

# Relationship of muscle power and bone mineral density with the $\alpha$ -actinin-3 R577X polymorphism in Japanese female athletes from different sport types

## An observational study

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### Abstract

The aim of this study was to clarify the relationships between muscle power and bone mineral density (BMD) and the  $\alpha$ -actinin-3 (*ACTN3*) R577X polymorphism in Japanese female collegiate athletes participating in sports with various mechanical-load characteristics. This study included 260 female collegiate athletes involved in 10 competitive sports and 26 controls (mean ages,  $19.2 \pm 1.2$  and  $19.7 \pm 1.3$  years, respectively). The sports were classified into 3 categories (low-impact, multidirectional, and high-impact) based on the exercise load characteristics. Data on sports participation and competition experience were obtained through a questionnaire-type survey. The maximum anaerobic power (MANP) test was performed to measure muscle power. The total body BMD was measured using dual-energy X-ray absorptiometry. The *ACTN3* R577X polymorphism (rs1815739) was analyzed using a TaqMan® assay. The multidirectional sports participants with the RR genotype of the *ACTN3* R577X polymorphism had a higher BMD than those with the RX and RX + XX genotypes ( $P = .018$  and  $P = .003$ , respectively). The RR genotype was also associated with a higher MANP than those with the RX + XX genotypes ( $P = .035$ ). No other variables related to BMD and MANP were significantly different. Our results suggest that the RR genotype may confer high trainability for BMD and muscle power in Japanese female collegiate athletes participating in multidirectional sport types. However, these associations were not found in the athletes participating in the low- and high-impact sport types.

**Abbreviations:** *ACTN3* =  $\alpha$ -actinin-3, BMD = bone mineral density, BMI = body mass index, DEXA = dual-energy X-ray absorptiometry, MANP = maximum anaerobic power, PCR = polymerase chain reaction.

**Keywords:** actinin, athlete, bone, female, genotype

## 1. Introduction

Recently, studies have increasingly examined individualized training methods.<sup>[1,2]</sup> These studies have primarily examined the individual differences in the response to exercise training, also termed trainability.<sup>[3]</sup> One genetic variant that determines trainability is the  $\alpha$ -actinin-3 (*ACTN3*) R577X polymorphism. *ACTN3*, which is normally expressed in fast-twitch skeletal muscle fibers, is the major structural component of the Z-line that anchors actin and plays a regulatory role in coordinating muscle fiber contraction.<sup>[4–6]</sup> The common null R577X polymorphism in the *ACTN3* gene converts the codon for arginine (R) at position 577 to a premature stop codon (X), resulting in complete deficiency of *ACTN3* in homozygote humans (*ACTN3* gene XX genotype).<sup>[6]</sup> *ACTN3* deficiency does not result in a disease phenotype, suggesting that *ACTN3* is not essential for

muscle development or normal cell function, and that  $\alpha$ -actinin-2 can provide functional compensation at least in fast muscle fibers.<sup>[7]</sup> This polymorphism was first associated with athletic performance in power-type sports.<sup>[8]</sup> In particular, the frequency of the *ACTN3* R577X genotypes in athletes participating in various types and levels of competitive sports was investigated. Frequencies of 50% for the RR genotype, 50% for the RX genotype, and 0% for the XX genotype were identified in sprint-/power-type Olympic-level athletes, while endurance-type Olympic-level athletes displayed frequencies of 30% for the RR genotype, 40% for the RX genotype, and 30% for the XX genotype. These findings suggested that having at least 1 R allele (RR and RX genotypes) are likely common in athletes who engage in sprint-/power-type sports. Subsequently, several studies reported associations between the *ACTN3* R577X polymorphism and

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muscle power,<sup>[9]</sup> sprint-/power-type sports performance,<sup>[10,11]</sup> muscle metabolism,<sup>[12,13]</sup> and muscle fiber composition.<sup>[14]</sup> These studies suggested that having at least 1 R allele is associated with high muscular strength. Additionally, the latest meta-analysis on the association between the *ACTN3* R577X polymorphism and elite power sports found that female power-type athletes have a higher R allele frequency than general populations.<sup>[15]</sup> Therefore, this genetic polymorphism may be related to the trainability of high muscle performance in female athletes.

