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Genetic associations of body composition, flexibility and injury risk with *ACE, ACTN3* and *COL5A1* polymorphisms in Korean ballerinas

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Jun Ho Kim, Eun Sun Jung, Chul-Hyun Kim, Hyeon Youn and Hwa Rye Kim. Genetic associations of body composition, flexibility and injury risk with *ACE, ACTN3* and *COL5A1* polymorphisms in Korean ballerinas. *JENB*., Vol. 18, No. 2, pp.205-214, 2014 [Purpose] The purpose of this study was to exam the association of body composition, flexibility, and injury risk to genetic polymorphisms including *ACE* ID, *ACTN3* RX, and *COL5A1* polymorphisms in ballet dancers in Korea. [Methods] For the purpose of this study, elite ballerinas (n = 97) and normal female adults (n = 203) aged 18 to 39 were recruited and these participants were tested for body weight, height, body fat, fat free mass, flexibility, injury risks on the joints and gene polymorphisms (*ACE, ACTN3, COL5A1* polymorphism). [Results] As results, the *ACE* DD genotype in ballerinas was associated with higher body fat and percentage of body fat than the *ACE* II and ID genotypes ($p < 0.05$). In the study on the *ACTN3* polymorphism and ballerinas, the XX genotype in ballerinas had lower body weight and lower fat-free mass than the RR and RX genotype ($p < 0.005$). Also, the means of sit and reach test for flexibility was lower in the *ACTN3* XX genotype of ballerinas than the RR and RX genotype of ballerinas ($p < 0.05$). Among the sports injuries, the ankle injury of the XX-genotyped ballerinas was in significantly more prevalence than the RR and XX-genotyped ballerinas ($p < 0.05$). According to the odd ratio analysis, XX-genotyped ballerinas have the injury risk on the ankle about 4.7 (95% CI: 1.6~13.4, $p < 0.05$) times more than the RR and RX-genotyped ballerinas. Meanwhile, the *COL5A1* polymorphism in ballerinas has no association with any factors including flexibility and injury risks. [Conclusion] In conclusion, *ACE* polymorphism and *ACTN3* polymorphism were associated with ballerinas' performance capacity; *COL5A1* was not associated with any factors of performance of Ballerinas. The results suggested that the *ACE* DD genotype is associated with high body fat, the *ACTN3* XX genotype is associated with low fat-free mass, low flexibility, and higher risk of ankle-joint injury. [Keyword] ballet, flexibility, body composition, injury risk, *ACE, ACTN3, COL5A1*

INTRODUCTION

Ballet is an art which expresses ideas and emotions with the movement of the body. Choreography of ballet requires high-intensity movement such as quick moving, jump, rotation and leap [32]. Especially, advanced movements and expressions of ballet require the deep and wide movement of arms and legs and their artistic expressions are using hyperextension and excessive range of motion of the body. As a result, flexibility of ballet dancers is a primary requirement among all the other physical-requirement. Also performing ballet requires high-intensity physical activities

and thus uses much energy and therefore the body of ballet dancers should be harmoniously composed with proper elements [3,13,22,28]. As a result, ballet dancers need harmonious body composition along with beautiful physical condition in order to perform artistic expressions with the movement of the body. Therefore, the dancer needs to have a developed physical capacity, especially flexibility and harmonious body composition along with beautiful physical condition, to successfully perform ballet [3,13,22,28]. To perform ballet successfully, the dancer needs both of the beautifully developed body and developed physical capacity and such are determined by environmental factors and genetic

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factors. Environmental factors include experience of participating in the practice and performance of ballet, dietary habits and physical activities and genetic factors can be determined by the genes received from the parent. The ratio between environmental factors and genetic factors to determine the performance of ballet has not been known yet but if we consider the fact that 35~85% of physical elements, which determine performance of athletes, are determined by genetic factors, ballet performance would be determined by genetic factors by 35% or higher [10,23,24,26,27]. Therefore, the ballet performance requires genetic factors to certain degree. For example, body composition, physical condition and especially flexibility, which are considered as important ones for ballet, are determined by genetic factors [27,28]. There is not enough reports on the genetic factors which can predict the ballet performance. But considering the fact that ballet is an artistic expression requiring great physical capacity and beautifully developed body, physical capacity that is required for ballet including, especially, flexibility-related genes, muscle strength and power-related genes, endurance-related genes and injury-related genes are actively studied with the recent advancement of science and biotechnology. And applying these physical capacity and injury-related genes to ballet, which expresses art with the body, can offer the information which can estimate and improve the ballet performance and prevent injuries. Physical capacity of individuals are determined by phenotype and such phenotype is considered as a result of interaction between genotype and the environment [11]. Therefore, the interpretation of genotype information of individuals is a proper method of finding out the potential of physical performance of the body and it will be an important information for assessing physical capacity and thus provides individualized training program or performance program for ballet dancers. Among currently known genes, a total of 120 candidate genes, which are related to physical capacity (cardiorespiratory endurance, muscle strength, muscle endurance, flexibility, body composition), are introduced [6,14]. However, most of them have low predictability regarding physical capacity and *ACE* ID polymorphism, *ACTN3* RX polymorphism and *COL5A1* polymorphism are strongly suggested as physical fitness-related genes [15]. Especially *ACE* ID polymorphism and *ACTN3* RX polymorphism are reported to have a characteristic of being able to predict body composition along with aerobic endurance and anaerobic muscle strength / power which are contradictory phenotype [2,6,14,17,19] and also *COL5A1* polymorphism is reported to be related to the prediction of joint injury along with flexibility by predicting ROM (range of motion) and endurance-based physical

