

Age differences in the relation between *ACTN3* R577X polymorphism and thigh-muscle cross-sectional area in women

Hirofumi Zempo, Kai Tanabe, Haruka Murakami, Motoyuki Iemitsu, Seiji Maeda, Shinya Kuno.

Authors' affiliations:

Hirofumi Zempo, Kai Tanabe, Seiji Maeda and Shinya Kuno.

Department of Sports Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, 1-1-1 Tennodai, Tsukuba, Ibaraki 305-8577, Japan.

Haruka Murakami. National Institute of Health and Nutrition, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8636, Japan.

Motoyuki Iemitsu. Faculty of Sport and Health Science, Ritsumeikan University, 1-1-1 Noji Higashi, Kusatsu, Shiga 525-8577, Japan.

Key words: aging, alpha-actinin 3, skeletal muscle (or musculoskeletal), human genetics, and population screening

Running title: Age differences in *ACTN3* variant and muscle CSA

Corresponding author:

Shinya Kuno, Ph.D.

Department of Sports Medicine, Graduate School of Comprehensive Human Sciences, University of Tsukuba, Tennodai, Tsukuba City, 305-8577 Ibaraki, Japan;

Tel: +81 29 853 7335, Fax: +81 29 853 6407, E-mail: kuno@taiiku.tsukuba.ac.jp

ABSTRACT

The relation between *ACTN3* R577X polymorphism and muscle mass in women has been reported, but its relation to age remains unclear. We investigated the relationship between *ACTN3* R577X polymorphism and muscle mass in both middle-aged and elderly women. Two age groups (middle-aged and older) were divided of 162 healthy, non-training Japanese women (mean \pm SE, 58.6 ± 0.8 yr). Their mid-thigh muscle cross-sectional area (CSA) was assessed using MRI, revealing no difference in thigh-muscle CSA among *ACTN3* R577X genotypes in the middle-aged group (XX, 87.3 ± 2.5 cm²; RR&RX, 86.1 ± 1.7 cm², $P = 0.7$). In contrast, the XX genotype in the older group had a smaller thigh-muscle CSA adjusted to body weight than the RR&RX genotype (XX, 67.8 ± 2.0 cm²; RR&RX, 72.5 ± 1.2 cm², $P < 0.05$). The present study showed an association between *ACTN3* R577X polymorphism and smaller thigh-muscle CSA in a group of elderly women, but not in a group of middle-aged women.

INTRODUCTION

Elderly populations are growing around the world because of increasing life expectancy. The United Nations reported that the number of people over 60 years old was 600 million in 2000. It is expected to rise to 2 billion by 2050 (United Nations, 2008). Japan has now entered the “super-aged society” stage, with elderly population constituting more than 23% of the population, which no other country in the world has ever experienced (Cabinet office Japan, 2008). Japan is increasingly confronting problems associated with its aging population. The rapidly increasing number of elderly people in need of care is especially important. About 4.8 million elderly people need care in Japan, of whom 65.9% are women (Ministry of Health, Labour and Welfare Japan, 2007).

Musculoskeletal disorders are a leading contributors to increased health care needs of elderly people. Sarcopenia characterized by a loss of skeletal muscle mass and strength concurrent with aging, is positively related to mobility disorders, increased risk of falls and fractures, and risk of death (Cruz-Jentoft et al., 2010). The estimated direct healthcare cost attributable to sarcopenia in the United States in 2000 was \$18.5 billion (Janssen et al., 2004). Therefore, developing appropriate prevention and treatment

strategies for sarcopenia is becoming an increasingly important public health concern. Sarcopenia characteristics include significantly less leg muscle mass than arm muscle mass (Janssen et al., 2000), and selective atrophy of type II muscle fibers (Kamel, 2003). Both genetic and environmental factors influence muscle mass. A twin report described that the lean body mass of postmenopausal women has a moderate genetic component with heritability estimates of 52% (Arden and Spector, 1997). However, the relation between individual variations of gene and muscle mass remains unclear.

