International Journal of Human Movement and Sports Sciences 11(4): 908-913, 2023 http://www.hrpub.org
DOI: 10.13189/saj.2023.110427

Association of ACTN3 R577X Genotype with Physical Fitness in Japanese Male College Students

Kazuhiro Matsui[1,*], Toshiyuki Kawamura[2], Akihiro Azuma[1], Christian Wisdom M. Valleser[3], Francis Carlos B. Diaz[3]

[1]Course of General Education, National Institute of Technology, Fukui College, Japan
[2]Department of Chemistry and Biology, National Institute of Technology, Fukui College, Japan
[3]College of Human Kinetics, University of the Philippines Diliman, Philippines

Received April 8, 2023; Revised June 12, 2023; Accepted July 20, 2023

Cite This Paper in the Following Citation Styles

(a): *[1] Kazuhiro Matsui, Toshiyuki Kawamura, Akihiro Azuma, Christian Wisdom M. Valleser, Francis Carlos B. Diaz , "Association of ACTN3 R577X Genotype with Physical Fitness in Japanese Male College Students," International Journal of Human Movement and Sports Sciences, Vol. 11, No. 4, pp. 908 - 913, 2023. DOI: 10.13189/saj.2023.110427.*

(b): *Kazuhiro Matsui, Toshiyuki Kawamura, Akihiro Azuma, Christian Wisdom M. Valleser, Francis Carlos B. Diaz (2023). Association of ACTN3 R577X Genotype with Physical Fitness in Japanese Male College Students. International Journal of Human Movement and Sports Sciences, 11(4), 908 - 913. DOI: 10.13189/saj.2023.110427.*

Abstract This study examined the association between the Japanese physical fitness test (field test) and ACTN3 genotypes in non-athlete male college students. Subjects were 123 healthy college male students (18.46 ± 0.61 years). DNAs were extracted by their biological samples, and genotypes (RR, RX, and XX types) of the ACTN3 gene were analyzed. The RR and the RX type were categorized as one group (R-allele group), and then there was a comparison of the physical fitness test items between the R-allele and the XX group. Eight physical fitness tests, standardized and applied to Japanese, were used to evaluate muscle strength, muscular endurance, flexibility, agility, aerobic endurance, and power. The genotypes of the subjects in this study were 23 for RR type, 61 for RX type, and 39 for XX type, respectively, and 84 subjects (RR type + RX type) were grouped as the R-allele group. The results showed significant differences in the 50-m run and the standing long jump between groups, which are considered sprint/power-related items (both $P < 0.05$), but not in the other items. Hence, the sprint/power-related physical fitness tests in the R-allele group were superior to those in the XX group. Moreover, the specificity of ACTN3 for sprint/power athletes, as observed in the laboratory tests, was also observed in the field tests for the Japanese general male college students. Physical fitness tests may be helpful to superiority in ACTN3-related aspects of physical activity for them.

Keywords ACTN3, Genotype, Physical Fitness Test, Field Test

1. Introduction

Among the factors determining exercise capacity, approximately 50% of endurance capacity [1], such as maximal oxygen uptake and muscle strength and power [2], are due to genetic factors. It is well known that there is a significant relationship between muscle fiber composition and adaptation to training or sports competition [3], and ACTN3, a gene of alpha actinin-3 protein expressed exclusively within the myotubes of type II fast-twitch muscle fibers, has recently (especially since 2000) attracted attention and been investigated in relation to muscle function. The ACTN 3 gene is not expressed when a nonsense base substitution on chromosome 11q13-q14 changes the 577th amino acid from R (arginine) to X (termination codon) (type XX). Many studies have shown that athletes with the R allele (type RR, type RX) have superior muscle function in sprint/power systems [4-8]. In particular, the relationship with speed rather than force is emphasized [9-12], with no R allele advantage for static muscle strength [13].