performance [1,4,7,8]. Genes related to physical capacity such as *ACE* ID, *ACTN3* RX, *COL5A1* polymorphism genes have a potential to be related to the genetic physical capacity and injury of the dancer. Also, when 3 genes are working as genes related to physical capacity and injury, there is a possibility of a correlation between ballet dancers and ballet performance.

But genetic relation on ballet dancers are not reported yet. Especially it is expected that finding and revealing the proper genes for ballet performance of ballerinas can bring the effect of predicting suitability for ballet, physical capacity and injury risk level but there is no study for them yet. Therefore we conducted our study to find out physical fitness-related genes related to ballerinas and to reveal the injury.

METHODS

Study subjects

Ballet dancers and normal female adults were recruited for the study. Their age was from 18 to 39 years old and the number of the female ballerinas was 97 and the number of normal female adults was 203. The subjects had body assessment, gene assessment, flexibility assessment and body composition assessment for the purpose of study. The tests were done on the same day on an empty stomach and were conducted while restricting excessive exercise, drinking and caffeine intake which affect the body composition. For test clothing, the tests were done after the subject changed to the test-clothing. The subjects were given the explanation on the goal and process of study and a consent on the study procedure was obtained before the study.

Study process

This study was done to find out the relation among flexibility, body composition and injury risk of ballet dancers and genes and to achieve the goal, proper subjects were recruited who can show typical body composition, flexibility and injury prevalence of ballet dancers. The subjects were ballerinas with 3 years or longer experience of ballet performance with the minimum of college education and no known disease that can alter the body composition and the body. For such criteria, patients with diabetes, kidney disease, metabolic disease and posture-related orthopedic skeletal muscle disease were excluded using basic survey. Therefore, the dancers with healthy-appearance without metabolic disease and posture-related skeletal muscle disease were selected. Selected subjects were recruited from April 2012 to

September 2012. The selected subjects were gathered on a test-site and listened to explanation on the study and signed a consent after fully understood the explanation. After that, we made them finish basic survey and injury-related test paper in order to assess body composition, posture-related disease, overall health, the level of stomach emptiness, water intake and exposure to dehydration. After that, they changed to test-clothing and body assessment was done to get height, weight and BMI. The body assessment was done by a qualified professional with an international certification. Body composition was tested using bioelectrical impedance analysis (BIA, S720, Biospace, Korea). BIA test measured bioelectrical impedance while having the subject standing on an empty stomach, passing electric charge from hands to feet. Basic tests on the dancers and the control group, injury survey, BIA test and flexibility test were done over the period of 3 months.

Genetic polymorphism analysis

Collecting blood and gDNA: For the analysis of genetic polymorphism, genomic DNA from leukocytes was extracted using Puregene® DNA Purification Kit (Catalog-D550, Gentra, USA). 5~15 μl of gDNA was extracted from 300 μl of whole blood. *ACE* polymorphism analysis: MGB TaqMan® SNP Genotyping assay method was used to analyze *ACE* ID polymorphism(rs1799752) from the extracted gDNA. 3 types of primers and 2 types of probes, developed by Koch and others (2005) were used for the analysis [12]. The nucleotide sequence is as follows. Primer *ACE*111: 5'-CCC-ATC-CTT-TCT-CCC-ATT-TCT-C-3', Primer *ACE*112: 5'-AGC-TGG-AAT-AAA-ATT-GGC-GAA-AC-3', Primer *ACE* 111: 5'-CCT-CCC-AAA-GTG-CTG-GGA-TTA-3', VIC-*ACE* 100: VIC-5'AGG-CGT-GAT-ACA-GTC-A-3'-MGB, FAM-*ACE*100: FAM-5'TGC-TGC-CTA-TAC-AGT-CA-3'MGB. I allele from *ACE* ID polymorphism was amplified by *ACE*111 (Forward), *ACE*112 (Reverse) and VIC probe among the aforementioned primer and probe and D allele was amplified by *ACE*111 (Forward), *ACE*113 (Reverse) and FAM probe. Gene amplification was done by formulating Master Mix for TaqMan Genotyping Assay with gDNA 10~50 ng, 150 pmol primer, 150 nM VIC probe, 50 nM FAM probe and amplifying with ABI 7900HT (Applied Biosystems Incooperate, U.S.A) for denaturation at 92℃ for 15 second, for annealing and extension at 57℃ for 1 minute by 35 cycles and then the type of gene was confirmed.

