

The COL5A1 genotype is associated with range of motion

Seung-Taek Lim¹, Chang-Sun Kim², Woo-Nam Kim¹ and Seok-Ki Min^{3*}

¹College of Sport Science, Dong-A University, Busan, Republic of Korea

²Department of Physical Education, Dongduk Women's University, Seoul, Republic of Korea

³Korea Institute of Sports Science (KISS), Seoul, Republic of Korea

(History: Received: 2015/04/30, Revised: 2015/05/25, Accepted:2015/05/27, Published: 2015/06/30)

Seung-Taek Lim, Chang-Sun Kim, Woo-Nam Kim and Seok-Ki Min. The COL5A1 genotype is associated with range of motion. *JENB.*, Vol. 19, No. 2, pp.49-53, 2015 [Purpose] The aim of our study was to investigate the association between COL5A1 genotype and Range of Motion as measured by the passive straight leg raise (SLR) and whole body joint laxity (WBJL) in Asian population. [Methods] One hundred and seventy seven participants including Korean and Japanese college students (male = 109, female = 68) participated in the study. Each subject performed the passive straight leg raise and whole body joint laxity test. Genotyping for the COL5A1 (rs 12722) polymorphism was performed using the TaqMan approach. The COL5A1 genotype exhibited a Hardy-Weinberg equilibrium distribution in our population. [Results] The physical parameters including height, weight, and BMI were higher in <90° group than >90° group. The SLR exhibited significant difference among the COL5A1 group. However, the WBJL did not differ significantly among the COL5A1 genotype, but significant difference was seen in CC genotype when compared to CT (2.99 ± 1.72) or TT (2.70 ± 1.52) genotype. [Conclusion] We concluded that COL5A1 gene polymorphism is associated with increased SLR ROM in Asian population. [Key words] COL5A1, genotype, ROM, SLR, flexibility

INTRODUCTION

Musculoskeletal injuries can be a result of participation in sports activities or carrying out physical activities for health benefits [1]. The causes of musculoskeletal injuries result from a complex interaction of multiple risk factors and events [2]. Range of motion (ROM) has been known as one of the risk factors for increased musculoskeletal injuries [3,4]. Musculoskeletal flexibility is defined as the ability to move a joint through its complete ROM, and therefore it is important to maintain flexibility as decreased ROM is a contributing risk factor for injuries [5].

Exercises to enhance flexibility can improve overall performance of athletes by preventing injuries with subsequent increase in ROM. Moreover, many athletes and others continue with their daily physical activities to increase the rate of performance in an event and for various health benefits; at the same time they are exposed to the risk of injuries caused due to fatigue. Arnason *et al.* [6] established that injuries occurring during the time of training or physical

activity were musculoskeletal injuries. Thus, it is important to increase flexibility or ROM for enhancement in performance and to prevent musculoskeletal injuries.

Type V collagen is a minor component of tissues containing type I collagen, such as skin, tendons, ligaments, bone, cornea, and blood vessels [7]. COL5 Va1 (COL5A1, SNP rs12722) has been located on chromosome 9 in humans [8], and SNP rs 12722 produces two alleles of the COL5A1 gene, namely, the COL5A1 rs 12722 C- and T-alleles. Recently, studies on COL5A1 have been represented 1) Ehlers-Danlos syndrome (EDS) and 2) injuries (running performance, achilles tendon injuries, and ROM). Most patients with classic EDS have disease-causing mutations within one copy of their COL5A1 genes, which results in a loss of function of the mutated gene and a 50% reduction in the production of type V collagen [9].

A common C to T single nucleotide polymorphism within the COL5A1 gene is associated with a number of sports injuries or performance-related phenotypes [10]. Posthumus *et al.* [11] suggested that the CC genotype of COL5A1 was under-represented in female subjects with anterior cruciate

* Corresponding author: Seok-Ki Min, Tel. 82-2-970-9555, Email. minseokki@kspo.or.kr

ligament ruptures. The causes of “increased risk” phenotype may be the result of increased musculotendinous stiffness and decreased ROM [12]. Moreover, Collins *et al.* [10] established that COL5A1 is independently associated with lower limb ROM in Caucasian subjects. Since the abnormal ROM has been reported to be associated with injuries, it is important to identify genetic factors for ROM.