More recent studies have also reported associations of the *ACTN3* R577X polymorphism with bone mineral density (BMD).<sup>[16–19]</sup> A study has reported that an *ACTN3*-knockout mouse displayed significantly reduced bone mass, with a reduced cortical bone volume and number of trabeculae.<sup>[16]</sup> Another study suggested that the *ACTN3* R577X polymorphism may be related to BMD in women, as it found that the RR genotype was associated with a higher BMD in older women than the RX and XX genotypes.<sup>[17]</sup> In addition, several studies have reported that the RR and RX genotypes are associated with a higher BMD than the XX genotype,<sup>[18,19]</sup> which suggest that carrying the *ACTN3* R577X R allele is associated with a higher BMD. However, there have been few study reports on the association of BMD with the *ACTN3* R577X polymorphism. To the best of our knowledge, there are no studies that have examined this association in Japanese female collegiate athletes.

In female athletes, low BMD is a particular problem because it may lead to serious injuries such as stress fractures among athletes; recently, it has become a point of focus in the literature.<sup>[20–24]</sup> Tenforde and Fredericson<sup>[25]</sup> reported that participation in aquatic sports, such as competitive swimming, negatively affected BMD and that female adolescent distance running was associated with suppressed bone mineral accrual, while participation in high-mechanical-load sports, such as jumping-type sports and judo, positively affected BMD. Kobayashi and Hwang have indicated that participation in sports with low-mechanical-load characteristics is a risk factor for low BMD in female athletes.<sup>[26]</sup> Thus, sport type classified according to impact-load characteristics is a determining factor in BMD.

Therefore, we aimed to investigate the relationships of muscle power and BMD with the *ACTN3* R577X polymorphism in Japanese female collegiate athletes participating in sports with various mechanical-load characteristics. We hypothesized that the RR genotype or R allele of the *ACTN3* R577X polymorphism might be advantageous to muscle power and BMD in Japanese female collegiate athletes who participate in any sport type.

## 2. Methods

### 2.1. Study design

A cross-sectional design was used to investigate the relationships of muscle power and BMD with the *ACTN3* R577X polymorphism in 260 female Japanese collegiate athletes and 26 controls. Each participant underwent a maximum anaerobic power (MAnP) test and a dual-energy X-ray absorptiometry (DEXA) scan. Each athlete completed a questionnaire on her competitive experience. DNA extracted from saliva was used to determine the *ACTN3* R577X genotype using polymerase chain reaction (PCR). These data were collected between 2018 and 2021. Participants with any type of injury or signs of infection were excluded from the study. All participants were informed of the benefits and risks of the investigation prior to signing an institutionally approved informed consent document to participate in the study. Additionally, we obtained written informed consent for participation as well as publication of their data. All the experiments reported in the manuscript were performed in accordance with the ethical standards of the Helsinki Declaration. The study was approved by the ethics review committee of Nippon Sport Science University (approval number, 019-G02; approval

date, May 8, 2017). For participants aged < 20 years, the experiments were performed with the consent of their parents (age range, 18–22 years).

### 2.2. Participants

We enrolled athletes who represented 10 competitive sports from a physical educational university (long-distance in track and field, n = 46; water polo, n = 31; lifesaving, n = 35; tennis, n = 27; soft tennis, n = 28; badminton, n = 9; handball, n = 28; judo, n = 13; jumping/throwing in track and field, n = 10; and weightlifting, n = 33) and healthy age-matched controls with no history of competitive sports participation. The athletes competed in various competitions, including international events and trained at least 5 days per week for at least 2 hours per day. Physical characteristics of the participants are shown in Table 1.

### 2.3. Procedures

**2.3.1. Questionnaire survey.** From the athletes, we obtained data on sports participation, competition experience, and age at menarche through a questionnaire-type survey at the start of the experiment.