ACTN3 R577X polymorphism analysis: MGB TaqMan® SNP Genotyping assay method was used to analyze *ACTN3* R577X polymorphism (rs1815739) from the extracted gDNA. For the primer to amply the gene, the custom-designed primer

TaqMan probes was made including SNP among primers which can recognize R allele and X allele by asking ABI company on ACTN nucleotide sequence obtained from Gene Bank. The nucleotide sequence is as follows. Forward: 5'ACG-ATC-AGT-TCA-AGG-CAA-GGC-AAC-ACT-3', Reverse: 5'-ACC-CTG-GAT-GCC-CAT-GAT-G-3', VIC (R-allele specific probe): 5'-TCG-CTC-TCG-GTC-CCA-TGA-TG-3'MGB, FAM (X-allele specific probe): 5'-TCG-CTC-TCG-GTC-AGC-3'MGB. Gene amplification was done by formulating Master Mix for TaqMan Genotyping Assay with gDNA 10~50 ng, 150 pmol primer, 70 nM VIC probe, 70 nM FAM probe and amplifying with ABI 7900HT (Applied Biosystems Incooperate, U.S.A) for denaturation at 92℃ for 15 second, for annealing and extension at 57℃ for 1 minute by 40 cycles and then the type of gene was confirmed.

Flexibility test

Flexibility of the dancers was assessed by Sit & Reach method and SRL (passive strait leg raise) methods. First, Sit & Reach was done by using a Sit & Reach Box, having a subject sitting on the floor with extended knees and straight upper body and arms located to the front and had them to move forward and measured the location of the point of maximally extended hand. The assessments were done 2 times and the higher score was used. For SRL, maximum extension and flexion of the right and the left were assessed using Gonia meter. For SRL assessment, the subject was remained in a lying position on the assessment table and the evaluator was holding ankle and lifting them up as much as possible and the moved angle of the joints was assessed. The assessments were done 2 times and the maximum value was used if the error was within 5% and the medium value of 3 assessments was used if the error was beyond 5%.

Data processing method

Data was built from the collected test results from August 2010 to September 2010 in the form that allows data input and analysis. *ACE*, *ACTN3* and *COL5A1* genotype distributions were obtained by frequency and percentage from the data from this study. HWE (Hardy-weingerg equilibrium) and the difference of distribution between groups were tested using cross analysis(χ^2). If there was a difference of distribution of genotype between groups by cross analysis, percentage test and Bonfferoni correction were done on the discrete data between 2 groups. If there was an area where examples were 5 or less from cross analysis, the Fisher test of accuracy was done. The difference verification on the measured values of the ballet dancer group and the control

group was done by independent T-test and the difference between physical capacity and body composition based on the type of gene was obtained by one-way analysis of variance. If there was the difference of variables, LSD was used for post-test. Also, logistic linear model was used to verify the odds ratio of the injury risk on genotype. Significance level for the difference verification was $p < 0.05$ (one-way verification) and SPSS 13.0 Statistical Package was used for all the analysis.

RESULTS

General characteristics of ballet dancers and normal adults

Table 1 is the general characteristics of 97 ballet dancers and 203 normal female adults. Compared to the control group, ballet dancers were significantly taller and weight was significantly lighter ($p < 0.05$). Especially regarding body composition, FFM and FFMI were lower compared to the control group and FM, FMI and PBF were significantly lower than the control group ($p < 0.05$). Also, BMI, which shows body size and weight, was significantly lower in ballet dancers compared to the control group ($p < 0.05$). Compared to that, Sit & Reach, which shows flexibility, was significantly higher in ballet dancers ($p < 0.05$).

Distribution of ACE ID, ACTN3 and COL5A1 genetic polymorphism in ballet dancers

Case vs. control group study was used to find out a correlation among flexibility, body composition, injury risk and gene from ballet dancers. For that purpose, the distribution on each genetic polymorphism was confirmed with normal adults as shown in <Table 2>. *ACE* ID

