Original Paper

Effects of home-based resistance training among elderly Japanese women with different *ACTN3*（R577X）genotypes

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Abstract

Immobility that causes the patient to become bedridden is a common health problem among elderly people that can be prevented by remaining physically active. However, elderly people show a wide range of responses to training. These large inter-individual differences may be explained by genetic factors. The *ACTN3*（R577X）gene, which encodes α-actinin-3 protein and is expressed only in type II fibers in human skeletal muscle, may be associated with physical performance. This study investigated the association between *ACTN3* polymorphism and physical fitness in elderly Japanese women. Forty-seven untrained elderly women participated for 2 months in a home-based resistance training program consisting of four different exercises to improve muscle strength and endurance of the trunk and lower limbs. The subjects were asked to perform at least three sets of 10 repetitions of each exercise each week. Body weight, percent body fat, grip strength, sit-ups, sit-and-reach, and one-leg standing with open eyes were measured before and after training. The *ACTN3* variant was genotyped using the TaqMan allele discrimination assay. In the 35 subjects（74.1 ± 5.8 yr）who completed the training, the genotype distribution was RR = 6, RX = 16, and XX = 13. No significant differences in physical characteristics or fitness level were found among genotypes before training. After training, sit-ups and one-leg standing with open eyes were significantly improved in the 577R allele group（RR and RX）, but not in the homozygous nonsense mutation group（XX）. Measurements of body weight, percent body fat, grip strength, and sit-and-reach did not differ significantly before and after training. Our study indicated an association between *ACTN3* polymorphism and training adaptation in elderly Japanese women. Further studies using much larger sample sizes and both genders are required to clarify the association between *ACTN3* polymorphism and training effects in elderly Japanese subjects.

Key words: α-actinin-3 protein, physical fitness test, trainability, skeletal muscle

1. Introduction

Physical performance is a complex human trait influenced by environmental factors such as diet, training, and genetic predisposition. Our growing understanding of the genetic basis of variation in movement and physical performance is evidenced by the extent of the human gene map for fitness and performance. The latest edition of this map[3] now includes 214 autosomal gene entries and quantitative trait loci plus seven others on the X chromosome, and 18 mitochondrial genes. Several genes are thought to play roles in physical performance; one gene potentially associated with physical performance is the *ACTN3* gene, which encodes the α-actinin-3 protein[12,13].

Alpha-actinins are important structural components of the Z-line[2], where they form crosslinks between the thin actin filaments and serve a static function in maintaining ordered myofibrillar arrays and a regulatory function in coordinating myofiber contraction[15]. In human skeletal muscle, the two

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isoforms, α-actinin-2 and α-actinin-3, are encoded by the genes *ACTN2* and *ACTN3*, respectively[15)18)]. Whereas *ACTN2* is expressed in all muscle fibers, *ACTN3* expression is restricted to fast (type II) fibers[19)23)]. Interestingly, homozygosity for a common nonsense polymorphism (R577X) in *ACTN3* results in complete deficiency of α-actinin-3 in ∼18% of Caucasians and ∼25% of Japanese subjects, with varying frequencies in other populations[15)26)]. This mutation seems not to compromise muscle function, suggesting that α-actinin-2 can compensate for the lack of α-actinin-3 protein.

The fully α-actinin-3-deficient genotype (XX) is markedly under-represented among elite sprint athletes[17)25)] and is also associated with reduced muscle strength and sprint performance in non-athletes[5)16)23)]. These previous studies suggested that α-actinin-3 deficiency has a detrimental effect on the function of fast skeletal muscle fibers. In contrast, the XX genotype was reported to be more common among endurance-trained athletes, indicating that lack of α-actinin-3 aids athletic performance in events requiring superior aerobic capacity[17)20)25)]. Previous studies in young adult non-athletes indicated that women with the XX genotype have lower baseline arm strength, but significantly greater increases in strength with strength training compared to the heterozygotes (RX)[5)]. However, recent data showed that α-actinin-3 deficiency does not influence the ability to generate explosive leg muscle power in young non-athletes[22)]. Controversy exists over whether the XX genotype is associated with higher knee extensor concentric peak power compared with R-homozygotes (RR) and RX-heterozygotes in older adults[7)]. These discrepancies between studies may be related to differences in age, gender, ethnic background, or fitness level of the selected subjects.