**2.3.2. Classification of sport types.** Considering the effect of the characteristics of different sport types on BMD, the 10 competitive sports were classified into 3 categories based on previous studies<sup>[23,25]</sup>: low-impact (athletes participating in long-distance in track and field, water polo, and lifesaving), multidirectional (tennis, soft tennis, badminton, and handball), and high-impact (judo, jumping/throwing in track and field, and weightlifting). Lifesaving, also known as rescue swimming, was classified as a low-impact-type sport because it was reported to be similar to competitive swimming in the previous study.<sup>[27]</sup> In addition, swimming was defined as a non-impact sport in a previous study,<sup>[23]</sup> but since lifesaving is a sport that combines swimming and running,<sup>[27]</sup> we decided to group long-distance and swimming events as low-impact in this study.

**2.3.3. DEXA scan.** The total body BMD, fat mass, and lean mass were measured by DEXA scanning (iDXA, GE Medical Systems Lunar, Madison, WI). DEXA scan preparation was as follows: having a normal meal on the day of the scan at least 2 hours prior to scanning and removing any piece of metal from the body, including jewelry and dental appliances. Scans were performed with the participant wearing plain underwear and a common inspection gown. All DEXA data were acquired by a single radiological technician.

**2.3.4. MAnP measurement.** MAnP was determined using a cycle ergometer (POWERMAX-VIII, COMBI, Tokyo, Japan)

**Table 1**  
Physical characteristics of the study participants.

Variable (units)	All athletes (n = 260)	Controls (n = 26)
Age (yrs)	19.2 ± 1.2	19.7 ± 1.3
Height (cm)	160.1 ± 5.0	161.2 ± 5.2
Weight (kg)	56.6 ± 6.9	54.3 ± 6.5
BMI (kg·m <sup>-2</sup> )	22.1 ± 2.5	20.9 ± 2.1
%Fat (%)	23.8 ± 4.1	29.2 ± 4.1
Fat mass (kg)	13.6 ± 3.6	16.0 ± 4.0
Lean mass (kg)	40.5 ± 4.0	35.9 ± 2.9
Menarche (yrs)	12.5 ± 1.7	12.6 ± 1.3
Total body BMD (g·cm <sup>-2</sup> )	1.202 ± 0.102	1.187 ± 0.062
Experience of competition (yrs)	7.8 ± 4.0	-

Values are presented as mean ± standard deviation.  
BMD = bone mineral density, BMI = body mass index.

as an indicator of muscle power. The MANP test consisted of 3 sets of 10 seconds full power pedaling with 2 minutes rest interval. First, participants warmed up for 5 minutes at 60 rpm with a 1 kg load (60 W), followed by a full power pedaling practice at 1 kg load. The MANP test was then performed. Toe clips and well fasted straps avoided losing the pedals. We instructed that during the pedaling, the participants had to stay seated on the saddle and were vigorously encouraged to reach the maximal pedaling rate as soon as possible. The first load of the test was determined by the participants' body weight (<50 kg = 2 kg, 50–69 kg = 3 kg, >70 kg = 4 kg load, respectively), the load of the second set was determined by the rpm of the first set (<150 rpm = +1 kg, 150–179 rpm = +2 kg, >180 rpm = +3 kg load, respectively), and the load of the third set was determined by the rpm of the second set (<130 rpm = +1 kg, 130–149 rpm = +2 kg, >150 rpm = +3 kg load, respectively). The peak velocity was noted and used to calculate the force-velocity relationship. MANP was the peak value of the power curve obtained from the regression line of the force-velocity relationship. Both absolute and relative values per body weight were used for the analysis.

**2.3.5. Genetic testing.** In this study, 2 mL of saliva was collected by using a self-collection kit (Oragene® DISCOVER, DNA Genotek, Ottawa, ON, Canada). Saliva samples were incubated at 55°C in a water incubator (SN-100SD, NISSIN, Tokyo, Japan) for 60 minutes. Afterward, DNA was extracted as per the manufacturer's instructions; a 500-µL saliva sample was transferred to microcentrifuge tubes and 20 µL (1/25th saliva volume) of Oragene DNA Purifier (PT-L2P, DNA Genotek, Ottawa, ON, Canada) was added. The samples were incubated on ice for 10 min and centrifuged at room temperature (15–30°C) for 5 minutes at 15,000 × g. The supernatant was transferred to a new tube, and an equal volume of 100% ethanol was added. The DNA was left to precipitate for 10 minutes; it was then pelleted by centrifugation for 2 minutes at 15,000 × g, and the supernatant was discarded. The DNA pellet was washed and resuspended in a 100-µL Tris-EDTA buffer. The presence of the *ACTN3* R577X genotype (rs1815739) was determined using the TaqMan™ SNP genotyping assay (Assay ID: C\_\_\_590093\_1\_) and a Real-Time PCR System (CFX96 Touch™ Real-Time PCR, Bio-Rad, Hercules, CA). We performed each PCR reaction in a 6-µL genotyping mixture containing 2.5 µL TaqMan Universal Master Mix II, 0.125 µL TaqMan SNP Genotyping Assay mix, 2.375 µL sterilized