Table 1. Female ballet dancers and the control group's general characteristics

	ballet dancers (n = 97)		the control group (n = 203)	
	Mean ± SD	Range	Mean ± SD	Range
Age (years)	20.9 ± 2.4***	18~37	25.3 ± 4.4	18~39
Height (cm)	163.4 ± 4.0***	154.0~175.2	157.0 ± 5.5	140.1~179.3
Weight (kg)	51.2 ± 4.7***	40.8~65.5	57.3 ± 6.8	38.7~79.2
FFM (kg)	38.0 ± 3.0***	32.2~47.3	39.3 ± 4.3	29.0~54.0
FM (kg)	13.2 ± 2.7***	7.0~22.0	18.0 ± 5.2	7.0~36.0
FFMI (kg/m²)	14.2 ± 1.0***	12.4~17.1	15.9 ± 1.5	13.1~20.7
FMI (kg/m²)	5.0 ± 1.0***	2.9~7.9	7.3 ± 2.2	2.9~14.9
BMI (kg/m²)	19.2 ± 1.6***	16.2~23.9	23.3 ± 2.8	17.0~32.5
PBF (%)	25.7 ± 3.6***	16.0~36.0	30.1 ± 6.4	15.0~51.1
Sit & Reach (cm)	26.8 ± 4.0***	12.0~36.2	15.5 ± 8.6	-10.0~31.0

FFM: fat-free mass (FFM), FM: fat mass (FM), FFMI: fat-free mass index (FFMI), FMI: fat mass index (FMI), BMI: body mass index (BMI), PBF: percentage of body fat (PBF), *** significantly difference at $p < 0.001$.

polymorphism's distribution from female ballet dancers was met the condition of HWE (F = 0.057, df = 1, p > 0.10) and also the control group was met the condition of HWE (F = 0.074, df = 1, p > 0.01). There was no difference of *ACE* ID polymorphism's distribution between 2 groups as shown with F = 1.488, df = 2, p = 0.475 from cross analysis of 2 groups on the type of genes from *ACE* ID polymorphism. *ACTN3* RX polymorphism's distribution of female ballet dancers met the condition of HWE (F = 1.314, df = 1, $p > 0.10$) and also the control group met the condition of HWE (F = 2.026, df = 1, $p > 0.01$). Therefore, the polymorphism distribution of ballet dancers and the control group was confirmed to have representativeness. It came out that the difference of polymorphism distribution and allele distribution of 2 groups was not significant when they met the condition of HWE. Cross analysis of 2 groups on *ACTN3* RX genotype showed F = 2.935, df = 2, $p = 0.230$ and the difference verification on allele showed F = 2.407, df = 2, $p = 0.121$. With such result,

Table 2. Ballet dancers' *ACE* ID, *ACTN3*, *COL5A1* genetic polymorphism-distribution

		Ballet Dancers (n = 97)			Controls (n = 203)			
Genotype	*ACE*	II	ID	DD	II	ID	DD	*p*-value
		29 (29.9%)	47 (49.3%)	21 (21.6%)	70 (34.5%)	100 (49.3%)	33 (16.3%)	.475
	ACTN3	RR	RX	XX	RR	RX	XX	*p*-value
		19 (19.6%)	54 (55.7%)	24 (24.7%)	58 (28.6%)	104 (51.2%)	41 (20.2%)	.230
	COL5A1	TT	TC	CC	TT	TC	CC	*p*-value
		4 (4.1%)	36 (37.1%)	57 (58.8%)	13 (6.4%)	66 (32.5%)	124 (61.1%)	.586
Allele	*ACE*	I allele		D allele	I allele		D allele	*p*-value
		105 (54.1%)		89 (45.9%)	240 (59.1%)		166 (40.9%)	.252
	ACTN3	R allele		X allele	R allele		X allele	*p*-value
		92 (47.4%)		102 (52.6%)	220 (54.2%)		186 (45.8%)	.121
	COL5A1	T allele		C allele	T allele		C allele	*p*-value
		44 (22.7%)		150 (77.3%)	92 (22.7%)		314 (77.3%)	.996

the difference of genotype and allele of 2 groups was not confirmed and thus we could not conclude that *ACTN3* RX polymorphism has special distribution for ballet. And *COL5A1* polymorphism's distribution met the condition of HWE for both of 2 groups (ballet dancers: F = 0.328, df = 1, $p > 0.10$; the control group: F = 1.065, df = 1, $p > 0.01$). Therefore, representativeness of genetic distribution of 2 groups were confirmed and thus analysis of genetic difference of 2 groups was possible. When cross analysis was conducted on the difference of genetic distribution of 2 groups, it came out as F = 1.070, df = 2, $p = 0.586$ and there was no difference on *COLA51* polymorphism's distribution from 2 groups. Also there was no difference of allele distribution between 2 groups (F = 0.001, df = 1, $p = 0.996$). Therefore, it was not confirmed whether *COLA51* polymorphism is related to flexibility and injury as a gene for ballet dancers.

