The ACTN3 XX Genotype’s Underrepresentation in Japanese Elite Wrestlers

Naoki Kikuchi, Dai Ueda, Seok-ki Min, Koichi Nakazato, and Shoji Igawa

Purpose: To examine the relationship between ACTN3 polymorphisms and level of athletic performance in Japanese wrestlers. Methods: The control group consisted of 243 healthy Japanese individuals. The authors divided 135 wrestlers into 3 groups based on their results in national or international competitions. They classified as elite 24 wrestlers who had placed in the top 8 in a world championship or participated in Olympic games, 28 wrestlers who had participated in world championships or become champions in Japan’s national championships were classified as subelite, and 83 wrestlers were classified as national (N-W, ie, national-level wrestler). In addition, the authors combined the elite and subelite to form the classification international-level wrestlers (I-W). Results: The frequency of the null X allele and the XX genotype were significantly lower in the I-W group than in the control group. However, there was no significant difference in ACTN3 genotype or allele frequency between the N-W and control groups. The frequency of the ACTN3 XX genotype in the elite groups was lower than that of all groups, and a linear tendency was observed between ACTN3 XX genotype and athletic status. Conclusions: In conclusion, the data indicated that ACTN3 polymorphisms were related to athletic performance in Japanese wrestlers.

Keywords: gene polymorphism, athlete, wrestling, athletic performance, power

Competitive wrestling requires high absolute and relative values of maximal strength, muscle power, and anaerobic metabolism.1,2 The Japanese national wrestling team won 12 medals (3 gold, 3 silver, and 6 bronze) in the Asian Games in 2010 and 6 medals (2 gold, 2 silver, and 2 bronze) in the Beijing Olympics in 2008. However, no genetic studies on Japanese power-oriented athletes have included wrestlers. Elite athletic performance is a complex phenotype determined by several environmental factors including diet, physical training, and social factors. Genetic factors may also contribute to interindividual differences in athletic performance, and a recent review reported that more than 200 kinds of gene variants are associated with fitness-related phenotypes.3 Above all, the α-actinin-3 (ACTN3) R577X polymorphism have been associated with muscle composition,4,5 muscle strength,5,6 and elite performance.7-9

α-Actinins are actin-binding proteins that constitute the major structural component of the Z-line in skeletal muscle.10 The 2 isoforms, α-actinin-2 and α-actinin-3, expressed in humans are encoded by their respective genes ACTN2 and ACTN3.11,12 ACTN3 is a fast-twitch-specific isofrom, expressed only in type II myofibers,12 and is known to be important for anchoring actin and playing a regulatory function in coordinating muscle-fiber contraction.13 ACTN3 protein is absent in approximately 18% of individuals of European descent, who are homozygous for the common allele that encodes a premature stop codon at the R577X polymorphism (rs1815739, C-to-T transition at position 1747 in exon 16) in the ACTN3 gene.12

MacArthur et al10 reported that ACTN3-knockout mice, animal models of α-actinin-3 deficiency, have skeletal muscle with higher oxidative capacity than the skeletal muscle of wild-type mice. Furthermore, ACTN3-knockout mice were able to run 33% farther than wild-type mice on a treadmill endurance test but had lower grip strength and lower muscle weights than the wild-type mice.

Previous studies in White humans found that the ACTN3 XX genotype might be negatively associated with power and sprint performance and benefit endurance performance.9,14-16 Shang et al14 reported that there was a positive relationship between frequency of ACTN3 XX genotype and level of athletic performance in Chinese female endurance athletes. In addition, Eynon et al15 reported that the ACTN3 genotype was associated with top-level performance in Israeli sprinters. However, no studies have investigated ACTN3 genotypes and allele frequencies among Japanese elite power-oriented athletes. We hypothesized that the frequency of ACTN3 XX genotype would be lower and associated with athletic status among Japanese power-oriented athletes.
The purpose of this study was to determine whether there is an association between ACTN3 polymorphisms and level of athletic performance among Japanese wrestler.

**Material and Methods**

**Subjects**

We enrolled 328 participants—135 Japanese male wrestlers and 243 controls (94 male and 149 female)—in this study. We divided the 135 wrestlers into 3 groups based on their results from national or international competitions. Wrestlers (n = 24) who had placed among the top 8 in world championships or participated in Olympic games were classified as elite, wrestlers (n = 28) who had participated in world championships or won a Japan national championship were classified as subelite, and wrestlers (n = 83) who competed at the national level were classified as national (N-W for national-level wrestler). In addition, we combined the elite and subelite into an international-level wrestlers (I-W) group. Participants were informed of the purpose and method of the study to ensure complete understanding, and each of them gave written informed consent. The study was approved by the ethics committee of Nippon Sport Science University in Japan (010-G01) and was in accordance with the Declaration of Helsinki for Human Research.