While many of the research reports mentioned above have been evident primarily in sprint/power athletes, there have been studies that ACTN3 is not associated with muscle power in the general population or in young non-athletes [4, 14]. If almost sprint/power athletes are assumed to have achieved excellent results and have originally genetically benefited from ACTN3 and have been trying their competitive activities. In that case, it is not surprising to find an ACTN3 advantage in athletes with the R allele. In the general population, functional specificity in fast-twitch muscle fibers may be silenced in terms of epigenetics even if they have the R allele [15]. With the mechanization and automation of life reducing the opportunities for daily life to involve physical labor, high sprint/power system ability may be an athletic ability that is not in demand in daily life. Additionally, in our survey of the preference for exercise (such as sprint or endurance) among the general student population, it was reported that many of those with the R allele (RR, RX) do not necessarily have a preference for sprint [16]. And it is possible that those with the R allele have not had the experience of physical training that would develop sprint/power abilities for the general population in their life. Furthermore, laboratory experiments have not adequately investigated the relationship between ACTN3 and motor function in the general population. In other words, the relationship between the ACTN3 gene and athletic performance in the general population is equally an open question.

On the other hand, a few reports have examined the relationship between the ACTN3 genotype and physical fitness in the general population in field tests (physical fitness tests) for minors [17, 18]. Physical fitness tests include tests that target muscle power (sprint/power) measures and other tests that capture overall physical fitness, such as muscle strength, flexibility, and general endurance. Although there is variety in their combinations, each test employs a simple method that can be administered even to people of low physical fitness, allowing for a large sample size and the ability to extract population trends. In the above two studies for minors, the sprint/power test items, the standing long jump and 40m sprint, showed strong associations with ACTN3, respectively. Thus, the specificity of the ACTN3 gene was observed even in field tests suitable for mass surveys of the general population, and it is adequate to examine the relationship between ACTN3 and athletic performance using a field test, which is a simple measurement method for a large number of general population subjects. Hence, investigating such among Japanese subjects will contribute to this field of knowledge. Moreover, Kikuchi et al. [19] and Kikuchi et al. [20] noted the association between ACTN3 and flexibility, suggesting that physical fitness tests could be applied to investigate physical fitness factors (athletic performance) related to ACTN3 across physical fitness in addition to sprinting/power-related items.

Therefore, this study aimed to determine the association of the ACTN3 R577 genotype with physical fitness in Japanese college male students.

2. Materials and Methods

2.1. Subjects

The subjects were 123 healthy college male students between the ages of 18 and 21 years who had no disabilities or medical history that could interfere with the measurement of physical fitness tests (mean age ± standard deviation: 18.5 ± 0.6 years, height: 170.9 ± 5.7 cm, weight: 61.6 ± 9.4 kg). The subjects were science and engineering students of the National Institute of Technology and did not include high-level athletes who had won prizes at national or international competitions. This study was approved in advance by The Research Ethics Committee for Human Subjects at the National Institute of Technology, Fukui College (Approval number: R4-5). Each participant voluntarily provided written informed consent before participating. It followed the ethical guidelines for Human Genome and Genetic Analysis Research of the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Ministry of Health, Labor and Welfare, and Ministry of Economy, Trade and Industry in Japan.

2.2. Analysis of ACTN3 Genotype

Genomic DNA was extracted from two or three hairs from a subject with fresh hair follicles using a DNA extraction kit (ISOHAIR, Nippon Gene Co., Ltd.). Then, following the method of Mills et al. (2001), the ACNT3 primer set (Forward primer: 5’CTGTTGCCTGTGGTAA GTGGG3’; Reverse primer: 5’TGGTCACAGTATGCAG GAGGG3’) and DNA polymerase (EX Taq, TaKaRa Bio Inc.), the PCR reaction process was repeated 40 times in three steps (94 ℃ for 30 s, 60 ℃ for 30 s, 72 ℃ for 40 s) [21]. The PCR products were separated by 3% agarose gel electrophoresis, and only the amplified product of 280 base pairs was extracted from the gel. PCR products were purified from the separated gels using a DNA purification kit (NucleoSpin Gel and PCR Clean-up, MACHEREY-NAGEL GmbH & Co. KG). The PCR product was sequenced by the Sanger method using the forward primer of the ACTN3 primer set described above. The genotypes RR, RX, and XX were determined by analyzing whether the 577th codon of ACTN3 encodes the arginine (R) or is a stop codon.