Body composition-distribution of ballet dancers based on ACE ID, ACTN3, COL5A1 genetic polymorphism

After comparing body composition of ballet dancers with normal adults, the difference of body composition within genetic polymorphism of ballet dancers was analyzed as <Table 3>. Ballet dancers had significantly lower score not only for FM ratio and FM but also FFM compared to the control group of women of the same age. In this case, *ACE* DD genotype ballet dancers had higher PBF and FM

compared to *ACE* II genotype when comparing body composition of ballet dancers per genotype. On the other hand, there was no difference of FFM of ballet dancers based on *ACE* polymorphism's genotype. Also the significantly lowest FFM was shown in XX genotype among *ACTN3* genotypes. On the other hand, there was no difference of body composition in *COL5A1* genetic polymorphism.

Flexibility of ballet dancers based on ACE ID, ACTN3, COL5A1 genetic polymorphism

The difference of flexibility between normal adults and ballet dancers were confirmed to analyze the genetic difference of flexibility of ballet dancers. As shown in <Table 4>, ballet dancers showed significantly more developed flexibility compared to the normal adults in all the genotypes. There was no difference of genotypes from *ACE* polymorphism and flexibility on *COL5A1* from the result of difference-analysis of genetic genotype from ballet dancers. But XX genotype of *ACTN3* gene showed significantly lower flexibility compared to genotype with R allele.

Injury risk of ballet dancers based on ACE ID, ACTN3, COL5A1 genetic polymorphism

Risk-prevalence of ballet dancers was assessed to analyze the injury risk of genes for ballet dancers. Among dancers,

Table 3. Ballet dancers' body composition based on *ACE* ID, *ACTN3*, *COL5A1* polymorphism

	Ballet Dancers (n = 97)			Controls (n = 203)		
	II (n = 29)	ID (n = 47)	DD (n = 21)	II (n = 54)	ID (n = 76)	DD (n = 21)
PBF(%)	24.6 ± 3.9^{a*}	26.0 ± 3.2^{ab*}	26.6 ± 3.8^{b*}	30.1 ± 6.4	31.1 ± 6.3	31.1 ± 7.0
BF (kg)	12.7 ± 2.9 ^{a*}	13.2 ± 2.4 ^{ab*}	14.1 ± 3.0 ^{b*}	18.1 ± 5.2	18.0 ± 5.0	18.0 ± 6.3
FFM (kg)	38.5 ± 3.3*	37.4 ± 2.7*	38.6 ± 3.1*	39.5 ± 3.8	39.4 ± 4.6	38.9 ± 4.6
FMI (kg)	4.7 ± 1.0 ^{a*}	5.0 ± 0.9 ^{ab*}	5.3 ± 1.1 ^{b*}	7.3 ± 2.2	7.3 ± 2.2	7.3 ± 2.6
FFMI (kg)	14.3 ± 1.0*	14.1 ± 1.0*	14.5 ± 1.0*	16.0 ± 1.4	16.0 ± 1.6	15.7 ± 1.3
	RR (n = 19)	RX (n = 54)	XX (n = 24)	RR (n = 45)	RX (n = 80)	XX (n = 26)
PBF(%)	26.2 ± 4.1*	26.2 ± 3.1*	25.3 ± 3.6*	30.5 ± 6.6	31.4 ± 6.4	30.9 ± 6.4
BF (kg)	13.7 ± 2.9*	13.9 ± 2.7*	12.8 ± 2.6*	17.9 ± 5.2	18.3 ± 5.2	17.6 ± 5.5
FFM (kg)	38.4 ± 3.1*	38.9 ± 3.2*	37.4 ± 2.8*	40.1 ± 4.7	39.1 ± 4.0	38.8 ± 4.6
FMI (kg)	5.1 ± 1.1*	5.2 ± 0.9*	4.8 ± 1.0*	7.2 ± 2.2	7.5 ± 2.2	7.1 ± 2.3
FFMI (kg)	14.2 ± 1.1^{ab*}	14.6 ± 0.9^{b*}	14.1 ± 0.9^{a*}	16.1 ± 1.5	15.9 ± 1.4	15.7 ± 1.8
	TT (n = 4)	TC (n = 36)	CC (n = 57)	TT (n = 13)	TC (n = 66)	CC (n = 124)
PBF(%)	25.7 ± 1.0	25.7 ± 3.3*	25.7 ± 3.9*	27.8 ± 6.4	31.7 ± 6.7	30.9 ± 6.3
BF (kg)	13.0 ± 1.6	13.3 ± 2.3*	13.2 ± 3.0*	16.9 ± 3.8	18.4 ± 5.3	17.9 ± 5.3
FFM (kg)	37.3 ± 2.3*	38.3 ± 3.2*	37.9 ± 2.9*	43.8 ± 4.3	38.9 ± 4.1	39.4 ± 4.4
FMI (kg)	5.1 ± 0.7*	4.9 ± 0.9*	5.0 ± 1.1*	6.9 ± 1.4	7.6 ± 2.4	7.3 ± 2.2
FFMI (kg)	14.6 ± 1.5*	14.2 ± 1.0*	14.2 ± 1.0*	18.0 ± 2.4	15.9 ± 1.5	15.9 ± 1.5