The actin-binding protein α -actinin is an important protein related to muscle contraction, it is a structural component of the Z-lines in skeletal muscle (Masaki et al., 1967). The α -actinin isoform, α -actinin 3 (ACTN3), is observed only in type II fibers (North et al., 1996 and 1999). However, one billion people are homozygous recessive for *ACTN3* (Chr. 11q13.2) R577X polymorphism (rs1815739), which causes complete deficiency of ACTN3 (XX genotype). Previous reports have described that *ACTN3* R577X polymorphism is related to muscle strength (Clarkson et al., 2005; Vincent et al., 2007; Walsh et al., 2008), walking ability (Delmonico et al., 2008), and athletic status (Yang et al., 2003). These findings also show that sex-specific genotype differences for *ACTN3* R577X polymorphism, specially

observed in women (Clarkson et al., 2005; Walsh et al., 2008; Delmonico et al., 2008; Yang et al., 2003). Few studies have investigated the relation between *ACTN3* R577X polymorphism and muscle mass in women (Clarkson et al., 2005; Walsh et al., 2008; Delmonico et al., 2008; Zempo et al., 2010; Delmonico et al., 2007). Walsh et al. (2008) demonstrated that the XX genotype has lower fat-free mass than the RR&RX genotype in women across the adult age span. Recently, we reported that the XX genotype has a smaller thigh-muscle cross-sectional area (CSA) than the RR&RX genotype in older women (Zempo et al., 2010). However, another report described that a younger cohort showed no link between *ACTN3* R577X polymorphism and arm muscle CSA (Clarkson et al., 2005). Consequently, the influence of *ACTN3* R577X polymorphism on muscle mass is observed in older women, not in younger women. These findings suggest that the influence of *ACTN3* R577X polymorphism on muscle mass change with ageing. Nevertheless, it remains unclear the association between *ACTN3* R577X polymorphism and age. Because of an accelerated loss of muscle mass occurs during middle-age in women (Maltais et al., 2009), we aim to investigate the relationship between *ACTN3* R577X polymorphism and muscle mass in both middle-aged and elderly women.

METHODS

Subjects

This cross-sectional study examined 162 healthy Japanese women (means \pm SE, 58.6 \pm 0.8 years). The subjects were non-smokers who did not have significant cardiovascular, metabolic or musculoskeletal disorders. None of the women had taken antidepressant, hypolipidemic or hormonal drugs. The study was approved by the Ethical Committees of the Institute of Health and Sport Sciences and the Institute of Clinical Medicine of the University of Tsukuba. The study conformed to the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all subjects before their inclusion in the study.

Genotyping

The genotype of *ACTN3* R577X polymorphism was identified as described previously (Zempo et al., 2010). Genomic DNA was prepared from EDTA-whole blood samples using a kit (QIAamp DNA Blood mini kit; Qiagen, Tokyo, Japan). Genotyping for *ACTN3* R577X polymorphism was determined by real-time polymerase chain

reaction assays using TaqMan probes (Pre-Designed SNP Genotyping assays; Applied Biosystems, Tokyo, Japan) and a sequence detector (ABI Prism 7700; Applied Biosystems, Foster City, CA). The oligonucleotide sequences were as follows:

ACTN3 forward: 5'-ACGATCAGTTCAAGGCAACACT-3'

ACTN3 reverse: 5'-ACCCTGGATGCCCCATGATG-3'

ACTN3/R probe: 5'-TCGCTCTCGGTCAGC-3'

ACTN3/X probe: 5'-CGCTCTCAGTCAGC-3'

Thigh-muscle cross-sectional area

Thigh-muscle CSA was determined by magnetic resonance imaging (MRI). Each subject's right foot was visualised by MRI (0.25 Tesla, AIRIS Mate; Hitachi Medical, Tokyo, Japan). All the cross-sectional MRI scans were obtained using a conventional T1-weighted spin-echo sequence (28 ms echo time, 360 ms repetition time), with a 10 mm slice thickness, a 20 mm intersection interval, a 240 mm field of view, 256×180 matrix, 90° flip angle and 4 signals on an average. Cross-sectional images of the thigh were obtained at 50 % of femur length, corresponding to the site of highest correlation thigh-muscle volume in middle-aged women (Cotofana et al., 2010). The MRI images were then analysed by manual planimetry using a hand-writable liquid