water, and 1 µL genomic DNA. The genotyping results were analyzed using the CFX Manager software version 2.1 (Bio-Rad, Hercules, CA).

## 2.4. Statistical analyses

The appropriate sample size was determined with reference to previous studies investigating the association between *ACTN3* R577X polymorphism and BMD.<sup>[17,19]</sup> Sample size estimation was calculated using G\*Power version 3.1.9.7. The estimation was based on the effect size of 0.4, alpha level of .05, and power of 0.80. We confirmed that the sample size was sufficient for the study design. All statistical analyses were performed using SPSS version 27.0 (IBM Corp., Armonk, NY). The level of significance was set as  $P < .05$ . Pearson's chi-square test was performed to confirm the conformance of the observed genotype frequencies with the Hardy–Weinberg equilibrium distribution. Pearson's correlation coefficient was used to investigate the relationship between MANP and total body BMD in participants in each sport type and in the controls. Comparisons of the physical characteristics, BMD, and MANP of the athletes according to the sport type and the controls and *ACTN3* R577X genotype were performed using a 1-way analysis of variance. If a significant effect was observed, the Bonferroni post hoc test for multiple comparisons was performed to identify significant differences among the mean values. Eta-squared ( $\eta^2$ ) effect sizes were classified as small < 0.06, moderate 0.06 to 0.14, and large > 0.14.<sup>[28]</sup> To compare detailed differences between the genotypes, an R-dominant model (RR + RX vs XX) and R-recessive model (RR vs RX + XX) were analyzed using an unpaired *t* test. Cohen's *d* effect sizes were interpreted as small < 0.5, medium 0.5 to 0.8, and large > 0.8.<sup>[28]</sup>

## 3. Results

### 3.1. Genotype frequency

The genotype frequencies of the *ACTN3* R577X polymorphism for athletes participating in each sport and each sport type and for the controls are shown in Table 2. The genotype frequencies were in Hardy–Weinberg equilibrium (low-impact,  $P = .902$ ; multidirectional,  $P = .836$ ; high-impact,  $P = .618$ ; controls,  $P = .945$ ).

**Table 2**  
Genotype frequencies of  $\alpha$ -actinin-3 R577X polymorphism.

Group	RR	RX	XX	RR + RX	RX + XX	HWE <i>P</i> value
Sport						
Long distance	11(24)	24(52)	11(24)	35(76)	35(76)	.768
Water polo	8(26)	15(48)	8(26)	23(72)	23(72)	.857
Lifesaving	3(9)	17(48)	15(43)	20(57)	32(91)	.551
Tennis	6(22)	14(52)	7(26)	20(74)	21(78)	.842
Soft tennis	6(21)	15(54)	7(25)	21(75)	22(79)	.700
Badminton	3(33)	5(56)	1(11)	8(89)	6(67)	.613
Hand ball	8(29)	11(39)	9(32)	19(68)	20(71)	.259
Judo	1(8)	8(61)	4(31)	9(69)	12(92)	.279
Jumping/throwing	4(40)	2(20)	4(40)	6(60)	6(60)	.058
Weightlifting	8(24)	16(49)	9(27)	24(73)	25(76)	.866
Impact type						
Low-impact	22(20)	56(50)	34(30)	78(70)	90(80)	.902
Multidirectional	23(25)	45(49)	24(26)	68(74)	69(75)	.836
High-impact	13(23)	26(47)	17(30)	39(70)	43(77)	.618
Controls	5(19)	13(50)	8(31)	18(69)	21(81)	.945

Data are number (%).