BF = Body fat, FFM = Fat-free mass, FMI = fat mass index, FFMI = fat-free mass index; Different superscript between groups means the significant difference between genotypes ($p < 0.05$).
Superscript a, b, c follow the order of lower to higher score (a < b < c). * = Statistically significant difference ($p < 0.05$) from the variable results of genotype of normal adults

Table 4. Flexibility based on *ACE* ID, *ACTN3*, *COL5A1* polymorphism of ballet dancers

	Ballet Dancers (n = 97)			Controls (n = 203)		
	II (n = 29)	ID (n = 47)	DD (n = 21)	II (n = 54)	ID (n = 76)	DD (n = 21)
Sit & Reach (cm)	27.4 ± 2.6*	26.8 ± 4.5*	25.7 ± 4.5*	16.8 ± 7.2	15.5 ± 9.0	11.9 ± 9.9
SRL-right-flexion (°)	105 ± 12	109 ± 11	106 ± 12	-	-	-
SRL-right-extension (°)	104 ± 12	106 ± 12	100 ± 10	-	-	-
SRL-left-flexion (°)	26 ± 10	27 ± 7	25 ± 6	-	-	-
SRL-right-extension (°)	30 ± 9	30 ± 7	29 ± 6	-	-	-
	RR (n = 19)	RX (n = 54)	XX (n = 24)	RR (n = 45)	RX (n = 80)	XX (n = 26)
Sit & Reach (cm)	27.5 ± 2.9^{b*}	27.2 ± 4.3^{b*}	25.1 ± 3.9^{a*}	15.8 ± 7.6	15.9 ± 9.5	14.8 ± 7.7
SRL-right-flexion (°)	107 ± 8.1	107 ± 11	107 ± 14	-	-	-
SRL-left-extension (°)	105 ± 11	104 ± 11	101 ± 13	-	-	-
SRL-right-flexion (°)	25 ± 7	27 ± 7	25 ± 8	-	-	-
SRL-left-extension (°)	29 ± 4	31 ± 8	28 ± 6.9	-	-	-
	TT (n = 4)	TC (n = 36)	CC (n = 57)	TT (n = 13)	TC (n = 66)	CC (n = 124)
Sit & Reach (cm)	22.5 ± 5.5*	26.4 ± 3.8*	27.3 ± 3.9*	18.5 ± 6.1	15.4 ± 8.6	15.9 ± 8.6
SRL-right-flexion (°)	106 ± 12	105 ± 14	108 ± 9	-	-	-
SRL-right-extension (°)	102 ± 18	102 ± 14	105 ± 10	-	-	-
SRL-left-flexion (°)	23 ± 2	25 ± 9	27 ± 7	-	-	-
SRL-right-extension (°)	25 ± 4	29 ± 8	27 ± 7	-	-	-

Different writings of superscript between groups mean the significant difference ($p < 0.05$) between genotypes within the group.
Superscript a, b, c follow the order of lower to higher score of variables (a < b < c). * = Statistically significant difference ($p < 0.05$) from the variable results of genotype of normal adults.

Table 5. Injury risk based on *ACE* ID, *ACTN3*, *COL5A1* polymorphism of ballet dancers

	Case (injury)			Controls (normal)			
	II	ID	DD	II	ID	DD	*p*-value
backache	7 (24.1%)	13 (27.7%)	3 (14.3%)	22 (75.9%)	34 (72.3%)	18 (85.7%)	.487
waist	10 (34.5%)	15 (31.9%)	7 (33.3%)	19 (65.5%)	32 (68.1%)	14 (66.7%)	.973
pelvis	5 (17.2%)	8 (17.0%)	3 (14.3%)	24 (82.8%)	39 (83.0%)	18 (85.7%)	.953
knee	11 (37.9%)	13 (27.7%)	6 (28.6%)	18 (62.1%)	34 (72.3%)	15 (71.4%)	.620
ankle	19 (65.5%)	35 (74.5%)	10 (47.6%)	10 (34.5%)	12 (25.5%)	11 (52.4%)	.097
	RR	RX	XX	RR	RX	XX	*p*-value
backache	4 (17.4%)	15 (65.6%)	4 (17.4%)	15 (20.3%)	39 (52.7%)	20 (27.0%)	.542
waist	8 (25.0%)	16 (50.0%)	8 (25.0%)	11 (16.9%)	38 (58.5%)	16 (24.5%)	.609
pelvis	3 (15.8%)	9 (16.7%)	4 (16.7%)	16 (84.2%)	45 (83.3%)	20 (83.3%)	.996
knee	7 (36.8%)	14 (25.9%)	9 (37.5%)	12 (63.2%)	40 (74.1%)	15 (62.5%)	.498
ankle	7 (36.8%)	39 (72.2%)	75 (75.0%)	12 (63.2%)	15 (27.8%)	6 (25.0%)	.011
	TT	TC	CC	TT	TC	CC	*p*-value
backache	0 (0.0%)	9 (69.2%)	4 (30.8%)	4 (5.4%)	27 (36.5%)	43 (58.1%)	.522
waist	1 (25.0%)	13 (36.1%)	18 68.4(%)	3 (75.0%)	23 (36.1%)	39 (68.4%)	.498
pelvis	1 (25.0%)	7 (19.4%)	8 (14.0%)	3 (75.0%)	29 (80.6%)	48 (86.0%)	.668
knee	0 (0.0%)	12 (33.3%)	18 (31.6%)	4 (100%)	24 (66.8%)	39 (68.4%)	.387
ankle	4 (6.3%)	24 (37.5%)	36 (56.2)	0 (0.0%)	12 (36.4%)	21 (63.6%)	.320