2.3. Physical Fitness Test Measurement

All subjects performed the New Physical Fitness Test developed by the MEXT, which included eight items (for 12 - 19 years old): grip strength (static or isometric strength), sit-ups (muscular endurance), sitting forward bending (flexibility), side-step (agility), 20-m shuttle run (aerobic endurance), 50-m run (speed or sprint ability), standing long jump (muscle power), and handball throwing (muscle power) [22]. The data used were the physical fitness results when the participants were 18 years old. The

50-m run and handball throwing are both items that assess muscle power. However, the former can be regarded as muscle power that reflects the running ability and the latter as throwing ability [22].

2.4. Analysis

The RR and RX genotypes of the subjects were grouped as the R-allele group, and various physical fitness test items were compared between the two groups, the R-allele group and the XX group.

In general, RR and RX types with the R allele are considered to be associated with fast and instantaneous athletic performance [23], and RR and RX types are known to be more common in sprint/power-oriented athletes [24-28]. Kikuchi et al. (2016) also compared 100-m best times and 400-m best times of RR and RX types as one group with XX types and found significant differences [29]. MacArthur and North (2007) also reported that alpha-actinin-3 deficiency (type XX) is likely to impair the performance of fast-twitch muscle fibers in some way in both superior and non-athletes [30]. Furthermore, the XX genotype has been reported to be associated with lower baseline muscle strength [31] and inferior sprint performance [26] in a non-athlete cohort study, respectively.

Based on these findings, the RR and RX types were designated as the R-allele group (RR + RX) in the present study and the R allele group and XX type were compared.

2.5. Statistics

An unpaired t-test was used to examine the difference in mean values in each item between the two groups. Cohen's d was adopted to calculate the effect size (ES). The level of statistical significance was set at 5%.

3. Results

The means and standard deviations of height, weight and body mass index (BMI; weight (kg)/height (m)^2) for the R-allele and XX groups are shown in Table 1. No significant differences were found between the two groups for any of the variables. The genotypes of the subjects in this study were 23 for RR type, 61 for RX type, and 39 for XX type, respectively, and 84 subjects (RR type + RX type) were grouped as the R-allele group. The means and standard deviations for each physical fitness test item for the R-allele group and XX type are shown in Table 2. As a result, the difference in means for all items except the 50-m run and the standing long jump was not significant, and the R-allele group was faster and greater in both the 50-m run ($P < 0.05$, ES = 0.450) and the standing long jump ($P < 0.05$, ES = 0.447) than the XX group.

Table 1. Means and standard deviations of physical characteristics in the R-allele and the XX groups

Variable	Group		P	ES
	R-allele (n = 84)	XX (n = 39)		
Height (cm)	171.0 ± 5.8	170.7 ± 5.9	0.751	0.062
Weight (kg)	62.7 ± 9.2	59.3 ± 9.3	0.060	0.369
BMI	21.5 ± 3.3	20.3 ± 3.0	0.076	0.337

Cohen's *d* was adopted to calculate the effect size (ES).

Table 2. Means and standard deviations of each variable of physical fitness test in the R-allele and the XX groups

Variable	Group		P	ES
	R-allele (n = 84)	XX (n = 39)		
Grip strength (kg)	42.5 ± 6.8	41.1 ± 7.7	0.319	0.203
Sit-up (times)	29.0 ± 5.1	28.5 ± 6.4	0.646	0.097
Sitting forward bending (cm)	51.9 ± 12.0	50.0 ± 13.0	0.429	0.158
Side-step (times)	57.0 ± 6.7	55.5 ± 5.4	0.222	0.224
20-m shuttle run (times)	80.5 ± 20.6	77.3 ± 23.4	0.446	0.155
50-m run (s)	7.60 ± 0.47	7.82 ± 0.58	0.033	0.450
Standing long jump (cm)	226.4 ± 21.6	216.4 ± 28.6	0.042	0.447
Handball throwing (m)	21.7 ± 4.9	20.9 ± 5.1	0.423	0.158

Cohen's *d* was adopted to calculate the effect size (ES).

