

Original Article

Role of polymorphisms in *COL9A1* gene in the development of congenital talipes equinovarus in a Chinese population

Huibin Li¹, Yongjian Sun², Fang Chen³, Feng Zhao¹, Jianhua Zhang¹, Jiani Wu¹, Yuchen Zhang⁴

¹Department of Burn and Plastic Surgery People's Hospital of Linyi City, Shandong, China; ²Department of Trauma Orthopedics, The Fifth Affiliated Hospital of Southern Medical University, Guangzhou, China; ³Department of Orthopedics, People's Hospital of Linyi City, Shandong, China; ⁴Department of Orthopedics, Shanghai Children's Medical Center Affiliated to Shanghai Jiaotong University, School of Medicine, Shanghai, China

Received December 5, 2015; Accepted March 24, 2016; Epub May 1, 2016; Published May 15, 2016

Abstract: In this case-control study, we conducted a case-control study to investigate the whether the two SNPs in *COL9A1* (rs1135056 and rs592121) could influence the development of congenital talipes equinovarus in a Chinese population. A total of 205 patients with congenital talipes equinovarus and 205 control subjects were consecutively selected between January 2012 and October 2014. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was taken to genotype *COL9A1* rs1135056 and rs592121. Unconditional logistic regression analysis was taken to analyze the correlation between *COL9A1* rs1135056 and rs592121 gene polymorphisms and risk of congenital talipes equinovarus. Using unconditional logistic regression analysis, we found that individuals with the GG genotype of rs1135056 was associated with an increased risk of congenital talipes equinovarus when compared to the AA genotype (OR=1.45, 95% CI=0.90-2.35). In dominant and recessive models, the rs1135056 polymorphism was associated with the development of congenital talipes equinovarus, and the adjusted ORs (95% CI) were 1.95 (1.13-3.78) and 1.59 (1.01-2.55). However, the rs592121 gene polymorphism did not influence the susceptibility to congenital talipes equinovarus. In conclusion, our study indicated that *COL9A1* rs1135056 polymorphism was associated with an increased risk of congenital talipes equinovarus in co-dominant, dominant and recessive models. Further studies with larger sample sizes must be conducted in the future to confirm this association.

Keywords: *COL9A1*, polymorphism, congenital talipes equinovarus

Introduction

Congenital talipes equinovarus is one of the most common congenital birth defects, and it is estimated that there are 1 per 1000 live births [1]. The clinical manifestations of this disease is the front-end adduction, ankle plantar flexion, calcaneal overpronate and tibial distal internal rotation [2]. The etiology of congenital talipes equinovarus is not well understood, and previous studies have suggested that about 25% of patients have a family history of talipes equinovarus, which suggests that a hereditary factor contribute to the development of this disease [3].

Type IX collagen is a trimer of three different gene products, including $\alpha 1$ (IX), $\alpha 2$ (IX) and $\alpha 3$

(IX) chains. This collagen are encoded by the *COL9A1*, *COL9A2*, and *COL9A3* genes, and it is quantitatively a minor component that is responsible for covalently cross-linking to the surface of type II collagen fibrils [4]. Previous experimental study has indicated that variations in collagen IX genes in humans and animals are correlated with the functional longevity of joint cartilages and are connected with osteochondropathy [5, 6]. *COL9A1* is reported to be associated with cartilage tissue, and has a role in the stability of the internal environment of articular cartilage [7]. Currently, only two studies reported the association between *COL9A1* gene polymorphism and development of congenital talipes equinovarus [8, 9]. In this case-control study, we conducted a case-control study to investigate whether the two SNPs

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Table 1. The primers, restriction enzymes and PCR products of COL9A1 rs1135056 and rs592121

SNP	Allele	Amino acid position	Primers (5'-3')	Restriction enzymes	Length of digested fragment
rs1135056	A/G	621	CTAGCATGGGCTCAAACA (Forward) CCTGGTCAGATGGGAAAT (Reverse)	Sam I	A allele: 160 bp; G allele: 127 bp
rs592121	A/G	339	TCTTGCTCTATTAGGGAT (Forward) TAATGTTAGTTGGCTTGC (Reverse)	Hae III	A allele: 102 bp; G allele: 50 bp

Table 2. Demographic characteristics of patients with congenital talipes equinovarus and control subjects

Variables	Patients	%	Controls	%	t-test or χ^2 test	P value
Age, years	5.36±6.71		5.75±7.24		0.57	0.29
Sex						
Females	85	41.46	85	42.44		
Males	120	58.54	120	57.56	0.00	1.00
Family history of congenital talipes equinovarus						
No	188	91.71	205	100.00		
Yes	17	8.29	0	0.00	17.74	<0.001
Maternal cigarette smoking						
No	196	95.61	200	97.56		
Yes	9	4.39	5	2.44	1.18	0.28
Maternal tobacco drinking						
No	187	91.22	195	95.12		
Yes	18	8.78	10	4.88	2.45	0.12
Maternal coffee consumption						
No	194	94.63	197	96.10		
Yes	11	5.37	8	3.90	1.40	0.48

in COL9A1 (rs1135056 and rs592121) could influence the development of congenital talipes equinovarus in a Chinese population.