those with injury to concerned body part were categorized into the case group and those without injury to concerned body part were categorized into the control group and then prevalence was compared based on genotype. And the result is listed in <Table 5>. There was no significant difference on injury from all the genotypes but the only exception was injury-frequency of ankle from *ACTN3* genotype, which was higher in XX genotype. Therefore, when the injury risk of ankle on *ACTN3* XX genotype was obtained with odds ratio, *ACTN3* XX genotype was 4.65 times significantly higher than *ACTN3* RR+RX genotype in terms of injury risk of ankle as shown in <Table 6>.

Table 6. Ankle injury's odds ratio based on *ACTN3* RX polymorphism from ballet dancers

		Genotype			Statistic value	
	RR	RX+XX	OR	F-value	df	*P*-value
ankle injury	7 (36.8%)	57 (73.1)	4.652	8.937	1	0.003
Normal	12 (63.2%)	12 (26.9%)	(CI: 1.6~13.4)			

DISCUSSION

Ballet uses developed flexibility and slim appearance to express high-intensity activities when using the body to express art. Therefore, dancers who are suitable for ballet need to have developed flexibility with the demand for little fat and FFM with strong muscle strength and muscle endurance. Environmental and genetic factors affect such characteristics of ballet dancers. This study was designed to find genes related to the characteristics of ballet. Also we tried to find the gene which is related to frequent joint injuries due to too much pressure on the joint during the high-intensity ballet performance with a slim appearance. For the first step, *ACE* ID polymorphism, *ACTN3* RX polymorphism and *COL5A1* polymorphism were selected as candidate genes related to ballet. And we hypothesized that *ACE* ID polymorphism, *ACTN3* RX polymorphism and *COL5A1* polymorphism were the related genes for successful ballet performance. Also these genes were hypothesized as the genes related to injury. And the basis behind that is that these 3 genes were strongly suggested as candidate genes that could related to muscle strength, body composition and injury [1,4,6,14,15,21]. We tried to find out whether these physical capacity and injury-related genes are related to ballet dancers. As a primary study for that, the case group vs. the control group study was done. The case group consisted of ballerinas and the control group consisted of normal female adults of the same age. There was no difference of genetic distribution between the case group and the control group from the result of comparing distribution of each gene by the study design. From the result of comparing the difference of flexibility, body composition and physical structure of each genotype of ballet dancers, *ACE* DD genotype ballerinas had genetic relation of having higher PBF and FM from *ACE* gene. From previous studies, a mechanism could be explained that when the activity of *ACE* protein is high, fat accumulation is increased and it becomes a risk factor for obesity [18,31]. High *ACE* activity brings the change of angiotensin I and angiotensin II on RAS (renin-angiotensin system) and thus maintains high level of activity in the blood and tissue [34]. Especially, the level of angiotensin II, which is not only circulatory RAS but also topical tissue which is high in fat tissue, increases fat accumulation and mobilization of blood flow and thus it was reported as a risk factor to increase blood pressure [5,29,30]. From recent studies on human beings and animals, high *ACE* level was highly related to BMI and increased *ACE* activity of fat tissue of animals had induced the accumulation of subcutaneous fat and visceral fat [5,9,29,30,34]. Therefore, *ACE* DD genotype in this study can be related to the mechanism inducing high PBF and FM by having increased angiotensin II, due to 2~3 times high activity of *ACE*, on fat tissue. Therefore, it can be explained that ballet dancers with *ACE* DD genotype can easily have higher PBF and FM. Also, based on the report that when *ACE* protein has high activity it increases fat accumulation and subsequent diabetes, ballet dancers prefer slim looks with little FM and FFM and in that case, DD genotype ballet dancers could have genetic difficulty in terms of having lesser FM [18,20,31]. And considering such result, it is estimated that a better strategy will be necessary for ballet dancer with *ACE* DD genotype to prevent fat accumulation. Especially, our study can suggest that additional aerobic exercise other than dance is necessary to promote the oxidation of fat to effectively reduce fat accumulation. On the other hand, body composition, flexibility and risk injury per *ACTN3* polymorphism from ballet dancers were related. XX genotype among *ACTN3* genotypes (RR, RX, XX) had low weight and it occurred because FFM was significantly lower from fat and FFM that compose weight. And XX genotyped ballerinas with low FFM had relatively low flexibility and high ankle injury risk. *ACTN3* gene is a gene expressing α-actinin-3, which consists of myomere of rapid muscle and RR and RX genotype from *ACTN3* polymorphism are expressing α-actinin-3 but XX genotype is a genotype which stops expression [17,20,25,]. α-actinin-3 has a characteristic of supporting myomere for the function of strong muscle contraction in rapid muscle. If α-actinin-3 is not expressed, then α-actinin-2 is expressed instead and rapid muscle becomes to possess the characteristic of slow muscle [16,17,20,25,].