4. Discussion

Comparison of physical fitness test items between the R allele and XX groups in this study revealed significant differences only for the 50-m run and the standing long jump ($P < 0.05$).

Many studies on the ACTN3 R577X genotype associated with muscle power have been reported in athletes from different countries [4-8]. These research results were experimentally derived from measuring muscle power as a laboratory test on athletes and were unrelated to the athlete's competition level [7]. Since power is expressed as the product of force and speed, the relationship between ACTN3 and the two (force and speed) has been investigated. Furthermore, some studies have indicated that the latter (speed) is more related to the ACTN3 than the former [9-12]. In other words, it has been pointed out that the influence of ACTN3 may be expressed in dynamic muscle strength rather than static muscle strength [11] and in sprinting rather than weightlifting [12]. Vincent et al. [9] also reported that relative quadriceps torque was as fast as 300 deg/s and reported that homozygosity for the R allele (R allele) was high. Thus, the relationship between ACTN3 and muscle power tends to emphasize the relationship with speed rather than force.

In this study, the physical fitness test items of the R-allele group that showed significant differences from the XX group were the 50-m run and the standing long jump, both of which are muscle power-related field tests that require higher speed. The superiority of these speed-related test items in the R allele group, in which the alpha-actinin 3 gene is specifically expressed in fast-twitch muscle fibers, is supported by many experimental studies described above. In addition, standing long jump in a physical fitness test in Han Chinese children (5.36 ±1.03 years old) [18; however, the SNP ID for ACTN3 is rs2290463] and 40-m sprint in a physical fitness test in Greek boys [17], both reported a strong association with ACTN3. Results of this study (i.e., the R-allele group was superior on the field items of standing long jump and short distance running) showed consistency with the studies mentioned above among non-athlete subjects.

In the 50-m run and the standing long jump tests, the lower limb and back muscle groups, which occupy the large muscle mass in the body, contribute to power [32]. Handball throw is another physical fitness test that evaluates muscle power. However, since the upper limb muscle groups contribute more to this test than the lower limb muscle groups, and since throwing experience and technique play a significant role, it is possible that the R allele group, which reflects the genetic characteristics of fast-twitch muscles, was not dominant in this test. Furthermore, none of the previous studies pointed out an association between handball throwing and ACTN3. Hence, the specificity of the R-allele group was observed in the power events in the performance of physical fitness test items, in which large muscle mass is mainly involved as a physical resource. However, the effect size (ES) of the 50-m run and the standing long jump, which showed significant differences between the R-allele and XX groups, was not significant ($P < 0.05$), suggesting that the influence of genetic factors may be smaller than that of environmental factors such as exercise experience. Therefore, a larger sample size is required to examine the difference accurately.

On the other hand, grip strength is a field test that reflects static voluntary muscle strength. The result that the R-allele group is not dominant in this test is supported by previous research that ACTN3 is more characteristic of dynamic muscle strength than static muscle strength [11] and that RR and RX groups are not dominant in the weight-bearing index (leg extension force divided by body weight) [13]. In addition, other items investigated in this study, i.e., sit-ups, sitting forward bending, side-steps, and 20-m shuttle run, are representative measures of muscular endurance, flexibility, agility, and aerobic capacity, respectively. Although there are several findings on the relationship between flexibility and aerobic capacity and ACTN3 genotype as laboratory tests [19, 20, 33-36], the association with ACTN3 has not been known in field tests yet. This study's main finding is that the specificity of ACTN3 for muscle power known from laboratory tests was also observed in field tests on the general population. Most of the subjects reported as laboratory tests for ACTN3 were elite athletes who were routinely exposed to high-intensity exercise stimuli (training). The observed superiority (XX type) of flexibility [19, 20] and aerobic capacity [33-37] in such subjects was not specific to the physical fitness tests in this study, and the association with ACTN3 genotypes was only observed in sprint/power test items (superiority in the R-allele group). As reported by Kawamura et al. (2021), there was often a mismatch between exercise preferences, such as sprint or endurance, and ACTN3 genotypes in the general population [16]. The specificity of the fitness tests may have been limited because they have not chosen a life of exposure to the rigorous physical training that would make them athletes. Paradoxically, it was found that sprint/power test items tended to be superior in the general population who were not exposed to high-intensity exercise stimuli. Hence, this suggests that the ACTN3 may be a valuable diagnosis for determining aptitude for physical fitness and selecting lifelong sports.