However, association between COL5A1 genotype and ROM in Asian people is still unclear. Therefore, the aim of our study was to investigate the association between COL5A1 genotype and flexibility as measured by the passive straight leg raise (SLR) and whole body joint laxity in general Asian college students.

METHODS

Participants

One hundred and seventy seven Korean and Japanese college students (male = 109, female = 68) were recruited for the present study (Table 1). The details along with the purpose and the methods of study were explained to the participants. The subjects also signed an informed consent form before participation.

Range of motion measurements

During muscle tightness measurement, the angle of each joint of the hamstrings and quadriceps were measured, by referring to the measurement method reported by previous study. We performed the SLR [13] test in participants, who had no preexistent or current injury in the examined leg. The SR and SLR measurement were assessed twice, and the average score was calculated.

In addition, we performed the whole body joint laxity (WBJL) [14], such as wrist, knee, spine, elbow, shoulder, ankle, and hip seven positions were tested. The total of positive position was regarded as the measured value.

DNA extraction and COL5A1 genotyping

Cotton swab (Single Wrapped, COPAN, CA, USA) derived

Table 1. The physical characteristic of the subjects

Subjects (n)	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m ²)
Male (n = 109)	23.39 ± 1.17	174.27 ± 6.79	75.56 ± 12.70	24.83 ± 3.65
Female (n = 68)	21.80 ± 0.45	161.85 ± 5.58	55.74 ± 6.00	21.24 ± 1.60
Total (n = 177)	23.18 ± 1.23	169.57 ± 8.76	68.06 ± 14.36	23.47 ± 3.50

Values are mean ± SD. BMI, body mass index

buccal cells were scraped from inner side of the cheek of participants and genomic DNA was extracted. After the cells were lysed in 400µl of DNA lysis solution and incubated at 95°C for 3 minutes. Typically, 400µl of DNA stabilizing solution was added to the samples and stored at 4°C until polymerase chain reaction (PCR) was carried out. Genotyping for the COL5A1 polymorphism was performed by real-time PCR using a TaqMan probe (rs12722, Pre-Designed SNP Genotyping assays). The PCR cycling reaction, using thermal cycler (7500, Applied Biosystem, CA, USA) were as follows: 95°C for 10 min, 40 cycles at 95°C for 15 sec and at 60°C for 1 min.

Statistical analysis

The SPSS statistical package version 19.0 for Windows (SPSS, Inc., Chicago, IL, USA) was used to perform all statistical evaluations. Allele frequencies were determined by gene counting. X² test was used to confirm that the observed genotype frequencies exhibited a Hardy-Weinberg equilibrium distribution. Further, we carried out an X² test for understanding association between SLR and COL5A1 allele and Fisher's exact test was used to evaluate association between SLR and COL5A1 genotypes. Differences in the WBJL results of ROM among the COL5A1 genotypes were tested using analysis of variance (with Tukey's multiple comparison test). The level of significance was set at α = 0.05.

RESULTS

COL5A1 polymorphism

The distribution of the COL5A1 polymorphism and allele are presented in Table 2. The distribution of COL5A1 gene in subjects was in the Hardy-Weinberg equilibrium (p = 0.159), and the results were analogous to that of the previous study in Asian population [15].