In this study, we focused on untrained elderly Japanese women. Immobility is a common health problem among elderly people that may cause them to become bedridden. Falls also lead to a deterioration of the health and physical status of elderly people[27)]. Although not all falls lead to injury, about 5 % of falls result in fractures, and 5–10% involve major injuries requiring medical care[1)]. In fact, falls have a causal role in 12% of all deaths and are the sixth leading cause of death among elderly individuals[1)]. Falls are closely related to the drop in muscle strength due to aging[9)]. Remaining physically active can help prevent falls among the elderly. Although muscle power has been shown to improve with strength training in the elderly, responses vary widely, even among people of similar characteristics performing the same training program[6)]. These large inter-individual differences, along with the high degree of skeletal muscle phenotype heritability, suggest that genetic factors may explain at least a portion of muscle responses to training.

The present study was performed to investigate the association between *ACTN3* genotype and the effects of resistance training in elderly Japanese women. As we were interested in exercise that could be performed easily and regularly to improve physical fitness, a home-based resistance training program was adopted in this study.

2. Materials and methods

2.1 Subjects

Forty-seven elderly women living in an urban community participated in this study, and 35 subjects (age: 74.1 ± 5.8 years; height: 149.2 ± 4.7 cm; weight: 51.6 ± 5.5 kg) completed the training. The subjects were recruited by announcements among the participants in health care prevention programs held in home health care support centers in Yokohama City, Japan. Subjects with blood pressure of 160 mmHg or higher were excluded. Those who were already taking medications prior to the start of the study were included in the analysis as long as medications and dosages were not changed during the study. All procedures described here were performed with the approval of the Juntendo University Human Ethics Committee and were in compliance with the Declaration of Helsinki. All of the subjects entering the study gave their written informed consent.

2.2 Genotyping

DNA was extracted from oral swabs as described previously[21]. Genotypes were determined using the Custom TaqMan SNP Genotyping Assay (Applied Biosystems, Tokyo, Japan). Briefly, primers and probes were CACGATCAGTTCAAGGCAACA (forward primer), CCCTGGATGCCCATGATG (reverse primer), Vic-CTGACCGAGAGCGA (probe for R allele), and Fam-AGGCTGAC-TGAGAGC (probe for X allele). Allelic discrimination was performed in triplicate using a 7300 Real-Time PCR System with TaqMan PCR Master Mix (Applied Biosystems). The thermal cycle protocol consisted of 80 cycles of 10 min at 95℃, 15 s at 95℃, and 1 min at 60℃ in a total reaction volume of 25 μL.

2.3 Experimental protocols

The experiment consisted of 2 months of home-based resistance training and physical fitness tests performed in before and after training periods. Before both physical fitness tests, subjects underwent a complete medical checkup. Body weight, percent body fat, grip strength, sit-ups, sit-and-reach, and one-leg standing with open eyes were measured. Physical fitness was determined according to the Japan fitness test of the Ministry of Education, Science, Sports and Culture, Japan.

2.4 Home-based resistance training program

The aim of the home-based resistance training performed in this study was to improve muscle strength and endurance of the trunk and lower limbs. As we focused on exercise that can be performed easily and regularly by elderly people, the training program was performed at home. In the fitness test before training, subjects learned how to perform the training. This training program consisted of four different exercises, performed without any equipment or extra weights: squat, leg extension, leg curl, and hip flexion (Fig. 1). To perform leg curl, the subjects stood by the chair, and held the seat back to support their body. Leg extension and hip flexion were performed while seated on the chair. The training period was set at 2 months, and subjects were asked to perform at least three sets of 10 repetitions of each exercise each week (> 3 days/week). No upper limit of training-session volume was set. At the fitness test after training, a self-reported training record was collected.

2.5 Statistical analysis

All data are expressed as means ± SD. One-way ANOVA was used to test for differences in physical characteristics among *ACTN3* genotypes. Student's paired *t*-test was used for comparison of before and after training values. All analyses were performed using GraphPad Prism 5 for Windows (GraphPad Software Inc., La Jolla, CA) and statistical significance was set at $p < 0.05$.

3. Results

3.1 Prevalence of R577X polymorphism

The genotype distribution among the 35 subjects who completed the training was RR = 6 (17.1%), RX = 16 (45.7%), and XX = 13 (37.1%), and the R and X allele frequencies were 40% and 60%, respectively. The XX genotype and X allele frequency were slightly higher than those reported previously[26].

3.2 Volume of training sessions

The volume of sessions during the 2-month home-based training program varied widely among subjects (Table 1). No significant differences in volume were found among the genotypes.

3.3 Physical characteristics and fitness

No significant differences among genotypes were found in age, height, body weight, or percent body fat (Table 2) before training. Similarly, grip strength, sit-ups, sit-and-reach, and one-leg standing with open eyes did not differ among genotypes, although a tendency for the order XX > RX > RR in one-leg standing with open eyes was found at before training (Fig. 2).