5. Conclusions

In this study, we examined the association between ACTN3 genotypes and physical fitness in general Japanese male college students from a similar viewpoint and found significant differences between the R-Allele and XX groups, in which RR and RX types were considered as one group, just in the 50m run and the standing long jump. Hence, the specificity of the ACTN3 genotypes (RR, RX), as observed in sprint/power athletes in the laboratory tests, can be captured in field test items in the general male student population. The results suggest that ACTN3 genotypes may be used to indicate superiority in sprint/power-related aspects of physical activity, not only in athletes but also in the general population. Genetic analysis should consider ethical and moral issues and should not restrict an individual's freedom of choice in exercise. In other words, the choice of sporting events based on individual interest and motivation should be respected. Genetic analysis is expected to be helpful as a source of information in this regard.

Acknowledgements

The physical fitness tests in this study were conducted with the cooperation of the physical education teachers at the institute to which the subjects belonged. And this work was supported by JSPS KAKENHI Grant Number JP21K06367.

REFERENCES

[1] E. Miyamoto-Mikami, H. Zempo, N. Fuku, N. Kikuchi, M. Miyachi, and H. Murakami, "Heritability estimates of endurance-related phenotypes: A systematic review and meta-analysis," *Scand J Med and Sci Sports,* vol. 28, no. 3 pp. 834-845, 2017, doi: https://doi.org/10.1111/sms.12958.

[2] H. Zempo, J. Suzuki, M. Ogawa, R. Watanabe, and M. Isobe, "A different role of angiotensin II type 1a receptor in the development and hypertrophy of plantaris muscle in mice," *J Appl Genet,* vol. 57, no. 1, pp. 91-97, 2016, doi: https://doi.org/10.1007/s13353-015-0291-8.

[3] J. R. Daugaard, and E. A. Richter, "Relationship between muscle fibre composition, glucose transporter protein 4 and exercise training: possible consequences in non-insulin-dependent diabetes mellitus," *Acta Physiol Scand,* vol. 171, no. 3 pp. 267-276, 2001, doi: https://doi.org/10.1046/j.1365-201x.2001.00829.x.

[4] T. Alfred, Y. Ben-Shlomo, R. Cooper, R. Hardy, C. Cooper, I. J. Deary, D. Gunnell, S. E. Harris, M. Kumari, R. M. Martin, C. N. Moran, Y. P. Pitsiladis, S.M. Ring, A. A. Sayer, G. D. Smith, J. M. Starr, D. Kuh, I. N. Day, and HALCyon study team, "ACTN3 genotype, athletic status, and life course physical capability: meta-analysis of the published literature and findings from nine studies," *Hum Mutat,* vol. 32, no. 9, pp. 1008-1018, 2011, doi: https://doi.org/10.1002/humu.21526.

[5] N. Kikuchi, K. Nakazato, S. K. Min, D. Ueda, and S. Igawa, "The ACTN3 R577X polymorphism is associated with muscle power in male Japanese athletes," *J Strength Cond Res,* vol. 28, no. 7, pp. 1783-1789, 2014, doi: https://doi.org/10.1519/jsc.0000000000000338.

[6] J. Orysiak, K. Busko, R. Michalski, J. Mazur-Różycka, J. Gajewski, J. Malczewska-Lenczowska, D. Sitkowski, and A. Pokrywka, "Relationship between ACTN3 R577X polymorphism and maximal power output in elite Polish athletes," *Medicina (Kaunas, Lithuania),* vol. 50, no. 5, pp. 303-308, 2014, doi: https://doi.org/10.1016/j.medici.2014.10.002.

