *CNTNAP2* genes encoding proteins involved in the axon pathfinding [19, 25-27]. Therefore, the authors of the first whole genome survey assumed that some common variants in genes encoding neural cell adhesion molecules and their interacting partners may contribute to AIS susceptibility [19].

Two independent case-control replication studies in Chinese population were reported but no significant association between AIS and *CHL1* (rs10510181, rs2055314, rs331894, rs2272524 and rs2272522) was found [28, 29].

The objective of the present case-control study is to examine the association between the *CHL1* (rs1400180) polymorphism and the progression of IS in Bulgarian population sample. In order to fulfill this aim, the following associations between the SNP and (i) curve severity (case-control study), (ii) curve pattern (case-only study) and (iii) brace treatment outcome (case-only study) were explored among Bulgarian patients.

2. Materials and Methods

All participants were included only after the subjects or their parents signed an informed consent approved by the University Ethics Committee.

2.1. Materials

A total of 105 ambulatory and hospitalised patients with IS and 210 unrelated gender-matched control subjects were selected by the orthopaedic physicians. Diagnosis was confirmed with radiographic methods. The mean value of Cobb angle was 54.6 ± 22.7. Secondary scoliosis was excluded. The mean age at the beginning of IS was 11.2 ± 3.1 years old. Female (n = 86) and male (n = 19) patients with primary scoliosis were included.

Radiological examination was not performed in the control group for the aims of the current study. Physical examination was performed to exclude mild scoliosis in the control group and previous available spinal roentgenographies were evaluated.

For the aims of the current case-control study all cases were divided into two groups according to the last Cobb angle measurement: progressive scoliosis group (Cobb angle > 40^0) and non-progressive or slowly progressive scoliosis group (Cobb angle < 40^0). The possible association between curve severity and *CHL1* was examined through comparison between each of these groups of progressive (n = 84) and non-progressive scoliosis (n = 62) and the control group (case-control study).

For the aims of the case-only study the cases were divided into three groups according to the type of the major curve: lumbar (n = 12), thoracolumbar (n = 31) and thoracic (n = 62) curve pattern. The possible association between curve pattern and *CHL1* was examined through comparison between these three groups (case-only study).

Additionally, progressive cases (n = 84) were separated into two groups: with (n = 49) and without (n = 34) previous bracing and the association between brace treatment outcome and *CHL1* was examined as these groups were compared to each other (case-only study).

2.2. Methods

Total DNA was isolated automatically from peripheral blood by using chemagic DNA Blood10k Kit (Chemagen, Baesweiler, Germany). The genotyping of *CHL1* (rs1400180 T/G) was carried out by using TaqMan SNP Genotyping Assay (Life Technologies, NY, USA) in a 7900HT Fast Real-time PCR System (Life Technologies, NY, USA). The results were analyzed 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 calculated (IBM SPSS 19.0, NY, USA).

3. Results

In the current association study we divided the cases in subgroups according to curve severity and then compared the genotype and allele frequencies of *CHL1*

(rs1400180) between the patient subgroup and the control group (case-control study). We also separated the cases in subgroups according to the brace treatment outcome and the curve pattern and then compared the genotype and allele frequencies of *CHL1* (rs1400180) between the different patient subgroups (case-only study). We performed comparisons between the groups by using genotypic (codominant, dominant and recessive) and allelic model. Genotypes were in Hardy-Weinberg equilibrium. The analysis was repeated for some samples that were not successfully genotyped at the first real-time amplification.

The distribution of genotypes/alleles of *CHL1* (rs1400180 T/G) were similar for the progressive and the non-progressive group compared to the control group ($p > 0.05$).

The results are summarised in Table 1.

The genotype and allele frequencies of *CHL1* (rs1400180 T/G) were also similar between the patient groups with different curve pattern ($p > 0.05$).

The results are summarised in Table 2.

The genotype and allele frequencies of *CHL1* (rs1400180 T/G) were also similar between the patient groups with and without previous bracing ($p > 0.05$).

The results are summarised in Table 3.

In conclusion, the *CHL1* gene polymorphism rs1400180 could not be associated with curve severity, curve pattern, bracing and finally with IS progression.

4. Discussion

A case-control study and a case-only study to investigate the association between *CHL1* (rs1400180) and IS progression were conducted in Bulgarian population.

There is evidence that curve severity and curve pattern can play a role in progression of IS. As a general rule, Cobb angles greater than 30^0 carry a substantial risk for deformity progression, and double and thoracic curve patterns carry a higher risk for progression than single, lumbar or thoracolumbar curves [30, 31]. Therefore, for the aims of the present association study we chose the curve severity (Cobb angle above and below 40^0) and the curve pattern as indicators of the IS progression. The exact value of Cobb angle is not very accurate assessment of the relationship between genotype and progression, as the average angle can reach significant values due to untimely treatment, i.e. it is influenced largely by external factors.

