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Original Article

Association between interleukin-6 gene polymorphism and idiopathic scoliosis: a meta-analysis

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Abstract: Idiopathic scoliosis (IS) is considered to be a multifactorial disease. Interleukin-6 (IL-6) is an important pro-inflammatory and anti-inflammatory cytokine. There is some controversy regarding whether IL-6 gene polymorphism (rs1800795 G/C) is associated with IS susceptibility. A systematic search in PubMed, EMBASE, and Cochrane CENTERAL database until the end of November, 2015 were carried out. Pooled ORs were performed for the allelic comparison (C vs. G) and genotypic comparisons of codominant (CC vs. GG and CG vs. GG), dominant (CC + CG vs. GG), and recessive (CC vs. CG + GG) genetic models. We calculated ORs and 95% CIs using random or fixed effects model. Newcastle-Ottawa Scale was used to evaluate the methodological quality, and Stata 11.0 was used to analyze data. The associations between IL-6 gene polymorphism and IS susceptibility were investigated. Five eligible case-control studies were included in our meta-analysis. All of the studies were of high methodological quality. There were a total of 2121 subjects, including 944 cases and 1177 controls, from Asia and Europe. The meta-analysis showed that no significant difference was detected in all genetic models ($P > 0.05$). However, the results of this meta-analysis should be interpreted with caution because of the heterogeneity among the studies, and additional high quality studies are still necessary to assist with this finding.

Keywords: Interleukin-6, polymorphism, rs1800795, idiopathic scoliosis, meta-analysis

Introduction

Idiopathic scoliosis (IS), as a complex three-dimensional structural deformity of the spine, is composed of thoracic hypokyphosis, lateral deviation, and axial rotation [1]. The prevalence of IS was reported to range from 1% to 3% in adolescents [2, 3]. Without proper treatment, patients with IS could have significant functional disabilities and severe cosmetic problems.

In spite of decades of investigation, the etiology and pathogenesis of IS has not been clearly elucidated. Some hypotheses have been reported, such as disproportional skeletal growth [4], osteopenia [5], deficiency or dysfunction in the melatonin signaling pathway [6, 7]. As reported by previous studies, there is a higher prevalence of IS in families than in the general population [8]. It seems that genetic factors play an important role in the development of IS [9, 10].

Recently, the analysis of possible associated genetic factors of IS has been focused on components of immune system, matrix remodeling factors, and the disturbance of hormonal regulation. Single nucleotide polymorphisms (SNPs) in the genes for estrogen receptor α [11], estrogen receptor β [12], matrix metalloproteinase-3 [13], and melatonin receptor 1B [14] have been reported to be associated with IS susceptibility. Interleukin-6 (IL-6), as a proinflammatory and anti-inflammatory cytokine, has also been investigated. It was reported that the nucleus pulposus of scoliotic discs responded to exogenous proinflammatory stimuli by secreting IL-6 [15]. The G/C polymorphism at 174 base pairs upstream of the transcription start site of the IL-6 gene was reported to be related with its expression [16].

Though a number of studies have been conducted to investigate the association between IL-6 gene SNP (rs1800795 G/C) and the risk of IS, the results were mixed and inconclusive. Up to now, no meta-analysis has investigated the association between them. Therefore, we performed this meta-analysis to determine the

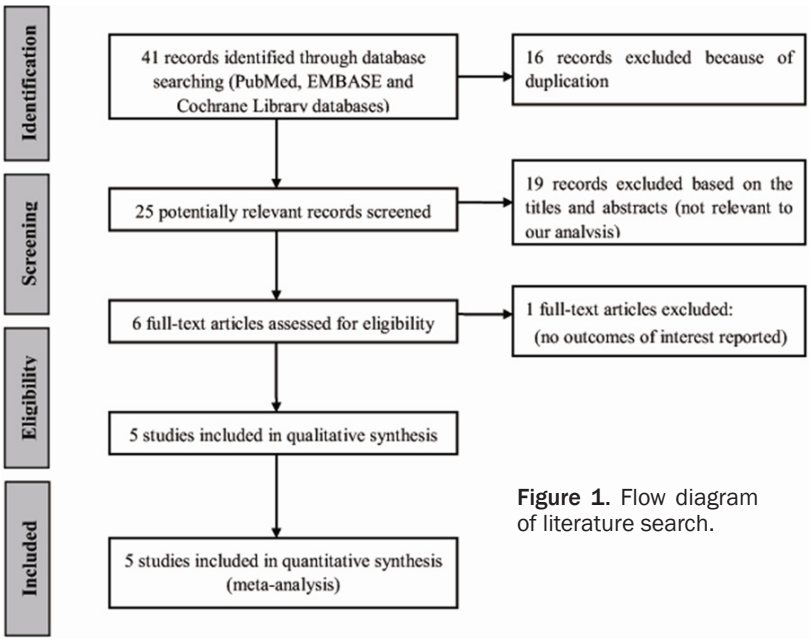

Figure 1. Flow diagram of literature search.

genetic association of the polymorphism of IL-6 gene (rs1800795 G/C) with IS.