crystal display (Cintiq 17SX; WACOM, Tokyo, Japan) and image analysis software (NIH Image ver. 1.62; National Institute of Health, USA) on a personal computer (Macintosh Power PC; Apple, Tokyo, Japan). The DICOM image data from MRI were converted into 652×652 pixels JPEG picture images. The MRI scans were assessed in a blind manner, i.e. the identity of the subject was not revealed. The regions of thigh-muscle CSA included quadriceps femoris, hamstrings and adductor magnus muscles. Thigh-muscle CSA was measured from the mean value of three traces.

Measurement of daily physical activity

Daily physical activity was measured using a uniaxial accelerometer (Life-Corder; Suzuken, Nagoya, Japan). All subjects wore the accelerometer on their waist continuously for 14 days, except during sleeping and bathing. The total physical energy expenditure (kcal) was calculated from the energy measured by the accelerometer combined with a questionnaire based on the compendium of physical activity (Ainsworth et al., 1993), which was administered to all subjects.

Measurements of blood components

Subjects were instructed to abstain from vigorous exercise and fast on the day

prior to the measurements. Plasma glucose concentrations and fasting serum concentrations of cholesterol and triglycerides were determined using standard enzymatic techniques.

Statistical analysis

Statistical analyses were performed using SPSS Base System 14.0 J for Windows (SPSS Japan, Tokyo, Japan). *ACTN3* R577X genotype distribution was evaluated for conformity with the Hardy-Weinberg equilibrium using the χ^2 test with one degree of freedom. Subjects were divided into two age groups (middle-aged and older) by median age (60 years). Differences in physical characteristics between the XX, RR and RX genotypes were tested using analysis of variance (ANOVA), and between the XX and RR&RX genotypes using unpaired t-tests. Thigh-muscle CSA was compared between the genotype groups using an analysis of covariance (ANCOVA) model with age and body weight. Values are expressed as mean \pm SE; $P < 0.05$ is considered to be statistically significant.

RESULTS

To clarify the influence of *ACTN3* R577X polymorphism and the effect of aging on muscle mass, we divided the subjects into the middle-aged ($n = 82$; mean 50.6 ± 0.9 years) and the older ($n = 80$; mean 66.8 ± 0.5 years) groups, with the dividing line set at the median value (60 years) of age. Table 1 shows the characteristics of the middle-aged group and older group. As the results, the older group had low body weight and thigh-muscle CSA, less physical activity, and higher blood glucose and triglyceride compared with the middle-aged group ($P < 0.05$).

The *ACTN3* R577X genotype distributions in the all subjects were 16%, 55% and 29%, RR, RX and XX respectively. The genotype frequencies were in Hardy-Weinberg equilibrium (χ^2 value = 2.24, $df = 1$, $P = 0.14$). Also, in the subgroups, the genotype frequencies were in Hardy-Weinberg equilibrium (middle-aged group: χ^2 value = 0.47, $P = 0.49$ and older group: χ^2 value = 2.09, $P = 0.15$). The characteristics of all subjects were not significant differences among *ACTN3* R577X genotypes. In the middle-aged group, no significant differences were found for all characteristics among *ACTN3* R577X genotypes (Table 2 and Figure 1). However, the older group indicated that only thigh-muscle CSA that was adjusted to age and body weight was different between the XX genotype and the RR&RX genotype (Figure 1). The other

characteristics in the older group did not indicate significant differences (Table 3).

DISCUSSION

This study investigated the relation between *ACTN3* R577X polymorphism and thigh-muscle CSA in different age groups of women. No significant differences were found between *ACTN3* R577X genotype in the middle-aged group. However, the older group showed that the XX genotype was associated with smaller thigh-muscle CSA compared to the RR&RX genotype. These findings suggest that the influence of *ACTN3* R577X polymorphism on muscle change with aging. Previous reports have described that gene polymorphisms are associated with age-related changes in left-ventricular mass (Peter et al., 2007) and carotid artery elasticity (Hanon et al., 2001). The present report is the first to describe that the relation between *ACTN3* R577X polymorphism and skeletal muscle is associated with age.