[7] N. Garatachea, Z. Verde, A. Santos-Lozano, T. Yvert, G. Rodriguez-Romo, F. J. Sarasa, S. Hernández-Sánchez, C. Santiago, and A. Lucia, "ACTN3 R577X polymorphism and explosive leg-muscle power in elite basketball players," *Int J Sports Physiol Perform,* vol. 9, no. 2, pp. 226-232, 2014, doi: https://doi.org/10.1123/ijspp.2012-0331.

[8] R. Yang, X. Shen, Y. Wang, S. Voisin, G. Cai, Y. Fu, W. Xu, N. Eynon, D.J. Bishop, and X. Yan, "ACTN3 R577X gene variant is associated with muscle-related phenotypes in elite Chinese sprint/power athletes," *J Strength Cond Res,* vol. 31, no. 4, pp. 1107-1115, 2017, doi: https://doi.org/10.1519/jsc.0000000000001558.

[9] B. Vincent, K. De Bock, M. Ramaekers, E. Van den Eede, M. Van Leemputte, P. Hespel, and M.A. Thomis, "ACTN3 (R577X) genotype is associated with fiber type distribution," *Physiol Genomics,* vol. 32, no. 1, pp. 58-63, 2007, doi: https://doi.org/10.1152/physiolgenomics.00173.2007.

[10] H. Kim, K.H. Song, and C.H. Kim, "The ACTN3 R577X variant in sprint and strength performance," *J Exerc Nutrition Biochem,* vol. 18, no. 4, pp. 347-353, 2014, doi: https://doi.org/10.5717/jenb.2014.18.4.347.

[11] J. Orysiak, K. Busko, J. Mazur-RóŻycka, R. Michalski, J. Gajewski, J. Malczewska-Lenczowska, and D. Sitkowski, "Relationship between ACTN3 R577X polymorphism and physical abilities in Polish athletes," *J Strength Cond Res,* vol. 29, no. 8, pp. 2333-2339, 2015, doi: https://doi.org/10.1519/jsc.0000000000000880.

[12] S. Ben-Zaken, A. Eliakim, D. Nemet, and Y. Meckel, "Genetic variability among power athletes: The stronger vs. the faster," *J Strength Cond Res,* vol. 33, no. 6, pp. 1505-1511, 2011, doi: https://doi.org/10.1519/jsc.0000000000001356.

[13] A. Azuma, T. Kawamura, and K. Matsui, "Is ACTN3 R577X Genotype Associated with Weight-Bearing Index in Male College Students?" *In W. Spratford, C. Coltman, N. Brown, & J. Warmenhoven (Eds.), Proceedings of the 39th International Conference on Biomechanics in Sports,* pp. 380-383, 2021, Retrieved from https://commons.nmu.edu/cgi/viewcontent.cgi?article=2253&context=isbs.

[14] C. Santiago, G. Rodríguez-Romo, F. Gómez-Gallego, M. González-Freire, T. Yvert, Z. Verde, F. Naclerio, S. Altmäe, J. Esteve-Lanao, J.R. Ruiz, and A. Lucia, "Is there an association between ACTN3 R577X polymorphism and muscle power phenotypes in young, non-athletic adults?" *Scand J Med Sci Sports 2021,* vol. 20, no. 5, pp. 771-778, 2010, doi: https://doi.org/10.1111/j.1600-0838.2009.01017.x.

[15] W. J. Świątowy, H. Drzewiecka, M. Kliber, M. Saccade, P. Karpiński, A. Pławski, and P. P. Jagodziński, "Physical activity and DNA methylation in humans," *Int J Mol Sci,* vol. 22, no. 23, p. 12989, 2021, doi: https://doi.org/10.3390/ijms222312989.

[16] T. Kawamura, A. Azuma, and K. Matsui, "Is ACTN3 R577X genotype associated with a preference in type of exercise such as sprint or endurance?" *Adv Phys Educ,* vol. 11, no. 2, pp. 268-275, 2021, doi: https://doi.org/10.4236/ape.2021.112022.