Body weight, percent body fat, grip strength, and sit-and-reach did not differ between before and af-

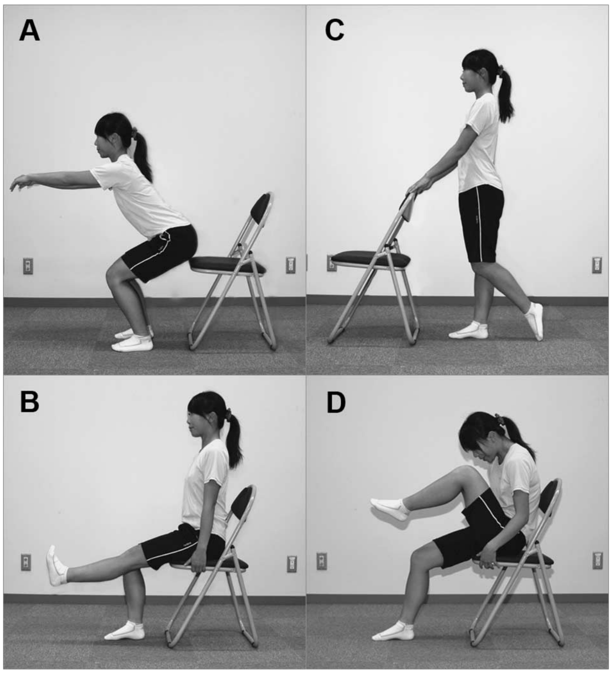

Fig. 1　The home-based resistance training program consisted of four different exercises: squat (A), leg extension (B), leg curl (C), and hip flexion (D), all performed without any exercise equipment or extra weights. When leg curl was performed, the subjects stood by the chair and held the seat back to support their body. Leg extension and hip flexion were performed while seated on the chair. Subjects were asked to perform at least three sets of 10 repetitions of each exercise per week.

Table 1　Volume of sessions of home–based resistance training according to α–actinin–3 (*ACTN3*) R577X genotype ($n=35$)

	XX ($n=13$)	RX ($n=16$)	RR ($n=6$)	RR + RX ($n=22$)
Squat	6.6 ± 2.8	6.4 ± 1.4	5.5 ± 1.9	6.1 ± 1.5
Leg extension	6.6 ± 3.0	6.4 ± 1.4	5.4 ± 1.9	6.1 ± 1.5
Leg curl	6.6 ± 3.3	6.3 ± 1.4	5.1 ± 2.0	6.0 ± 1.6
Hip flexion	6.0 ± 2.7	6.3 ± 1.4	4.8 ± 2.3	5.9 ± 1.8

Values are means ± SD (sets・week^{-1}).

Table 2　Baseline characteristics of subjects according to α–actinin–3 (*ACTN3*) R577X genotype ($n=35$)

	XX ($n=13$)	RX ($n=16$)	RR ($n=6$)	RR + RX ($n=22$)
Age, y	73.7 ± 5.9	74.7 ± 6.4	73.5 ± 4.2	74.4 ± 5.8
Height, cm	149.5 ± 4.7	149.4 ± 4.5	147.7 ± 5.6	147.7 ± 4.8
Weight, kg	52.6 ± 5.8	51.0 ± 5.6	51.0 ± 4.8	51.0 ± 5.3
Body fat, %	35.5 ± 3.9	34.0 ± 3.7	35.3 ± 2.4	34.4 ± 3.4

Values are means ± SD.