The obtained results indicated that *CHL1* (rs1400180) could not be associated with the curve severity or curve pattern of IS among Bulgarian patients. The observed differences in the results between our study in Eastern European population group and the study in North American population sample [19] could be explained with few differences. First, the study conducted by Sharma et al. [2013] included only AIS patients with Cobb angle above 10^0 and investigated the genetic predisposition to AIS [19] while the current study selected patients with adolescent as well as cases with infantile and juvenile idiopathic scoliosis and separate them into progressive with Cobb angle above 40^0 and non-progressive with Cobb angle below 40^0 and investigated the association with IS progression. In addition to different selection criteria, the study design was different. Sharma et al. found a significant association with *CHL1* (rs1400180) in the discovery cohort of trios (affected child and two parents) [19] unlike the present association study including case-unrelated control study and case-only study. Additionally, genotype and allele frequencies can differ in various population and ethnic groups [11]. The major limitation of our study is the relatively small sample sizes which could affect the statistical power of the results. Replication population-based as well as family-based studies will be necessary to explore the potential relationship between the *CHL1* gene and IS predisposition and progression in different population groups.

There are many studies that demonstrate the effectiveness of bracing in the treatment of scoliosis in immature patients [32-34]. Patients who present with Cobb angles of 20-29^0 should be braced according to the risk, patients with Cobb angles of 30^0 or greater should be braced while patients with Cobb angle < 20^0 and significant thoracic rotation or flatback could be considered for nighttime bracing [35]. The effectiveness of brace treatment could be affected by insufficiently studied factors [36]. Several clinical and radiographic markers as Risser sign, curve severity and curve pattern were found to be associated with the prediction of brace treatment final outcome [37-39]. Xu et al. [2011] demonstrated that genetic factors could also have an influence on the outcome of brace treatment [36].

The association between *CHL1* gene and bracing was not investigated. We grouped the cases according to the brace treatment outcome – success or failure and then compared the genotype and allele frequencies in the different subgroups of patients (case-only study) as we applied genotypic as well as allelic model. As a result from the analysis, *CHL1* (rs1400180) could not be associated with the brace treatment final outcome.

The statistical analysis of data indicated that the genetic variant rs1400180 of the *CHL1* gene is not correlated to the clinical prognostic markers: curve severity, curve pattern or bracing. Therefore, the examined polymorphic variant could not be considered as a genetic modifying factor for IS.

Table 1. Odds ratios of genotypes and alleles of *CHL1* (rs1400180 T/G) in groups with different curve severity.

Subgroup	Genotype, Allele	p	OR [95% CI]
Progressive IS Cobb angle > 40^0 (n_1=84, n_2=210)	TT / GG	1	1.01 [0.48-2.12]
	TT / TG + GG	0.73	1.11 [0.62-1.98]
	TT + TG / GG	0.78	0.92 [0.49-1.71]
	T / G	1	1.01 [0.71-1.44]
Non- or slowly progressive IS Cobb angle < 40^0 (n_1=21, n_2=210)	TT / GG	1	0.99 [0.28-3.47]
	TT / TG + GG	0.79	1.25 [0.46-3.38]
	TT + TG / GG	0.78	0.8 [0.28-2.31]
	T / G	1	1.01 [0.54-1.91]

Table 2. Odds ratios of genotypes and alleles of *CHL1* (rs1400180 T/G) in groups with different curve pattern.

Curve pattern	Genotype, Allele	p	OR [95% CI]
Thoracic/ thoracolumbar + lumbar (n_1=62, n_2=43)	TT / GG	0.79	0.86 [0.28-2.64]
	TT / TG + GG	0.81	0.90 [0.37-2.16]
	TT + TG / GG	0.84	0.91 [0.35-2.34]
	T / G	0.79	0.93 [0.53-1.61]
thoracolumbar /thoracic + lumbar (n_1=31, n_2=74)	TT / GG	0.89	0.91 [0.27-3.06]
	TT / TG + GG	0.89	0.94 [0.36-2.44]
	TT + TG / GG	0.92	0.95 [0.35-2.59]
	T / G	0.89	0.96 [0.53-1.73]
Lumbar/ thoracolumbar + thoracic (n_1=12, n_2=93)	TT / GG	0.68	1.75 [0.29-10.5]
	TT / TG + GG	0.73	1.44 [0.40-5.21]
	TT + TG / GG	0.73	1.46 [0.30-7.18]
	T / G	0.67	1.31 [0.55-3.10]

Table 3. Odds ratios of genotypes and alleles of *CHL1* (rs1400180 T/G) in the progressive group (with and without bracing).

Progressive group	Genotype, Allele	p	OR [95% CI]
Previous bracing/ without bracing (n_1=49, n_2=34)	TT / GG	0.5	0.63 [0.16-2.43]
	TT / TG + GG	0.75	1.17 [0.43-3.24]
	TT + TG / GG	0.2	0.48 [0.15-1.50]
	T / G	0.58	0.84 [0.45-1.56]

5. Conclusions

This association study revealed no significant association between *CHL1* (rs1400180) and IS progression in Bulgarian population sample.

The molecular marker *CHL1* (rs1400180) could not be considered as a prognostic marker for IS progression in Bulgarian population.

These results could not exclude a role of the same polymorphic marker in the etiology and progression of IS in other population groups.

The identification of molecular markers could be a useful means for a more accurate prognosis of the risk for a rapid progression of the curve. That would permit early stage treatment of the patient with less invasive procedures.

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