Materials and methods

Literature search

To assemble all relevant studies, a systematic research of Pubmed, Embase and Cochrane CENTRAL database were performed on studies published until November, 2015. The following keyword search strings were used: (scoliosis OR AIS OR IS) AND (interleukin OR IL6 OR IL-6) AND (polymorphism OR mutation). No language or publication date restrictions were applied. The reference lists of all the full text papers were also examined to identify potentially eligible studies.

Inclusion and exclusion criteria

Studies were included for review if they met the following inclusion criteria: (a) case-control design; (b) IS diagnosed on the basis of clinical and/or radiologic examinations; (c) studies investigated the association of IL-6 gene polymorphism (rs1800795 G/C) with IS; (d) sufficient data could be acquired to calculated the odds ratios (ORs) and 95% confidence intervals (CIs). Studies providing insufficient data, contrary to the inclusion criteria, and duplicate publications were excluded.

Data extraction and outcome measures

Data were extracted by two reviewers independently, with any disagreements resolved by discussion and consensus. The following contents were collected: author name, publication year, country where the study was conducted, subject characteristics (ie, number of cases and controls, age, gender and Cobb angle), genotyping method, and allele/genotype frequencies.

Assessment of methodological quality

The methodological quality of included studies was assessed using a modified version of the Newcastle-Ottawa Scale (NOS) for observational studies [17]. The quality score ranges from 0 to 9, and studies with a score of 5 or higher were considered as high methodological quality. The quality was assessed independently by two reviewers, and disagreements between reviewers were resolved by discussion until consensus was reached.

Statistical analysis

We calculated ORs and 95% CIs to evaluate the strength of the association between IL-6 gene polymorphism (rs1800795 G/C) and IS susceptibility. The allele C was considered as the risk allele. Pooled ORs were performed for the allelic comparison (C vs. G) and genotypic comparisons of codominant (CC vs. GG and CG vs. GG), dominant (CC + CG vs. GG), and recessive (CC vs. CG + GG) genetic models. Hardy-Weinberg equilibrium (HWE) was evaluated for each study by chi-square test in control groups. The heterogeneity was tested by a chi-square-based Q statistic test. The effect of heterogeneity was quantified by I^2 value as well as P value. If $I^2 > 50\%$ and $P < 0.10$, we think that an obvious heterogeneity existed, and ORs should be pooled by random effect model; otherwise, a fixed effect model was used. Subgroup analysis was conducted on basis of the ethnicity. Sensitivity analysis was also performed to check the

Table 1. General characteristics of studies included in this meta-analysis

Author	Year	Country	Age (year)	Gender	Cobb angle (°)	Case	Control	Method	Quality
Aulisa	2007	Italy	NR	NR	25-125[a]	53	206	PCR	5
Lee	2010	Korea	11-14[a]	Female	16-69[a]	198	120	PCR	5
Liu	2010	China	11-19[a]	Female	20-55[a]	487	494	PCR-RFLP	6
Morocz	2011	Hungary	16.8 (3.1)[b]	Female, male	64.7 (19.2)[b]	126	197	PCR-RFLP	5
Nikolova	2015	Bulgaria	11.2 (3.1)[b]	Female, male	54.6 (23.2)[b]	80	160	PCR-RFLP	6

NR, not report; PCR, polymerase chain reaction; RFLP, restriction fragment length polymorphism. [a], range; [b], mean (stand deviation).