Material and methods

Subjects

A total of 205 patients with congenital talipes equinovarus were consecutively selected from the Department of Burn & Plastic Surgery in People's Hospital of Linyi City between January 2012 and October 2014. All the patients with congenital talipes equinovarus were confirmed by X-ray or surgery. The exclusion criteria were patients who had neuromuscular or other recognizable syndromes involving clubfoot.

During the same period time, a total of 205 subjects without any foot deformities were selected from the outpatient clinic patients in the People's Hospital of Linyi City. Each control

subject was matched with one patient by sex and age (± 5 years). At recruitment, all participants were asked to provide 5 ml peripheral venous blood for DNA extraction. Moreover, the demographic and clinical characteristics were collected from medical records, including sex, age, family history of congenital talipes equinovarus, maternal cigarette, maternal alcohol and maternal coffee consumption.

A signed informed consent forms were obtained from enrolled individuals prior to their participation in the study. The study protocol was approved by the Clinical Research Ethics Committee of People's Hospital of Linyi City. Ethical approval for

this study was based on the standards of the Declaration of Helsinki.

DNA extraction and genotyping

The blood samples were collected in ethylene diamine tetra-acetic acid (EDTA)-coated tubes and stored at -20°C until use. Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was taken to genotype COL9A1 rs1135056 and rs592121. The primers of rs1135056 and rs592121 were designed using the Primer 5.0 software (PREMIER Biosoft Ltd. Palo Alto, USA). The primers, restriction enzymes and PCR products were shown in **Table 1**. The reaction conditions were performed as follows: one cycle of DNA denaturation at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 50 s, 54°C annealing step for 50 s, extension at 72°C for 50 s, with a final extension step of 5 min at 72°C . The PCR products were confirmed by electrophoresis in

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Table 3. Genotype distributions of COL9A1 rs1135056 and rs592121 in study subjects

SNPs	Patients	%	Controls	%	χ^2 test	P value	P for HWE	
							In patients	In controls
rs1135056								
AA	57	27.80	79	38.54				
AG	86	41.95	82	40.00				
GG	62	30.24	44	21.46	6.71	0.04	0.02	0.01
rs592121								
GG	60	29.27	65	31.71				
GA	93	45.37	91	44.39				
AA	52	25.37	49	23.90	0.31	0.86	0.19	0.13

Compared to the controls, patients were more likely to have a family history of congenital talipes equinovarus ($\chi^2=17.74$, $P<0.001$).

The genotype distributions of COL9A1 rs1135056 and rs592121 were presented in **Table 3**. By chi-square test, there was significant difference in the genotype frequencies of AA, AG and GG in COL9A1 rs1135056 between patients with congenital talipes equinovarus and control subjects ($\chi^2=6.71$, $P=0.04$), but no significant difference was found in the genotype distributions of COL9A1 rs592121 ($\chi^2=0.31$, $P=0.86$). Moreover, the genotype distributions of rs592121 conformed to the Hardy-Weinberg equilibrium in patients and controls ($P>0.05$), while those of rs1135056 did not ($P<0.05$).

Using unconditional logistic regression analysis, we found that individuals with the GG genotype of rs1135056 were associated with an increased risk of congenital talipes equinovarus when compared to the AA genotype (OR=1.95, 95% CI=1.13-3.78) (**Table 4**). In dominant model, the AG+GG genotype was associated with the development of congenital talipes equinovarus compared to the AA genotype, and the adjusted OR (95% CI) was 1.63 (1.05-2.52). In recessive model, the GG genotype was correlated with an increased risk of congenital talipes equinovarus (OR=1.59, 95% CI=1.01-2.55), compared to the AA+AG genotype. However, the rs592121 gene polymorphism did not influence the susceptibility to congenital talipes equinovarus.

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Discussion

The development of congenital talipes equinovarus is caused by multiple environmental and lifestyle factors, such as skeletons dysplasia of bone neuromuscular disease, soft tissue contracture, vascular anomalies, intrauterine growth retardation and maternal smoking [10]. The COL9A1 is found in 1989 by Kimura et al., and Warman et al. reported that the COL9A1 is

a 2% agarose gel stained with ethidium bromide and visualized under UV light.

Statistical analysis

The demographic characteristics of patients with congenital talipes equinovarus and control subjects were compared by *t*-test and chi-square test. The goodness-of-fit χ^2 -test was taken to analyze whether the COL9A1 rs1135056 and rs592121 genotype distributions were deviated from the Hardy-Weinberg equilibrium (HWE). Unconditional logistic regression analysis was taken to analyze the correlation between COL9A1 rs1135056 and rs592121 gene polymorphisms and risk of congenital talipes equinovarus. Odds ratios (ORs) and their 95% confidence interval (95% CI) were calculated. Statistical analysis was performed using the SPSS 16.0 package (SPSS Inc., Chicago, IL, USA). All tests were two-sided, and $P<0.05$ was considered significantly different.