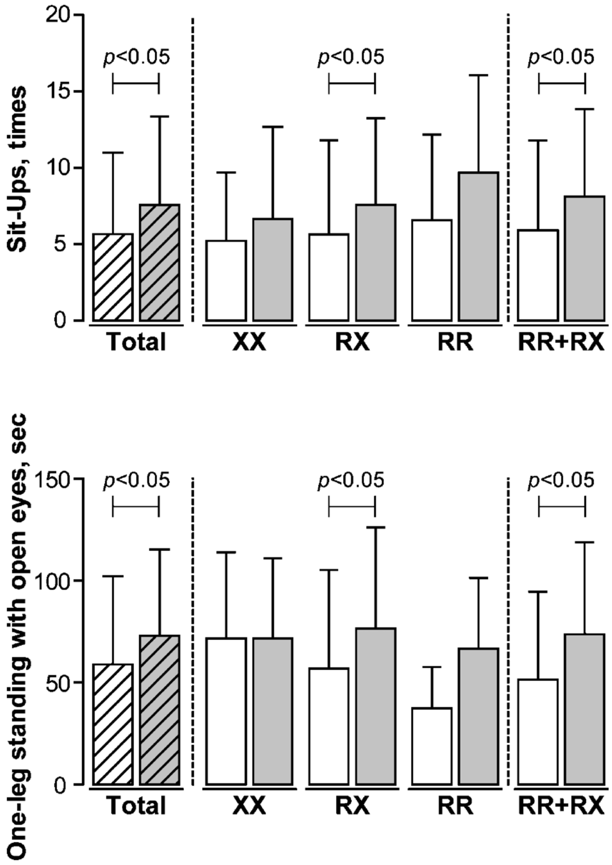

Fig. 2 The results for sit-ups (upper) and one-leg standing with open eyes (lower) according to α-actinin-3 (*ACTN3*) R577X genotype in before and after training periods. Open bars, before training; gray bars, after training. The R-allele group (RR + RX) showed significant improvement after training, whereas no significant differences were found between before and after training in the XX genotype.

ter training. However, sit-ups ($p < 0.01$) and one-leg standing with open eyes ($p < 0.05$) were increased after training in all subjects. These performances were increased significantly in the RX genotype (sit-ups: $p < 0.01$; one-leg standing with open eyes: $p < 0.05$), but not for other groups. The R-allele group (RR + RX) showed significant increases in sit-ups and one-leg standing with open eyes after training ($p < 0.01$), but no significant difference was observed in the XX genotype.

4. Discussion

The aim of the resistance training program performed in this study was to improve muscle strength and endurance of the trunk and lower limbs. As we were interested in exercise that could be performed easily and regularly to improve physical fitness and that would resolve the actual problem of subjects' having difficulties with mobility, a home-based training program was used. Our results showed that the resistance training program improved muscle strength and endurance. The self-reported results indicated that 91.4% of subjects fulfilled the study requirements: *i.e.*, performed three sets or more per week of each of the four different exercises, although training volume and frequency varied among subjects. According to the results of the questionnaire survey, more than 80% of subjects felt some positive changes in their mobility, and more than 95% of subjects intended to continue our training program even after the experimental period. None of the subjects complained about negative effects of our training program. However, 12 subjects could not complete the 2-month training (one had a fracture while walking; reasons were not given for the other 11). Thus, although further effort may be required to produce a training program to maintain motivation among the subjects, our results suggested the effectiveness of regular exercise programs for elderly women.

Falls are closely related to the drop in muscle strength and muscle mass gradually decline with aging[9)11)]. Delmonico *et al*.[8)] demonstrated over a 5-year period that women with XX genotype 70–79 years old had a ～35％ greater risk of persistent lower extremity limitation compared to those with the RR genotype. Walsh *et al*.[24)] reported that women deficient in α-actinin-3 appear to be at a disadvantage for muscular strength. These findings suggested that the muscle strength in elderly women with the XX genotype may be lower. However, in the present study, no significant differences were found in the results of physical fitness tests before training among the genotypes, although the subjects with the RR genotype tended to have shorter one-leg standing times. Further studies are needed to clarify these contradictory observations in much larger sample sizes.

After a 2-month home-based resistance training program, sit-ups and one-leg standing with open eyes were improved, suggesting that this training program improved muscle strength and endurance. We considered that no significant changes was observed in grip strength and sit-and reach since the aim of training performed in this study was to improve muscle strength and endurance of the trunk and lower limbs. Although the differences between genotypes were not significant, our results supported those from a previous investigation by Delmonico *et al*.[7)], which found greater improvement in fitness in the R allele group. The results may have been clearer if subjects had trained using a high-intensity protocol (*e.g.*, using free weights). Vincent *et al*.[23)] reported that cross-sectional area and number of type IIx fibers were greater in the RR than in the XX genotype. In addition, a study in *ACTN3* knockout mice indicated that α-actinin-3 deficiency is associated with reduced fast fiber diameter and altered contractile properties[4)14)]. Responses to resistance exercise of type I and type II fibers were not different in the elderly[10)]. However, type II fibers produce greater force and exhibit greater responsiveness than type I fibers. Thus, we speculated that the higher percentage of type II fibers resulted in a greater response to training in the R allele group.

Many studies have examined *ACTN3*, but most findings are controversial. These discrepancies between studies may be related to the differences in the age, gender, ethnic background, or fitness level of the selected subjects. Further studies are needed in much larger study populations of elderly Japanese subjects.

5. Conclusion

In conclusion, our study indicated an association between *ACTN3* R577X polymorphism and training adaptation in elderly Japanese women. The baseline of the physical characteristics and the results of physical fitness test were not different among genotypes before training. The 2-month home-based resistance training program improved sit-ups and one-leg standing with open eyes in the *ACTN3* R577 allele group, but not in 577X homozygotes. Further studies in much larger sample sizes and both genders are required to clarify the association between *ACTN3* R577X polymorphism and effects of training in Japanese elderly subjects.

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