Physical characteristics of the SLR and COL5A1 gene

The characteristics of the subjects according to each

Table 2. Distribution of COL5A1 genotype in all subjects

	Genotype frequency, % (n)			Allele frequency, % (n)	
	CC	CT	TT	C	T
Subjects (n = 177)	55.3 (98)	40.7 (72)	4.0 (7)	77.2 (268)	22.8 (79)

COL5A1 gene distribution of subjects was in Hardy-Weinberg equilibrium (p = 0.159)

Table 3. The characteristic of the subjects according to each genotype and SLR

a. According to each genotype

	COL5A1 polymorphism (score)		
	CC	CT	TT
Height (cm)	168.91 ± 8.49	169.81 ± 9.15	176.14 ± 6.12
Weight (kg)	66.63 ± 13.33	69.53 ± 15.60	72.74 ± 14.63
BMI (kg/m ²)	23.16 ± 3.03	23.90 ± 4.00	23.48 ± 4.54

Values are mean ± SD. BMI; body mass index
There was no significant difference among the groups

b. According to the passive straight leg raise

	Height (cm)	Weight (kg)	BMI (kg/m ²)
SLR > 90°	168.18 ± 9.14	65.46 ± 13.63	22.91 ± 2.92
SLR < 90°	173.60 ± 5.61***	75.07 ± 13.73***	24.91 ± 4.37**

Values are mean ± SD. BMI; body mass index, SLR; the passive straight leg raise

p-value was analyzed by One-way ANOVA, ** p < 0.01, *** p < 0.001

Table 4. Range of motion based on COL5A1 polymorphism

a. The passive straight leg raise

	COL5A1 polymorphism (%)				
	CC	CT	TT	C	T
SLR > 90° (n)	61.11 (77)	38.10 (48)	0.79 (1)	80.16 (202)	19.84 (50)
SLR < 90° (n)	41.17 (21)	47.06 (24)	11.77 (6)	64.71 (66)	35.29 (36)
p-value		0.001*		0.002 [#]	

SLR; the passive straight leg raise

* Fisher's exact test

[#] X² test

b. Whole body joint laxity

	COL5A1 polymorphism (score)		
	CC	CT	TT
WBJL	3.47 ± 1.51	2.99 ± 1.72	2.70 ± 1.52
p-value		0.126	

Values are mean ± SD. WBJL; whole body joint laxity

p-value was analyzed by One-way ANOVA

genotypes and SLR are shown in Table 3. There was no significant difference among the groups with respect to genotypes (Table 3a). However, significant difference was noted between the relative SLR and characteristics of the subjects. The physical parameters including height, weight, and BMI were higher in < 90° group than > 90° group (Table 3b).

Relationship of COL5A1 polymorphism with ROM

The SLR and WBJL results of the ROM with respect to COL5A1 genotype and the C-dominant model (CC and CT genotype) and T-dominant (TT and CT genotype) model in subjects are shown in Table 4. The relative SLR differed

significantly among the COL5A1 genotype (Table 4a). However, no significant difference was seen in the WBJL among the COL5A1 genotype (Table 4b), but significant difference was seen in CC genotype when compared to CT (2.99 ± 1.72) or TT (2.70 ± 1.52) genotype.

DISCUSSION

The present study is the first of its type to investigate the association between COL5A1 genotype and ROM in general Asian college students. The main finding of the study was the presence of association between SLR ROM and COL5A1. Moreover, an increasing trend in WBJL ROM was noted in subjects with COL5A1 CC genotype, which was not significant. The physical parameters including height, weight, and BMI were higher in < 90° group than > 90° group. Previously, association between COL5A1 genotypes and straight leg raise ROM in Caucasian population has been reported [16]. In addition, a positive correlation between COL5A1 genotype and ROM has been established in a group of South African participants [1]. An overexpression in CC genotype has been reported in individuals with greater ROM [17]. CC genotype collagen fiber containing large irregular and small fibrils. These mutations cause Ehlers-Danlos Syndrome (EDS), which has been demonstrated to have a detrimental effect on the habitual level of physical activity within the patients. Also, the study demonstrates presence of a mixture of large and small irregular fibrils in EDS. In addition, TT genotype collagen fibers containing smaller more densely packed fibrils have been reported. The fibrils, which are proposed to have an increased stiffness and/or creep inhibition, are associated with reduced joint ROM, increased risk for specific musculoskeletal soft tissue injuries, and faster endurance running performance. [10]. COL5A1 gene encodes α1 (V) chain of type V collagen and a mutation in this gene, which causes haploinsufficiency, results in 50% reduction of type V collagen, and leads to poorly organized fibrils, decreased tensile strength, and reduced stiffness of connective [11]. It has been suggested that due to reported association with tendons, individuals with a TT genotype would have less extensible tendon structures, resulting in a lower range of motion (ROM) as compared to individuals with at least one copy of the C allele [10].