Therefore, high power from genetic capacity could be gained based on *ACTN3* genotype along with endurance and in some studies, FFM level came out different due to the adjusted muscle shape [17,20,25,31]. Also this study showed that XX genotype in ballet dancers, who tend to have lower fat and FFM compared to normal adults, had much lower FFM with lower flexibility and thus higher risk of injury was

there. When *ACTN3* was initially discovered, XX genotype was considered as a gene related to hereditary disease of muscle since there was no expression of a-actinin-3 [20,25], but from the studies afterwards revealed that even there was no expression of a-actinin-3 in rapid muscle, a-actinin-2 would be expressed instead in rapid muscle and there was no correlation with muscle disease [20,25,31]. But when a-actnin-2 was expressed instead of a-actinin-3 in rapid muscle, the contractility and the size of muscle fiber was decreased and also cell metabolism, which has advantage in endurance, was developed [15,16,17]. In other words, aerobic metabolism related substances were increased and also showed a mechanism of protein-increase related to aerobic metabolism. Such mechanism showed that the metabolism of rapid muscle became developed closer to slow muscle and also rapid muscle's size got decreased. Such mechanism is related to a mechanism which can bring the decrease of FFM and also possibly bring excessive burden on rapid muscle easily with physical activities that require powerful action in a quick moment such as in sports game or ballet and thus as a result, muscle strength for the wider range of joint movement is decreased and therefore it is potentially limiting to flexibility's development. Also we think that such limitation of decreased muscle strength and restricted flexibility-development of rapid muscle bring more burden during quick jump, rotation and the change of action and therefore contribute to the increase of injury of skeletal muscle. Therefore more efforts and strategy will be necessary for ballet dancers with *ACTN3* genotype, which cannot express a-actinin-3 in rapid muscle, to develop flexibility and also injury risk should be prevented along with the improvement of ballet performance with muscle strength-increasing program. Considering that ballet requires slim looks with strong physical capacity to perform high-intensity performance, ballet dancers with XX genotype should be more careful. In other words, ballet dancers with XX genotype would need additional muscle strengthening exercise and with such, the improvement of ballet performance and injury prevention can be achieved. On the other hand, the hypothesis of this study was that *COL5A1* polymorphism is a gene related to ballet and it can predict high flexibility and injury. But the study result showed that there was no difference of genetic distribution between ballet dancers and the control group and also genetic relation could not be found when flexibility and the injury level per genotype from ballet dancers were analyzed. Such results indicate that *COL5A1* gene is not applicable for Koreans since the previous studies were done on Caucasian population. One of the reasons of such conclusion is that genetic distribution of *COL5A1* of ballet

dancers or normal adults (the control group) of this study showed different genetic distribution compared to genetic distribution of *COL5A1* in Caucasian population. In other words, *COL5A1*'s distribution was generated based on the characteristics of Koreans and it would be difficult to apply the result from Caucasian population to Koreans. To sum it up, *COL5A1* genetic polymorphism came out with no relation to flexibility and ankle injury in Koreans contrary to the studies on Caucasian population. The relation of flexibility, body composition, injury risk based on genotype of *ACTN3* gene should be studied in detail for future studies with intervention-programs and prospective studies.

CONCLUSION

From the result of this study, *ACE* DD genotype from *ACE* ID polymorphism was related to higher PBF for ballet dancers and *ACTN3* XX genotype from *ACTN3* RX polymorphism was related to low FFM and flexibility for ballet dancers. *ACTN3* gene came out as a gene related to injury when checking out the genes potentially related to injury and people with *ACTN3* XX genotype has 4 times higher risk of ankle injury during ballet compared to the people with different genotypes.

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