High-impact = athletes participating in judo, jumping/throwing, and weightlifting, HWE = Hardy–Weinberg equilibrium, Low-impact = athletes participating in long distance, water polo, and lifesaving, Multidirectional = athletes participating in tennis, soft tennis, badminton, and hand ball.

### 3.2. Physical characteristics and MAnP of each sport type

The physical characteristics and MAnP of athletes competing in each sport type are shown in Table 3. Regarding physical characteristics, high-impact athletes had significantly higher weight, body mass index (BMI), lean mass, and total body BMD than the athletes competing in other sport types and the controls, while low-impact athletes had significantly lower %fat and fat mass than the athletes competing in other sport types and the controls (All  $P < .05$ ).

In muscle power, high-impact athletes had significantly greater absolute MAnP than the athletes competing in other sport types and the controls (All  $P < .05$ ).

### 3.3. Relationship between MAnP and BMD

The absolute MAnP values were significantly positively correlated with BMD in low-impact ( $P < .001$ ) and multidirectional ( $P < .001$ ) sports athletes, and controls ( $P = .046$ ), but not in high-impact sports athletes ( $P = .240$ ). The relative MAnP values were significantly positively correlated with BMD in low-impact ( $P = .045$ ), but not in multidirectional, high-impact sports athletes, and controls ( $P = .470$ ,  $P = .435$ ,  $P = .272$ , respectively) (Table 4).

### 3.4. Comparison of BMD and MAnP according to ACTN3 R577X polymorphism in each sport type

Multidirectional sports athletes with the RR genotype of the ACTN3 R577X polymorphism had higher BMD than those with the RX and RX + XX genotypes ( $P = .018$  and  $P = .003$ , respectively). No significant differences were observed in the other sport types (Table 5).

Multidirectional sports athletes with the RR genotype had a higher MAnP value than those with the RX + XX genotypes ( $P = .035$ ). No other variables related to MAnP were significantly different (Table 6).

## 4. Discussion

We investigated the relationship of muscle power and BMD with the ACTN3 R577X genotype in Japanese female collegiate athletes participating in sports with various impact levels.

The main findings in our study were that the RR genotype of the ACTN3 R577X polymorphism was associated with a higher BMD than the RX and RX + XX genotypes in

multidirectional sports athletes. In addition, the RR genotype was associated with a higher MAnP than the RX + XX genotypes. This suggests that the RR genotype may confer high trainability for BMD and muscle power in athletes participating in multidirectional sports. To date, the association of the ACTN3 R577X polymorphism with various phenotypes has been reported, and several studies have highlighted the effects of the R allele.<sup>[9,11,12,14]</sup> Many recent studies have also reported that the ACTN3 R577X polymorphism is associated with BMD.<sup>[17]</sup> Further, a study has reported that the RR genotype is associated with a significantly higher femur BMD than the XX genotype in Korean individuals.<sup>[19]</sup> Another study investigated muscle strength, bone mass, and BMD according to the ACTN3 R577X polymorphism in various types of athletes and in people who performed heavy resistance training at least 5 days per week and found that those with the RR + RX genotype has a higher BMD than those with the XX genotype.<sup>[18]</sup> A study has reported that ACTN3 is expressed in osteoblasts.<sup>[16]</sup> ACTN3 knockout mice presented lower BMD and bone formation rates per unit of bone surface when compared to wildtype littermates, suggesting that the lack of ACTN3 is associated with disruptions in mineralization and resorption.<sup>[16]</sup> In addition, ACTN3 deficiency (XX genotype) has higher values of serum bone remodeling markers than R allele carriers at rest in humans.<sup>[29]</sup> Moreover, some studies have reported that muscle power is positively correlated with bone strength and density.<sup>[30,31]</sup> In this study, athletes with the RR genotype participating in multidirectional sports had the highest BMD and MAnP. These findings suggest that the higher power exhibited by athletes with the RR genotype practicing multidirectional sports may favorably affect BMD. However, the reason for the low BMD in athletes participating in multidirectional sports with RX genotype and the biological mechanism related to the ACTN3 R577X polymorphism and BMD are unclear. Further research is required to investigate the relationship between ACTN3 expression and bone metabolism.