The reduction in ROM leads to concomitant reduction in physical activity [18], or an alteration in collagen integrity with increasing age [19]. Brown *et al.* [1] reported that SR ROM tended to increase with age in those subjects with COL5A1 CC genotype, while there was little or no correlation

in the other two genotypes. Bertuzzi *et al.* [20] reported no significant differences between COL5A1 genotypes for ROM in physically active men. Saunders *et al.* [21] stated that COL5A1 genotypes might interact with age for ROM in physically active subjects. On the other hand, Goncalves-Neto *et al.* [22] and Satomi *et al.* [23] reported that damaged and pathological tendons contained relatively higher proportion of collagen type V, and these alterations were accompanied by a reduction in type I collagen. Thus, according to COL5A1 genotype might be increase rate of injuries in elderly. However, in the present study, subjects were young college students, and there were significant differences in the three COL5A1 genotypes with SLR.

The present study has some limitations. We did not consider the levels of physical activity and exercise. We assumed that there were no significant differences in physical activity levels and exercise experienced. However, physical activity and exercise levels should be considered in future studies. Another limitation is that sample size was relatively small, and further studies with larger populations are required to validate our findings. The primary aim of the study was not intended to investigate gender-specific genetic risk factors, and therefore further research is required.

In conclusion, our data indicates association between the COL5A1 genotypes and SLR in young Asian population. CC genotype of COL5A1 has been found to be associated with increased SLR ROM. Although present study shown that did not significantly in WBJL but increased tended to CC genotype. Therefore, present findings provide preliminary evidence on association of COL5A1 genotype with ROM in Asian population.

ACKNOWLEDGMENTS

This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2014S1A5B5A07039021).