Previous reports have described the relation between *ACTN3* R577X polymorphism and muscle mass in women (Walsh et al., 2008; Zempo et al., 2010).

These findings are supported by findings related to *Actn3* knockout mice, which simulating the human XX genotype (MacArthur et al., 2008). The present study investigated that relation in middle-aged group, but no relation was found. In addition, Clarkson et al. (2005) reported that no relation exist between *ACTN3* R577X polymorphism and arm muscle CSA in younger women. These suggest the possibility that the relation between *ACTN3* R577X polymorphism and muscle mass does not emerge in younger and middle-aged women. In contrast, Walsh et al. demonstrated that the XX genotype had lower fat-free mass than the RR&RX genotype in women across the adult age span (22-90 years old) (Walsh et al., 2008). Their study measured whole and leg fat-free mass as muscle mass using dual-energy X-ray absorptiometry. Therefore, the differences of the measurement methods of muscle mass and measurement area of body might influence the results. Delmonico et al. assessed longitudinal mid-thigh muscle CSA changes in *ACTN3* R577X polymorphism in 70-79 years old Caucasian women (Delmonico et al., 2008) but found no differences of baseline and change CSA after 5 years. Instead, the differences were apparently attributable to ethnic group, body size, living habits, and other factors. More studies using common measurements of various subjects are needed.

The mechanisms by which *ACTN3* R577X polymorphism affects muscle mass remain unknown. We assume that *ACTN3* R577X polymorphism affects sarcopenia. A major characteristic of sarcopenia is selective atrophy of type II muscle fibers (Kamel, 2003). Although type II fibers express a large amount of ACTN3, the XX genotype has been shown to result in loss of ACTN3. Alternatively, type II fibers of the XX genotype replace ACTN3 with ACTN2 (North et al., 1996). In fact, ACTN2 is a closely related homolog of ACTN3 that is expressed in all muscle fibers. Previous studies showed that loss of ACTN3 (or Actn3) affects muscle mass in humans and mice (Walsh et al., 2008; Zempo et al., 2010; MacArthur et al., 2008). These findings suggest that ACTN2 is insufficient as a replacement of ACTN3 in the XX genotype. Therefore, it is predicted that atrophy occurs easily in the type II fiber solely comprising ACTN2. Vincent et al. reported that the type II fiber diameter of the XX genotype is comparable with that of the RR genotype in younger adults (Vincent et al., 2007). Nevertheless, it is not clear that muscle fiber characteristics of the XX genotype in older adults remain unchanged. More research is necessary to elucidate the relation between *ACTN3* R577X polymorphism and muscle.

Results of previous studies have shown that sex-specific genotype differences

for *ACTN3* R577X polymorphism are particularly prevalent among women (Clarkson et al., 2005; Walsh et al., 2008; Delmonico et al., 2008; Yang et al., 2003). Results of those studies present the possibility that the sex-hormone influences the phenotypes. In general, sex hormones decrease after menopause in women. Another report described that, in women, an accelerated loss of muscle mass and strength occurs around the time of menopause (Maltais et al., 2009). Therefore, the influence of *ACTN3* R577X polymorphism on muscle might arise depending on the postmenopause.

We also measured physical activity and health status (disease and medications). It is important to ask patients about resistance training habits because resistance training increases muscle mass (Hunter et al., 2000). We determined physical activity based on both actual measurements using an accelerometer and using a questionnaire. Results showed that subjects performed standard physical activity (Zhang et al., 2003) and did not exercise regularly using resistance training. Although antihypertension medication and dyslipidemia-improving drugs can alter muscle mass (Harper and Jacobson, 2010; Di Bari et al., 2004), the subjects of our study used no such drugs. The subjects of this study were regarded as healthy, implying that their results are applicable to the general population.