Table 2. Frequency of genotypes and the results of Hardy-Weinberg equilibrium

Study	Year	Ethnicity	Case					Control					P_H
			C	G	CC	CG	GG	C	G	CC	CG	GG	
Aulisa	2007	Caucasian	28	78	3	22	28	214	198	62	90	54	0.20
Lee	2010	Asian	1	395	0	1	197	1	239	0	1	119	0.99
Liu	2010	Asian	0	974	0	0	487	0	988	0	0	494	-
Morocz	2011	Caucasian	117	135	25	67	34	169	225	36	97	64	0.99
Nikolova	2015	Caucasian	47	113	9	29	42	146	174	42	62	56	0.02

P_H, *P* for Hardy-Weinberg equilibrium in control group.

robustness of the results by excluding the HWE-deviated studies for both the overall and subgroup analyses. Funnel plots were examined for the evidence of publication bias if sufficient numbers of studies were included for meta-analysis ($n \geq 10$). All statistical analyses were performed using STATA, version 11.0 (StataCorp, College Station, TX). A *P* value < 0.05 was regarded as statistically significant, except where otherwise specified.

Results

Literature search results

A total of 41 records were selected from databases, but 36 records were excluded, of which 16 were duplicate ones, 19 were not relevant to our study, and 1 had no interested outcomes. Finally, 5 eligible case-control studies were included in our meta-analysis [13, 18-21]. **Figure 1** provides a summary of the study identification and selection process.

Study characteristics

The details of studies are listed in **Table 1**. The 5 high methodological quality studies recruited a total of 2121 subjects (944 cases and 1177 controls) from Asia [19, 20] and Europe [13, 18, 21]. The distribution of genotypes in the control groups of included studies did not deviate significantly from HWE except for one study [21]. Different genotyping methods were utilized including polymerase chain reaction (PCR) [18, 19] and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) [13, 20, 21]. Two studies recruited only female in cases and controls [19, 20], whereas another 2 studies included both female and male in studies [13, 21]. Based on the NOS score, 2 studies scored 6 [20, 21], and 3 studies scored 5 [13, 18, 19]. All studies were of high quality. Characteristics of included studies in the meta-analysis are presented in **Table 1**. The distributions for allele and genotype frequency of IL-6 gene SNP were demonstrated in **Table 2**.

Main results

Association between IL-6 gene polymorphism and IS susceptibility was analyzed. All of the 5 studies were available for data analysis. Significant heterogeneity was identified by Q-test and I^2 statistic in all of the genetic models; therefore only random-effects model was used. Overall, there is no significant association identified in any genetic model ($P > 0.05$) (**Table 3**).

Subgroup analysis

Subgroup analyses were conducted according to the ethnicity. Of the 5 studies, 3 were conducted in Caucasian population. In the Caucasian subgroup, significant heterogeneity

Table 3. Results on the association between the interleukin-6 gene polymorphism (rs 1800795 G/C) and idiopathic scoliosis

Comparison	Pooled OR	LL 95% CI	UL 95% CI	*P* value	Q-test (*P*)	I^2 (%)
Overall						
Allelic contrast (C/G)	0.59	0.29	1.19	0.14	< 0.01	86.2
Co-dominant model (CG/GG)	0.74	0.43	1.29	0.29	0.09	54.4
Co-dominant model (CC/GG)	0.35	0.08	1.55	0.17	< 0.01	88.4
Dominant model (CC + CG/GG)	0.60	0.28	1.29	0.19	< 0.01	78.3
Recessive model (CC/CG + GG)	0.42	0.13	1.33	0.14	< 0.01	83.8
Caucasian subgroup						
Allelic contrast (C/G)	0.58	0.28	1.24	0.16	< 0.01	90.8
Co-dominant model (CG/GG)	0.74	0.40	1.37	0.34	0.04	69.4
Co-dominant model (CC/GG)	0.35	0.08	1.55	0.17	< 0.01	88.4
Dominant model (CC + CG/GG)	0.60	0.26	1.37	0.22	< 0.01	85.5
Recessive model (CC/CG + GG)	0.42	0.13	1.33	0.14	< 0.01	83.8

OR, odds ratio; LL, lower limit; UL upper limit; CI confidence interval.

was identified by Q-test and I^2 statistic in all of the genetic models; therefore only random-effects model was used. Overall, there is no significant association identified in any genetic model. ($P > 0.05$) (**Table 3**).

There were 2 studies conducted in Asian. However, it was improper to pool the genetic effects of the IL-6 gene polymorphism because of its very low frequency in the Asian population. In the study conducted by Lee et al., there were only one patient with CG genotype in both case and control groups, and no one showed CC genotype in both case and control groups [19]. In the study conducted by Liu et al., all patients were GG genotype, and no one showed CG or GG genotypes [20].