The association between the RR genotype and BMD was not observed in the low-impact and high-impact sports participants in our study. This suggests that there may be differences in the trainability effects of the ACTN3 R577X polymorphism in terms of BMD and muscle power. Even if low-impact sports participants have different levels of expression of proteins such as ACTN3 in fast muscle fibers, it is possible that endurance training will not sufficiently stimulate those fast muscle fibers. In high-impact sports, bone formation may be promoted due to the high mechanical load of specific exercises regardless of

**Table 3**

Physical characteristics and maximum anaerobic power in participants who play each sport type and controls.

Variable	Low-impact (n = 112)	Multidirectional (n = 92)	High-impact (n = 56)	Controls (n = 26)	ANOVA P value	$\eta^2$
Physical characteristics						
Height (cm)	160.3 ± 4.5***	161.3 ± 5.2***	157.7 ± 5.1***	161.2 ± 5.2***	<.001	0.07
Weight (kg)	53.8 ± 7.0***	57.5 ± 4.8***	60.7 ± 7.3***	54.3 ± 6.5***	<.001	0.15
BMI (kg·m <sup>-2</sup> )	20.9 ± 2.4***	22.1 ± 1.6***	24.4 ± 2.5***	20.9 ± 2.1***	<.001	0.27
%Fat (%)	22.5 ± 4.5***	24.5 ± 3.3***	25.1 ± 3.5***	29.2 ± 4.1***	<.001	0.19
Fat mass (kg)	12.3 ± 3.8***	14.1 ± 2.6*	15.4 ± 3.6*	16.0 ± 4.0*	<.001	0.13
Lean mass (kg)	39.2 ± 3.8***	40.8 ± 3.4***	42.6 ± 4.2***	35.9 ± 2.9***	<.001	0.20
Total body BMD (g·cm <sup>-2</sup> )	1.144 ± 0.084***	1.216 ± 0.083***	1.294 ± 0.089***	1.187 ± 0.062***	<.001	0.31
Muscle power						
MAnP (W)	538.3 ± 113.0***	614.3 ± 93.8***	666.9 ± 113.7***	494.2 ± 99.9***	<.001	0.22
MAnP (W·BW <sup>-1</sup> )	10.0 ± 1.3***	10.7 ± 1.4***	11.0 ± 1.4***	9.1 ± 1.7***	<.001	0.14

Values are presented as mean ± standard deviation.

ANOVA = analysis of variance, BMD = bone mineral density, BMI = body mass index, BW = body weight, High-impact = athletes participating in judo, jumping/throwing, and weightlifting, Low-impact = athletes participating in long distance, water polo, and lifesaving, MAnP = maximum anaerobic power, Multidirectional = athletes participating in tennis, soft tennis, badminton, and hand ball, W = watt.

\*Compared with low-impact ( $P < .05$ )

\*\*Compared with multidirectional ( $P < .05$ )

\*\*\*Compared with high-impact ( $P < .05$ )

\*\*\*\*Compared with controls ( $P < .05$ )

muscle power exertion; high muscle power and BMD would thus not necessarily be associated. Additionally, it is unclear why MAnP, which is important in high-impact sports, was not associated with the *ACTN3* R577X polymorphism. Further studies that evaluate different types of sports, rather than simply increasing the number of participants in the study, are needed.

When comparing body composition among the sport types in this study, low-impact sports were associated with a lower body weight, BMI, fat percentage, fat mass, lean mass, and BMD than multidirectional and high-impact sports. The low body weight and fat percentage among athletes participating in low-impact sports may be attributed to the inclusion of long-distance runners. Nattiv et al<sup>[32]</sup> indicated that participation in long-distance events has imposed a high risk of developing the female athlete

triad (i.e., low energy availability, amenorrhea, and osteoporosis), possibly because athletes and coaches expect leanness to improve competitive performance.<sup>[33]</sup> Compared to other sport types, the significantly lower BMD associated with low-impact sports may be related to athletes who play water polo and life-saving sports. Aquatic sports such as swimming and water polo are not associated with improvements in BMD, suggesting that loading characteristics may have a particularly important effect on skeletal health.<sup>[23,25]</sup> Therefore, low-impact sports athletes may need to consider employing reinforcement exercises and adequate nutritional intake to increase bone mass. This is also important for maintaining health after retirement from competition for athletes, and for non-athletes.