[17] C. N. Moran, N. Yang, M. E. Bailey, A. Tsiokanos, A. Jamurtas, D. G. MacArthur, K. North, Y. P. Pitsiladis, and R. H. Wilson, “Association analysis of the ACTN3 R577X polymorphism and complex quantitative body composition and performance phenotypes in adolescent Greeks,” *Eur J Hum Genet,* vol. 15, no. 1, pp. 88-93, 2007, doi: https://doi.org/10.1038/sj.ejhg.5201724.

[18] Q. Zhang, Y. Cao, J. Chen, J. Shen, D. Ke, X. Wang, J. Ji, Y. Xu, W. Zhang, Y. Shen, D. Wang, D. Pan, Z. Wang, Y. Shi, S. Cheng, Y. Zhao, and D. Lu, ”ACTN3 is associated with children's physical fitness in Han Chinese,” *Mol Genet Genomics,* vol. 294, no. 1, pp. 47-56, 2019, doi: https://doi.org/10.1007/s00438-018-1485-7.

[19] N. Kikuchi, H. Zempo, N. Fuku, H. Murakami, M. Sakamaki-Sunaga, T. Okamoto, K. Nakazato, and M.Miyachi, “Association between ACTN3 R577X polymorphism and trunk flexibility in 2 different cohorts,” *Int J Sports Med,* vol. 38, no. 5, pp. 402-406, 2017, doi: https://doi.org/10.1055/s-0042-118649.

[20] N. Kikuchi, Y. Tsuchiya, K. Nakazato, N. Ishii, and E. Ochi, “Effects of the ACTN3 R577X Genotype on the muscular strength and range of motion before and after eccentric contractions of the elbow flexors,” *Int J Sports Med,* vol. 39, no. 2, pp. 148-153, 2018, doi: https://doi.org/10.1055/s-0043-120762.

[21] M. Mills, N. Yang, R. Weinberger, D. L. Vander Woude, A. H. Beggs, S. Easteal, and K. North, “Differential expression of the actin-binding proteins, alpha-actinin-2 and -3, in different species: implications for the evolution of functional redundancy,” *Hum Mol Genet*, vol. 10, no. 13, pp. 1335-1346, 2001, doi: https://doi.org/10.1093/hmg/10.13.1335.

[22] Japan Sports Agency, “Physical Fitness and Motor Ability Survey Report 2021,” *Japan Sports Agency,* 2022. (in Japanese)

[23] D. Saito, N. Fuku, E. Mikami, T. Kawahara, H. Tanaka, M. Higuchi, and M. Tanaka, “The ACTN3 R577X nonsense allele is under-represented in elite-level Japanese endurance runners,” *Jpn J Phys Fitness Sports Med,* vol. 60, no. 4, pp. 443-451, 2011, doi: https://doi.org/10.7600/jspfsm.60.443.

[24] L. L. Chiu, Y. F. Wu, M. T. Tang, H. C. Yu, L. L. Hsieh, and S. S. Hsieh, “ACTN3 genotype and swimming performance in Taiwan,” *Int J Sports Med,* vol. 32, no. 6, pp. 476-480, 2011, doi: https://doi.org/10.1055/s-0030-1263115.

[25] A. M. Druzhevskaya, I. I. Ahmetov, I. V. Astratenkova, and V. A. Rogozkin, “Association of the ACTN3 R577X polymorphism with power athlete status in Russians,” *Eur J Appl Physiol,* vol. 103, no. 6, pp. 631-634, 2008, doi: https://doi.org/10.1007/s00421-008-0763-1.

[26] A. K. Niemi, and K. Majamaa, “Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes,” *Eur J Hum Genet,* vol. 13, no. 8, pp. 965-969, 2005, doi: https://doi.org/10.1038/sj.ejhg.5201438.

[27] I. D. Papadimitriou, C. Papadopoulos, A. Kouvatsi, and C. Triantaphyllidis, “The ACTN3 gene in elite Greek track and field athletes,” *Int J Sports Med,* vol. 29, no. 4, pp. 352-355, 2008, doi: https://doi.org/10.1055/s-2007-965339.