Results

The demographic data of included subjects were described in **Table 2**. The mean age of patients with congenital talipes equinovarus and control subjects were 5.36 ± 6.71 and 5.75 ± 7.24 years, respectively. There were respectively 85 (41.46%) females and 120 (58.54%) males in patients and control subjects. By *t*-test or χ^2 test, there was no significantly different between patients with congenital talipes equinovarus and control subjects in terms of age ($t=0.57$, $P=0.29$), sex ($t=0.00$, $P=1.00$), maternal cigarette smoking ($\chi^2=1.18$, $P=0.28$), tobacco drinking ($\chi^2=2.45$, $P=0.12$) and coffee consumption ($\chi^2=1.40$, $P=0.48$).

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Table 4. Association between *COL9A1* rs1135056 and rs592121 polymorphisms and risk of congenital talipes equinovarus

	SNPs	Patients	%	Controls	%	OR (95% CI) ¹	P value
rs1135056							
Co-dominant	AA	57	27.81	79	38.54	Ref.	-
	AG	86	41.95	82	40.00	1.45 (0.90-2.35)	0.11
	GG	62	30.24	44	21.46	1.95 (1.13-3.78)	0.01
Dominant	AA	57	27.81	79	38.54	Ref.	-
	AG+GG	148	72.19	126	61.46	1.63 (1.05-2.52)	0.02
Recessive	AA+AG	143	69.76	161	78.54	Ref.	-
	GG	62	30.24	44	21.46	1.59 (1.01-2.55)	0.04
rs592121							
Co-dominant	GG	60	29.27	65	31.71	Ref.	-
	GA	93	45.37	91	44.39	1.11 (0.69-1.79)	0.66
	AA	52	25.36	49	23.90	1.15 (0.66-2.01)	0.6
Dominant	GG	60	29.27	65	31.71	Ref.	-
	GA+AA	145	70.73	140	68.29	1.12 (0.72-1.75)	0.59
Recessive	GG+GA	153	74.64	156	76.10	Ref.	-
	AA	52	25.36	49	23.90	1.08 (0.67-1.74)	0.73

¹Adjusted for age, sex and family history of congenital talipes equinovarus.

located in 6q12-q13. This gene has two transcripts, and the two full lengths of cDNA are 3704 bp and 2985 bp, and they are 38 exon and 32 exons, respectively [11]. *COL9A1* plays an important role in combining matrix metalloproteinases, inhibiting growth factors and the surface of the cartilage cell membrane receptor, protecting and stabilizing the articular cartilage [12]. Currently, it is reported that *COL9A1* gene expression could contribute to the mild cartilage dysplasia [12]. In our study, we conducted a case-control study to investigate the association between *COL9A1* polymorphism and development of congenital talipes equinovarus, and our study indicated that *COL9A1* rs1135056 polymorphism was associated with an increased risk of this disease in co-dominant, dominant and recessive models.

Previous studies have reported that the *COL9A1* rs1135056 polymorphism was correlated with several kinds of diseases, such as degenerative lumbar spinal stenosis, primary osteoarthritis, osteoarthritis, hip osteoarthritis and multiple epiphyseal dysplasia mutations [6, 13-16]. Noponen-Hietala et al. conducted a study in a Finnish population, and they found that mutation in *COL9A1* had an important role in the pathogenesis of lumbar spinal stenosis [13]. Alizadeh et al. conducted a study in a Netherlandish population, and they found that

COL9A1 gene was close to the susceptibility for hip osteoarthritis [16]. Czarny-Ratajczak et al. reported that mutations in *COL9A1* could cause multiple epiphyseal dysplasia in a Finnish population [6].

Currently, only two studies reported the association between *COL9A1* polymorphism and development of congenital talipes equinovarus [8, 9]. Liu et al. performed a study in a Chinese population, and they reported that the expression of *COL9A1* on mRNA levels was significant correlated with higher in patients with congenital talipes equinovarus than in normal person [8]. Liu et al. also conducted a study in a Chinese population with 25 children with congenital talipes equinovarus and 5 normal controls, and they found that *COL9A1* rs1135056 polymorphism was associated with the pathogenesis of this disease [9]. In our study, we found that *COL9A1* rs1135056 polymorphism was associated with an increased risk of congenital talipes equinovarus. Further studies are greatly needed to confirm the findings of our study.

In conclusion, our study indicated that *COL9A1* rs1135056 polymorphism was associated with an increased risk of congenital talipes equinovarus in co-dominant, dominant and recessive models, but the rs592121 polymorphism did not contribute to the risk of this disease. Further

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studies with larger sample sizes must be conducted in the future to confirm this association.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Fang Chen, Department of Orthopedics, People's Hospital of Linyi City, No. 27, Jiefang Road, Lanshan District, Linyi, Shandong, China. Tel: +86-0539-8012810; Fax: +86-0539-8012810; E-mail: fangchenyl@sina.com; wangzhigangfg@163.com

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