Sensitive analysis and publication bias

The exclusion of one study, which was deviated from HWE, did not affect the meta-analysis outcomes in any genetic model. Publication bias was not assessed owing to the limited number of included studies (< 10).

Discussion

IL-6 is an important proinflammatory and anti-inflammatory cytokine [22, 23]. In a previous study, Burke et al. cultivated human disc tissue from scoliosis patients, analyzed the medium for the production of proinflammatory mediators, and concluded that both scoliotic and degenerate human nucleus pulposus can respond to an exogenous proinflammatory stimulus by secreting increased amounts of IL-6 and other cytokine [15]. In the year of 2007, Aulisa et al reported that the polymorphism in the promoter region of IL-6 gene determined the biologic activities of IL-6. In their case-control study, they demonstrated a strong association between the IL-6 gene polymorphism and IS [18]. This study is important because their result provide a support for the "disc degeneration-inflammation" hypothesis for the etiology of IS, which means that the primary defect in IS could be the intervertebral disc degeneration.

However, after several years, the study conducted by Liu et al. did not replicate the previous result [20]. In their study, no significant differences were found for allele and genotype frequencies of the polymorphism of IL-6 gene between cases or controls. Sometimes, a single study may be unable to detect a true association due to the relatively small sample size. Meta-analysis, on the other side, can increase the statistical power of the association analysis and obtain more precise estimates of effect by pooling the results of several studies [24, 25].

In this meta-analysis, five eligible case-control studies were analyzed, including 944 cases and 1177 controls for IL-6 gene polymorphism. The results showed that there is no significant association identified in any genetic model, which means we cannot draw a definite conclusion that IL-6 gene polymorphism is associated with the susceptibility of IS yet.

As the five studies were conducted in different countries, we assumed that ethnicity may have an effect on the final result. Thus, we further

performed a subgroup analysis to decrease the influence of ethnicity on the final outcomes. In the Caucasian subgroup, the pooled result did not detect a significant association either, even if two studies concluded that IL-6 gene polymorphism could increase the risk of IS. This result could be due to some reasons. For example, IL-6 is involved in too many functions, such as the activation of the immune system, the regulation of metabolism, regenerative processes, and the maintenance of bone homeostasis [26], false-positive association could be found in a single genetic study. On the other hand, a positive association could be overlooked in a meta-analysis because significant heterogeneity can decrease the sensitivity of a meta-analysis study.

In the Asian subgroup, two studies conducted in Koreans and Chinese showed very rare allele frequencies in both groups. It is reported that the allele frequencies of IL-6 gene SNP in Caucasians are much higher than those in Asian [27]. We think that the IL-6 polymorphism is unlikely to be contributing significantly to IS susceptibility in Asian population because of its low frequency. With this study, however, we cannot exclude the role of this SNP in other populations and more replication studies in other ethnic group are essentially needed.

What's more, we only analyzed SNP of IL-6 gene in the current study. Since IS is a complex disease with numerous predisposing genes, the genetic variations may have minor effect when considered in isolation. Though there was a difficult analytical challenge, the detection and characterization of the interactions among multiple genetic and environmental factors can help in better understanding the pathogenesis of IS [13]. Thus, in the further study, it is necessary to identify and analyze numerous susceptibility factors in multiplex haplotype combination.

This meta-analysis was conducted in a strict and comprehensive process, but there are still some potential limitations that should be noticed. First, patients included in this meta-analysis had different baseline characteristics, such as initial Cobb angle, genotyping methods and quality of study, which may lead to heterogeneity and have an effect on the final results. Second, we did not conduct subgroup analysis for every confounding factor, because of the relatively small number of included studies and a lack of unified criterion for recruitment amongst the different studies. Third, potential for selection bias cannot be adequately assessed, as case selection was not well described in some included studies and control selection was described in less detail compared to cases. This methodological flaw decreased the quality of studies to some extent although the overall quality of these studies was high. Finally, the statistical efficacy may be decreased by the finite number of included studies. The small sample size of some pooled studies may also exert an influence on the stability of the results, especially in the subgroup analysis. Consequently, the quantitative results of this review should be interpreted with caution.

Despite the limitations mentioned above, this study is clinically valuable to some extent. The present systematic review provides a comprehensive examination of available evidence on the association of the SNP (rs1800795 G/C) in IL-6 gene with IS in diverse ethnic populations. Meta-analysis found a lack of significant association between this SNP and risk of IS. Considering the limitations listed above, additional high quality studies are still necessary to assist with this finding.

Disclosure of conflict of interest

None.

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