[28] E. Mikami, N. Fuku, H. Takahashi, N. Ohiwa, Y. P. Pitsiladis, M. Higuchi, T. Kawahara, and M. Tanaka, “Polymorphisms in the control region of mitochondrial DNA associated with elite Japanese athlete status,” *Scand J Med Sci Sports,* vol. 23, no. 5, pp. 593-599, 2013, doi: https://doi.org/10.1111/j.1600-0838.2011.01424.x.

[29] N. Kikuchi, E. Miyamoto-Mikami, H. Murakami, T. Nakamura, S.K. Min, M. Mizuno, H. Naito, M. Miyachi, K. Nakazato, and N. Fuku, “ACTN3 R577X genotype and athletic performance in a large cohort of Japanese athletes,” *Eur J Sport Sci*, vol. 16, no. 6, pp. 694-701, 2016, doi: https://doi.org/10.1080/17461391.2015.1071879.

[30] D. G. MacArthur, and K. N. North, “ACTN3: A genetic influence on muscle function and athletic performance,” *Exerc Sport Sci Rev,* vol. 35, no. 1, pp. 30-34, 2007, doi: https://doi.org/10.1097/jes.0b013e31802d8874.

[31] P. M. Clarkson, J. M. Devaney, H. Gordish-Dressman, P. D. Thompson, M. J. Hubal, M. Urso, T. B. Price, T. J. Angelopoulos, P. M. Gordon, N. M. Moyna, L. S. Pescatello, P. S. Visich, R. F. Zoeller, R. L. Seip, and E. P. Hoffman, “ACTN3 genotype is associated with increases in muscle strength in response to resistance training in women,” *J Appl Physiol,* vol. 99, no. 1, pp. 154-163, 2005, doi: https://doi.org/10.1152/japplphysiol.01139.2004.

[32] T. Korff, S. L. Horne, S. J. Cullen, and A. J. Blazevich, “Development of lower limb stiffness and its contribution to maximum vertical jumping power during adolescence,” *J Exp Biol* vol. 212, no. 22, pp. 3737-3742, 2009, doi: https://doi.org/10.1242/jeb.033191.

[33] N. Yang, D. G. MacArthur, J. P. Gulbin, A. G. Hahn, A. H. Beggs, S. Easteal, and K. North, “ACTN3 genotype is associated with human elite athletic performance,” *Am J Hum Genet,* vol. 73, no. 3, pp. 627-631, 2003, doi: https://doi.org/10.1086/377590.

[34] A. Lucia, F. Gómez-Gallego, C. Santiago, F. Bandrés, C. Earnest, M. Rabadán, J. M. Alonso, J. Hoyos, A. Córdova, G. Villa, and C. Foster, “ACTN3 genotype in professional endurance cyclists,” *Int J Sports Med,* vol. 27, no. 11, pp. 880-884, 2006, doi: https://doi.org/10.1055/s-2006-923862.

[35] E. M. Pimenta, D. B. Coelho, C. E. Veneroso, E. J. Barros Coelho, I. R. Cruz, R. F. Morandi, G. De A. Pussieldi, M. R. Carvalho, E. S. Garcia, and J. A. De Paz Fernández, “Effect of ACTN3 gene on strength and endurance in soccer players,” *J Strength Cond Res,* vol. 27, no. 12, pp. 3286-3292, 2013, doi: https://doi.org/10.1519/jsc.0b013e3182915e66.

[36] Romero-Blanco, M. J. Artiga-González, A. Gómez-Cabello, S. Vila-Maldonado, J. A. Casajús, I. Ara, and S. Aznar, “Strength and endurance training in older women in relation to ACTN3 R577X and ACE I/D polymorphisms,” *Int J Environ Res Public Health,* vol. 17, no. 4, p. 1236, 2020, doi: https://doi.org/10.3390/ijerph17041236.

[37] X. Shang, C. Huang, Q. Chang, L. Zhang, and T. Huang, “Association between the ACTN3 R577X polymorphism and female endurance athletes in China,” *Int J Sports Med,* vol. 31, no. 12, pp. 913-916, 2010, doi: https://doi.org/10.1055/s-0030-1265176.