The high-impact sports in the present study included jumping/throwing in track and field, judo, and weightlifting. These sports were likely associated with the highest MAnP values because they require instantaneous high-level exertion. Meanwhile, low-impact sports athletes likely had lower MAnP due to an adaptation to long-term exercise performance. Multidirectional sports, which were positioned between high-impact and low-impact sports in terms of MAnP, involved ball sports such as tennis, badminton, and handball. Thus, these sports require competitive characteristics involving both anaerobic and aerobic power, explaining their intermediate measurements.<sup>[34-36]</sup>

We classified sports into 3 types and investigated the relationships between MAnP and BMD. We identified positive correlations between the absolute values of MAnP and BMD in low-impact and multidirectional sports. This may be because high muscle mass increases muscle strength and power exertion, and because increased muscle mass is generally associated with high bone mass and BMD.<sup>[37-39]</sup> Although some reports indicate that MAnP is associated with BMD, a study reported a positive

**Table 4**  
Relationship between maximum anaerobic power and bone mineral density in each sport type.

Variable	MAnP (W)		MAnP (W-BW <sup>-1</sup> )	
	r	P value	r	P value
Total body BMD (g·cm <sup>-2</sup> )				
Low-impact (n = 112)	0.352	<.001	0.190	.045
Multidirectional (n = 92)	0.350	<.001	0.076	.470
High-impact (n = 56)	0.160	.240	-0.106	.435
Controls (n = 26)	0.395	.046	0.224	.272

BMD = bone mineral density, BW = body weight, High-impact = athletes participating in judo, jumping/throwing, and weightlifting, Low-impact = athletes participating in long distance, water polo, and lifesaving, MAnP = maximum anaerobic power, Multidirectional = athletes participating in tennis, soft tennis, badminton, and hand ball, W = watt.

**Table 5**  
Comparison of total body bone mineral density among participants with  $\alpha$ -actinin-3 R577X polymorphism.

Variable	RR	RX	XX	ANOVA		T test			T test		
				P-value	$\eta^2$	RR + RX	P value	d	RX + XX	P value	d
Total body BMD (g/cm <sup>2</sup> )											
Low-impact	1.138 ± 0.092	1.138 ± 0.084	1.158 ± 0.078	.516	0.01	1.138 ± 0.086	.249	0.24	1.146 ± 0.082	.710	0.09
Multidirectional	1.258 ± 0.089	1.199 ± 0.081*	1.207 ± 0.071	.018	0.09	1.219 ± 0.088	.537	0.15	1.202 ± 0.077*	.003	0.69
High-impact	1.288 ± 0.116	1.297 ± 0.087	1.294 ± 0.070	.956	0.01	1.294 ± 0.096	.990	0.01	1.296 ± 0.080	.782	0.09
Controls	1.202 ± 0.041	1.174 ± 0.075	1.200 ± 0.050	.565	0.05	1.182 ± 0.067	.496	0.29	1.184 ± 0.066	.575	0.28

Values are presented as mean ± standard deviation.

BMD = bone mineral density; ANOVA = analysis of variance; Low-impact = athletes participating in long distance, water polo, and lifesaving; Multidirectional = athletes participating in tennis, soft tennis, badminton, and hand ball; High-impact = athletes participating in judo, jumping/throwing, and weightlifting.

\*Compared with RR (P < .05).