This investigation was limited because the study was conducted using a cross-sectional design. The best method of determining the influence of *ACTN3* R577X polymorphism on muscle mass with aging may be longitudinal examination. The relations described herein will be clarified further by performing such a longitudinal examination. Moreover, we did not conduct survey of menopause status and nutritional intake which influences muscle mass. Future studies should include these factors which vary with increase in age.

In conclusion, this present study showed an association between *ACTN3* R577X polymorphism and smaller thigh-muscle CSA in a group of elderly women, but not in a group of middle-aged women. The results suggest that the influence of *ACTN3* R577X polymorphism on muscle change with ageing. For individuals with XX genotype, precautionary measure to prevent or minimize muscle loss should be advised before they become older adults.

AUTHOR DISCLOSURE STATEMENT

No competing financial interests exist.

ACKNOWLEDGEMENT

This work was supported by two grants from the Ministry of Education, Culture, Sports, Science and Technology Special Coordination Fund (Promoting Science and Technology and Health, 1999-2004) and the Ministry of Health, Labour and Welfare (Labour Sciences Research Grants, 2006-2008), Japan.

REFERENCES

Cabinet office Japan (2008) 1-1 Situation on aging. In: Annual Report on the Aging Society 2008. 2008; pp 2-10. Available at <http://www8.cao.go.jp/kourei/english/annualreport/2008/pdf/p2-10.pdf>, accessed Jan 6 2011.

Ministry of Health, Labour and Welfare Japan (2007) National livelihood survey, 2007 edition. Available at <http://www.mhlw.go.jp/toukei/list/20-19-1.html>, (Japanese) accessed Jan 6 2011.

United Nations (2008) World population prospects, the 2008 revision. Available at http://esa.un.org/unpd/wpp2008/pdf/WPP2008_PressRelease.pdf, accessed Jan 6 2011.

Ainsworth BE, Haskell WL, Leon AS, et al. (1993) Compendium of physical activities: classification of energy costs of human physical activities. *Med Sci Sports Exerc* 25:71-80.

Arden NK, Spector TD (1997) Genetic influences on muscle strength, lean body mass, and bone mineral density: a twin study. *J Bone Miner Res* 12:2076-2081.

Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. (2010) Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* 39:412-423.

Clarkson PM, Devaney JM, Gordish-Dressman H, et al. (2005) ACTN3 genotype is associated with increases in muscle strength in response to resistance training in women. *J Appl Physiol* 99:154-163.

Cotofana S, Hudelmaier M, Wirth W, et al. (2010). Correlation between single-slice

muscle anatomical cross-sectional area and muscle volume in thigh extensors, flexors and adductors of perimenopausal women. *Eur J Appl Physiol* 110:91-97.

Delmonico MJ, Kostek MC, Doldo NA, et al. (2007) Alpha-actinin-3 (ACTN3) R577X polymorphism influences knee extensor peak power response to strength training in older men and women. *J Gerontol A Biol Sci Med Sci* 62:206-212.

Delmonico MJ, Zmuda JM, Taylor BC, et al. (2008) Association of the ACTN3 genotype and physical functioning with age in older adults. *J Gerontol A Biol Sci Med Sci* 63:1227-1234.

Di Bari M, van de Poll-Franse LV, Onder G, et al. (2004) Antihypertensive medications and differences in muscle mass in older persons: the Health, Aging and Body Composition Study. *J Am Geriatr Soc* 52:961-966.

Hanon O, Luong V, Mourad JJ, et al. (2001) Aging, carotid artery distensibility, and the Ser422Gly elastin gene polymorphism in humans. *Hypertension* 38:1185-1189.

Harper CR, Jacobson TA (2010) Evidence-based management of statin myopathy. *Curr Atheroscler Rep* 12:322-330.

Hunter GR, Wetzstein CJ, Fields DA, et al. (2000) Resistance training increases total energy expenditure and free-living physical activity in older adults. *J Appl Physiol* 89:977-984.