REFERENCES

- [1] Brown JC, Miller CJ, Schwellnus MP, Collins M. Range of motion measurements diverge with increasing age for COL5A1 genotypes. *Scand J Med Sci Sports*. 2011;21(6): e266-72.
- [2] Bahr R, Holme I. Risk factors for sports injuries--a methodological approach. *Br J Sports Med*. 2003;37(5): 384-92.
- [3] Amako M, Oda T, Masuoka K, Yokoi H, Campisi P. Effect of static stretching on prevention of injuries for military recruits. *Mil Med*. 2003;168(6):442-6.
- [4] Witvrouw E, Mahieu N, Danneels L, McNair P. Stretching and injury prevention: an obscure relationship. *Sports Med*. 2004;34(7):443-9.
- [5] Whaley MH. ACSM guidelines for exercise testing and prescription. Philadelphia; Lippincott Williams & Wilkins. 2006, 1-366.
- [6] Arnason A, Sigurdsson SB, Gudmundsson A, Holme I, Engebretsen L, Bahr R. Risk factors for injuries in football. *Am J Sports Med*. 2004;32(1 Suppl):5S-16S.
- [7] Viglio S, Zoppi N, Sangalli A, Gallanti A, Barlati S, Mottes M, Colombi M, Valli M. Rescue of migratory defects of Ehlers-Danlos syndrome fibroblasts in vitro by type V collagen but not insulin-like binding protein-1. *J Invest Dermatol*. 2008;128(8):1915-9.
- [8] September AV, Cook J, Handley CJ, van der Merwe L, Schwellnus MP, Collins M. Variants within the COL5A1 gene are associated with Achilles tendinopathy in two populations. *Br J Sports Med*. 2009;43(5):357-65.
- [9] Malfait F, Wenstrup RJ, De Paepe A. Clinical and genetic aspects of Ehlers-Danlos syndrome, classic type. *Genet Med*. 2010;12:597Y605.
- [10] Collins M, Posthumus M. Type V collagen genotype and exercise-related phenotype relationships: a novel hypothesis. *Exerc Sport Sci Rev*. 2011;39(4):191-8.
- [11] Posthumus M, September AV, O'Cuinneagain D, van der Merwe W, Schwellnus MP, Collins M. The COL5A1 gene is associated with increased risk of anterior cruciate ligament ruptures in female participants. *Am J Sports Med*. 2009;37(11):2234-40.
- [12] Posthumus M, Schwellnus MP, Collins M. The COL5A1 gene: a novel marker of endurance running performance. *Med Sci Sports Exerc*. 2011;43(4):584-9.
- [13] Göeken LN, Hof AL. Instrumental straight-leg raising: results in healthy subjects. *Arch Phys Med Rehabil*. 1993;74(2):194-203.
- [14] Torii S. Management and prevention for injuries of adolescent athletes in track and field. *Orthop Traumatol*. 2000;43:1311-8.
- [15] Kim JH, Jung ES, Kim CH, Youn H, Kim HR. Genetic associations of body composition, flexibility and injury risk with ACE, ACTN3 and COL5A1 polymorphisms in Korean ballerinas. *J Exerc Nutrition Biochem*. 2014; 18(2):205-14.
- [16] Collins M, Mokone GG, September AV, van der Merwe L, Schwellnus MP. The COL5A1 genotype is associated with range of motion measurements. *Scand J Med Sci*

- Sports. 2009;19(6):803-10.
- [17] Brown JC, Miller CJ, Posthumus M, Schweltnus MP, Collins M. The COL5A1 gene, ultra-marathon running performance, and range of motion. *Int J Sports Physiol Perform*. 2011;6(4):485-96.
- [18] Corbin CB. Flexibility. *Clin Sports Med*. 1984;3:101-17.
- [19] Pollock M, Gaesser G, Butcher J, Despres J-P, Dishman R, Franklin B, Ewing G. American College of Sports Medicine Position Stand. The recommended quantity and quality of exercise for developing and maintaining cardiorespiratory and muscular fitness, and flexibility in healthy adults. *Med Sci Sports Exerc*. 1998;30:975-91.
- [20] Bertuzzi R, Pasqua LA, Bueno S, Lima-Silva AE, Matsuda M, Marquezini M, Saldiva PH. Is the COL5A1 rs12722 gene polymorphism associated with running economy? *PLoS One*. 2014;4:9(9):e106581.
- [21] Saunders CJ, van der Merwe L, Cook J, Handley CJ, Collins M, September AV. Extracellular matrix proteins interact with cell-signaling pathways in modifying risk of achilles tendinopathy. *J Orthop Res*. 2015; doi: 10.1002.
- [22] Goncalves-Neto J, Witzel SS, Teodoro WR, Carvalho-Junior AE, Fernandes TD, Yoshinari HH. Changes in collagen matrix composition in human posterior tibial tendon dysfunction. *Joint Bone Spine*. 2002;69:189-94.
- [23] Satomi E, Teodoro WR, Parra ER, Fernandes TD, Velosa APP, Capelozzi VL, Yoshinari NH. Changes in histoanatomical distribution of type I, III and V collagen promote adaptative remodeling in posterior tibial tendon rupture. *Clinics*. 2008;63:9-14.
- [24] Fletcher B, Hanson J, Page N, Pine K. FIT - Do something different a new behavioral program for sustained weight loss. *Swiss Journal of Psychology*. 2011;70(1): 25-34.
- [25] Sairanen E, Lappalainen R, Lapveteläinen A, Tolvanen A, Karhunen L. Flexibility in weight management. *Eat Behav*. 2014;15(2):218-24.