**Table 6**  
Comparison of maximum anaerobic power among participants with  $\alpha$ -actinin-3 R577X polymorphism.

Variable	RR	RX	XX	ANOVA		T test			T test		
				P value	$\eta^2$	RR + RX	P value	d	RX + XX	P value	d
MAnP (W)											
Low-impact	541.5 ± 118.4	521.6 ± 108.4	564.0 ± 115.4	.225	0.03	527.2 ± 110.9	.114	0.33	537.6 ± 112.4	.885	0.03
Multidirectional	649.9 ± 81.8	602.8 ± 92.6	601.8 ± 101.6	.110	0.05	618.7 ± 91.3	.451	0.18	602.5 ± 95.1*	.035	0.52
High-impact	672.9 ± 104.0	676.7 ± 110.9	647.4 ± 128.5	.703	0.01	675.4 ± 107.3	.402	0.25	665.1 ± 117.5	.830	0.07
Controls	553.8 ± 138.8	475.9 ± 80.2	486.5 ± 101.8	.336	0.09	497.6 ± 101.8	.800	0.11	480.0 ± 86.7	.140	0.76
MAnP (W/BW)											
Low-impact	10.2 ± 1.4	9.8 ± 1.3	10.0 ± 1.3	.498	0.01	10.0 ± 1.3	.837	0.04	9.9 ± 1.3	.301	0.25
Multidirectional	11.0 ± 1.3	10.7 ± 1.3	10.4 ± 1.5	.275	0.03	10.8 ± 1.3	.224	0.29	10.6 ± 1.4	.161	0.34
High-impact	11.3 ± 1.4	11.0 ± 1.5	10.7 ± 1.1	.460	0.03	11.1 ± 1.5	.275	0.32	10.9 ± 1.3	.335	0.31
Controls	9.9 ± 2.9	8.9 ± 1.2	8.9 ± 1.4	.528	0.05	9.2 ± 1.8	.686	0.17	8.9 ± 1.3	.253	0.56

Values are presented as mean ± standard deviation.

MAnP = maximum anaerobic power; W = watt; BW = body weight; ANOVA = analysis of variance; Low-impact = athletes participating in long distance, water polo, and lifesaving; Multidirectional = athletes participating in tennis, soft tennis, badminton, and hand ball; High-impact = athletes participating in judo, jumping/throwing, and weightlifting.

\*Compared with RR (P < .05).

correlation between anaerobic power in a Wingate test and BMD in wrestlers.<sup>[31]</sup> A study examined the effects of power training that involved raising a barbell as quickly as possible and strength training that involved raising the barbell slowly and reported that the effect on BMD was significantly higher in the power training group.<sup>[40]</sup> They concluded that loading amplitude, which was higher in the power training group, plays an important role in bone anti-resorptive effects. Thus, BMD can be improved with training that increases muscle power exertion. However, this relationship was not observed in the high-impact sports participants in our study. This might be due to the characteristics of the different sport types. That is, regardless of the MAnP of each athlete, such as those participating in jumping/throwing, judo, and weightlifting, specific actions such as lifting or receiving a load heavier than one's own weight may strongly influence the promotion of bone formation. We speculate that this is the reason for the significantly higher BMD in high-impact than in multi-impact and low-impact sports participants in this study. Accordingly, BMD enhancement due to increased muscle power may differ between sport types.

There are some limitations to our study. First, the sample size of the control group was relatively small. A larger sample size might have allowed us to detect more sufficient statistical power and thus increase the reliability of the study. Second, our results suggested that the effects of the *ACTN3* R577X polymorphism on muscle power and BMD depend on sport type; thus, further studies should investigate the effect of the *ACTN3* R577X polymorphism on athletes in each competitive sport. Third, we focused on a single polymorphism that has been reported to be associated with BMD in recent years, while other bone metabolism-related gene polymorphisms, such as the vitamin D receptor and estrogen receptor genes, may be associated with BMD in female athletes.<sup>[26]</sup> Therefore, research combining multiple genetic polymorphisms has the potential to reveal more detailed bone responses. In the future, large research studies to characterize the effects of multiple genetic polymorphisms in female athletes are necessary.

In conclusion, to the best of our knowledge, no previous study has investigated the relationship between muscle power and BMD and the *ACTN3* R577X polymorphism in Japanese female collegiate athletes. In our study, multidirectional sports participants with the RR genotype demonstrated a higher BMD than the RX and RX + XX genotypes. In addition, the RR genotype was associated with a higher MAnP than those with the RX + XX genotypes. This suggests that the RR genotype may confer high trainability for BMD and muscle power in Japanese female collegiate athletes participating in multidirectional sport type. However, these associations were not found in the athletes participating in the low- and high-impact sport types.

## Author contributions

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