- Janssen I, Heymsfield SB, Wang ZM, et al. (2000) Skeletal muscle mass and distribution in 468 men and women aged 18-88 yr. *J Appl Physiol* 89:81-88.
- Janssen I, Shepard DS, Katzmarzyk PT, et al. (2004) The healthcare costs of sarcopenia in the United States. *J Am Geriatr Soc* 52:80-85.
- Kamel HK (2003) Sarcopenia and aging. *Nutr Rev* 61:157-167.
- MacArthur DG, Seto JT, Chan S, (2008) An Actn3 knockout mouse provides mechanistic insights into the association between alpha-actinin-3 deficiency and human athletic performance. *Hum Mol Genet* 17:1076-1086.
- Maltais ML, Desroches J, Dionne IJ (2009) Changes in muscle mass and strength after menopause. *J Musculoskelet Neuronal Interact* 9:186-1897.
- Masaki T, Endo M, Ebashi S (1967) Localization of 6S component of a alpha-actinin at Z-band. *J Biochem* 62:630-632.
- North KN, Beggs AH (1996) Deficiency of a skeletal muscle isoform of alpha-actinin (alpha-actinin-3) in merosin-positive congenital muscular dystrophy. *Neuromuscul Disord* 6:229-235.
- North KN, Yang N, Wattanasirichaigoon D, et al. (1999) A common nonsense mutation results in alpha-actinin-3 deficiency in the general population. *Nat Genet* 21:353-354.

Peter I, Huggins GS, Shearman AM, et al. (2007) Age-related changes in echocardiographic measurements: association with variation in the estrogen receptor-alpha gene. *Hypertension* 49:1000-1006.

Vincent B, De Bock K, Ramaekers M, et al. (2007) ACTN3 (R577X) genotype is associated with fiber type distribution. *Physiol Genomics* 32:58-63.

Walsh S, Liu D, Metter EJ, et al. (2008) ACTN3 genotype is associated with muscle phenotypes in women across the adult age span. *J Appl Physiol* 105:1486-1491.

Yang N, MacArthur DG, Gulbin JP, et al. (2003) ACTN3 genotype is associated with human elite athletic performance. *Am J Hum Genet* 73:627-631.

Zempo H, Tanabe K, Murakami H, et al. (2010) ACTN3 polymorphism affects thigh-muscle area. *Int J Sports Med* 31:138-142.

Zhang JG, Ohta T, Ishikawa-Takata K, et al. (2003) Effects of daily activity recorded by pedometer on peak oxygen consumption (VO₂peak), ventilatory threshold and leg extension power in 30- to 69-year-old Japanese without exercise habit. *Eur J Appl Physiol* 90:109-113.

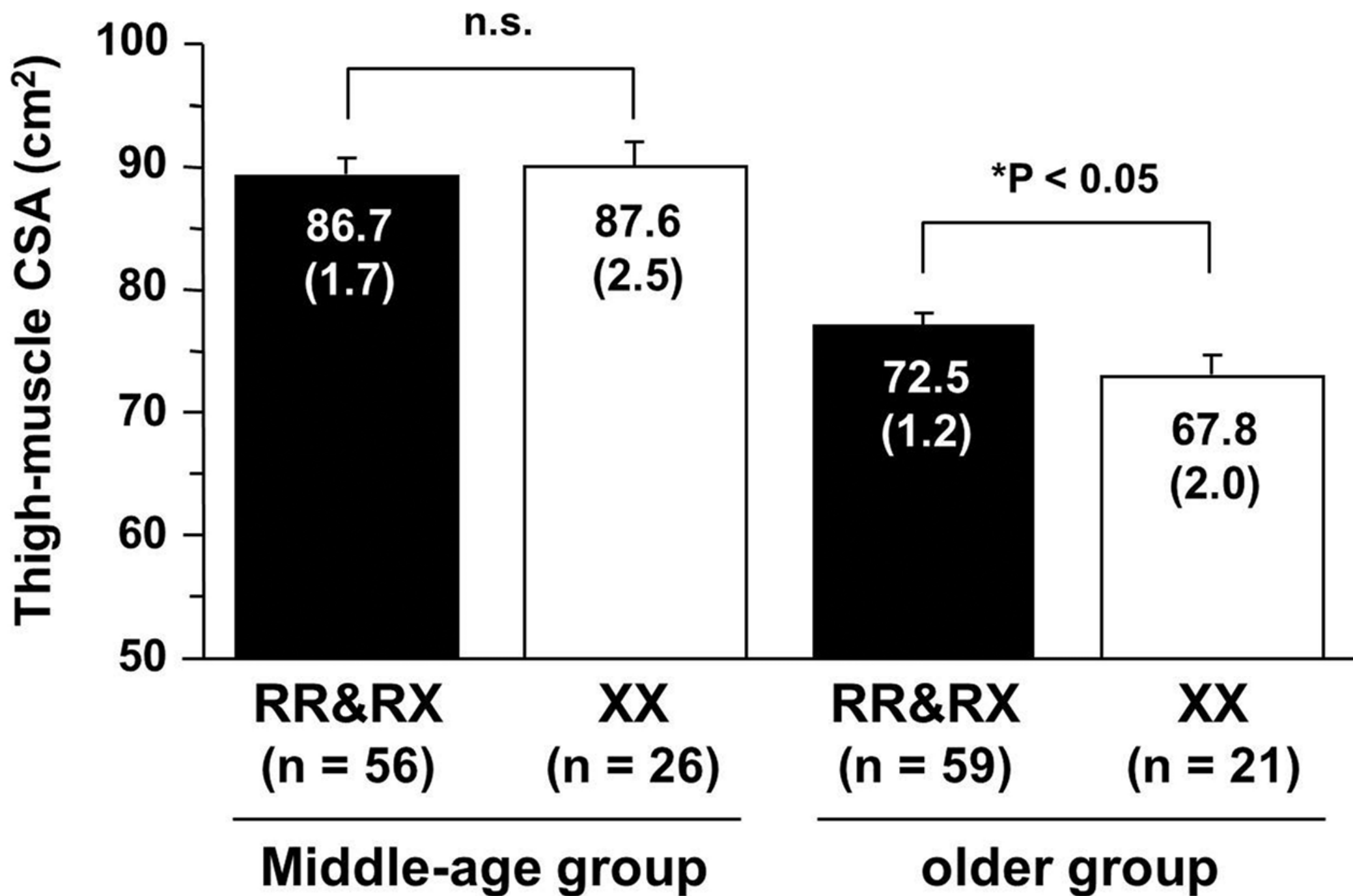


Table 1. Characteristics of the subjects by age group.

	All subjects (n =162)	Middle-aged (n = 82)	Older (n = 80)	P value (middle-aged vs. older)
Age (years old)	58.6 ± 0.8	50.6 ± 0.9	66.8 ± 0.5	0.001*
Height (cm)	153.1 ± 0.5	155.8 ± 0.7	150.3 ± 0.7	0.001*
Body weight (kg)	54.0 ± 0.6	55.9 ± 0.9	52.0 ± 0.7	0.001*
BMI (kg/m ²)	23.0 ± 0.2	23.0 ± 0.3	23.0 ± 0.3	0.967
Thigh-muscle CSA (cm ²)	79.0 ± 1.2	86.5 ± 1.7	71.3 ± 1.2	0.001*
Physical activity (kcal/day)	196.8 ± 8.1	227.0 ± 12.5	165.9 ± 9.0	0.001*
Blood components				
Glucose (mg/dl)	98.0 ± 1.1	96.7 ± 1.0	99.3 ± 2.0	0.252
Total cho (mg/dl)	214.4 ± 2.8	208.4 ± 4.1	220.5 ± 3.8	0.030*
HDL-cho (mg/dl)	60.6 ± 1.0	60.3 ± 1.5	61.0 ± 1.3	0.740
LDL-cho (mg/dl)	130.0 ± 2.4	126.6 ± 3.6	133.5 ± 3.2	0.156
Triglycerides (mg/dl)	97.5 ± 4.5	89.6 ± 6.5	105.5 ± 6.1	0.079

Values are means ± SE. BMI, Body mass index; CSA, cross-sectional area. cho, cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein. *P < 0.05.

Table 2. Characteristics of the subject by *ACTN3* R577X genotype in middle-aged group.

	RR ^(a)	RX ^(b)	RR&RX ^(c)	XX ^(d)	P Value	P Value
	(n = 13)	(n = 43)	(n = 56)	(n = 26)	(a vs. b vs. d)	(c vs. d)
Age (years old)	51.2 ± 2.2	51.0 ± 1.4	51.0 ± 1.2	49.7 ± 1.6	0.814	0.522
Height (cm)	155.8 ± 1.5	156.1 ± 0.9	156.0 ± 0.8	155.4 ± 1.2	0.895	0.662
Body weight (kg)	55.2 ± 3.3	56.5 ± 1.1	56.2 ± 1.1	55.1 ± 1.5	0.745	0.561
BMI (kg/m ²)	22.7 ± 1.2	23.2 ± 0.5	23.1 ± 0.5	22.8 ± 0.5	0.777	0.634
Physical activity (kcal/day)	197.7 ± 25.8	235.5 ± 19.4	226.7 ± 16.1	225.6 ± 18.5	0.573	0.966
Blood components						
Glucose (mg/dl)	93.8 ± 2.4	98.0 ± 1.4	97.0 ± 1.2	96.0 ± 1.7	0.293	0.618
Total cho (mg/dl)	192.5 ± 9.8	212.8 ± 5.7	208.1 ± 5.0	209.0 ± 7.1	0.221	0.916
HDL-cho (mg/dl)	64.5 ± 3.7	58.2 ± 1.9	59.6 ± 1.7	61.7 ± 3.0	0.282	0.518
LDL-cho (mg/dl)	108.5 ± 8.4	130.0 ± 5.2	125.0 ± 4.6	130.0 ± 5.9	0.093	0.528
Triglycerides (mg/dl)	64.8 ± 8.4	103.8 ± 11.0	94.7 ± 8.9	78.7 ± 7.4	0.058	0.256

Values are means ± SE. BMI, Body mass index; CSA, cross-sectional area. cho, cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.

Table 3. Characteristics of the subject by *ACTN3* R577X genotype in older group.

	RR ^(a)	RX ^(b)	RR&RX ^(c)	XX ^(d)	P Value	P Value
	(n = 13)	(n = 46)	(n = 59)	(n = 21)	(a vs. b vs. d)	(c vs. d)
Age (years old)	67.0 ± 1.2	66.6 ± 0.7	66.7 ± 0.6	67.0 ± 1.0	0.933	0.813
Height (cm)	149.9 ± 1.8	150.6 ± 0.9	150.4 ± 0.8	149.9 ± 1.2	0.881	0.729
Body weight (kg)	51.5 ± 1.8	51.8 ± 1.0	51.7 ± 0.8	52.9 ± 1.4	0.783	0.489
BMI (kg/m ²)	22.9 ± 0.6	22.8 ± 0.4	22.9 ± 0.3	23.5 ± 0.6	0.564	0.285
Physical activity (kcal/day)	178.7 ± 20.5	167.0 ± 11.8	169.6 ± 10.2	155.5 ± 19.1	0.714	0.494
Blood components						
Glucose (mg/dl)	96.1 ± 2.0	96.1 ± 1.1	96.1 ± 1.0	108.2 ± 7.0	0.259	0.103
Total cho (mg/dl)	224.9 ± 7.5	216.7 ± 5.4	218.5 ± 4.5	226.0 ± 6.8	0.508	0.382
HDL-cho (mg/dl)	58.1 ± 4.3	62.8 ± 1.7	61.8 ± 1.6	58.6 ± 2.3	0.260	0.298
LDL-cho (mg/dl)	132.9 ± 6.8	129.8 ± 4.4	130.5 ± 3.7	142.0 ± 6.2	0.275	0.115
Triglycerides (mg/dl)	125.2 ± 23.2	102.0 ± 7.5	107.1 ± 7.8	101.0 ± 8.4	0.369	0.665

Values are means ± SE. BMI, Body mass index; CSA, cross-sectional area. cho, cholesterol; HDL, high density lipoprotein; LDL, low density lipoprotein.