**Genotyping**

Genotyping of DNA polymorphism in the ACTN3 gene was performed for 135 Japanese wrestlers and 243 controls. DNA samples were obtained from subjects’ buccal cells by rubbing the inner surface of their mouths with cotton swabs. Genotyping for the ACTN3 R577X polymorphism was determined by real-time polymerase chain reaction (PCR) assays using a TaqMan probe (rs1815739, predesigned SNP genotyping assays, Applied Biosystems, USA). The PCR cycling reactions were performed using a heating block (CFD-3120J1, BioRad, Hercules, CA) as follows: first cycle, 95°C for 10 minutes; 39 cycles, 92°C for 15 seconds, 60°C for 1 minute, melting curve 65°C to 95°C.17

**Data Analysis**

The SPSS statistical package, version 16.0 for Windows, was used to perform all statistical evaluations. Allele frequencies were determined by gene counting. Pearson χ² test and Fischer exact test were used to confirm that the observed genotype frequencies were in Hardy–Weinberg equilibrium and to compare the ACTN3 R577X alleles and genotype frequencies between groups of wrestlers and controls. Logistic-regression analysis was used to determine whether there would be a linear trend in the ACTN3 XX genotype frequency among the groups with different athletic status. The level of significance was set at P < .05.

**Table 1 Subject Characteristics (Wrestlers), Mean ± SD**

<table>
<thead>
<tr>
<th></th>
<th>I-W (n = 52)</th>
<th>N-W (n = 83)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>24.2 ± 4.1**</td>
<td>19.7 ± 1.26**</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170.3 ± 7.5</td>
<td>169.1 ± 6.8</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>74.4 ± 14.2</td>
<td>70.9 ± 10.8</td>
</tr>
<tr>
<td>Body-mass index (kg/m²)</td>
<td>25.5 ± 3.1</td>
<td>24.7 ± 2.7</td>
</tr>
<tr>
<td>Athletic experience (y)</td>
<td>11.2 ± 4.6**</td>
<td>6.7 ± 3.6**</td>
</tr>
</tbody>
</table>

Abbreviations: I-W, international-level wrestlers; N-W, national-level wrestlers.

**Results**

Subject characteristics and competitive experience are presented in Table 1. In comparison with I-W group, the age and competitive experience of the N-W group were higher.

ACTN3 genotype distribution among controls and wrestlers was in Hardy–Weinberg equilibrium (controls, χ² = .368, P = .544; all wrestlers, χ² = .019, df = 2, P = .870). Genotype distribution among controls (RR 25%, RX 46%, and XX 29%) was similar to those observed in reported groups of Japanese populations.18,19

ACTN3 genotype and allele frequency among wrestlers are presented in Table 2. The frequency of the ACTN3 R allele was significantly higher in all wrestlers than in controls (P = .033). When the wrestlers were separated based on athletic status (I-W vs N-W), ACTN3 XX genotype and X allele frequency were significantly lower in I-W than in controls (genotype, P = .022; allele, P = .022). However, there was no significant difference between the N-W group and the controls.

ACTN3 XX genotype frequency was significantly correlated with elite wrestler status (Figure 1). We found an inverse linear correlation between the frequency of the ACTN3 XX genotype and level of athletic status (P = .014 for linear trend). The odds ratio of ACTN3 XX genotype frequencies compared with controls was 4.54 (95% confidence interval [CI] 1.04–19.82), 2.74 (95% CI 0.83–7.40), and 1.08 (95% CI 0.62–1.87) for elite, subelite, and national, respectively.

**Discussion**

To the best of our knowledge, this study is the first to investigate the distribution of the ACTN3 genotype and allele frequencies among Japanese power-oriented athletes. Our main finding was the relationship between ACTN3 polymorphisms and level of athletic performance in Japanese wrestlers. We found an inverse linear correlation between the frequency of the ACTN3 XX genotype
Our data indicated that the ACTN3 RR and RX genotypes are associated with athletic performance of power-oriented athletes in Japanese populations and are consistent with findings from several previous studies.9,14,15,20,21 The first study on the influence of the ACTN3 genotype on elite athletic performance was conducted by Yang et al,9 who found that only 6% of elite sprinters and none of the top-level sprinters had the ACTN3 XX genotype. Consistent with this were other reports of associations between the RR or RX genotype and elite performance in Finnish,20 Greek,21 Russian,15 and Israeli14 elite sprint and power athletes. In addition, Santiago et al22 reported that elite soccer players tend to have the ACTN3 RR genotype. We similarly found that the frequency of the XX genotype was significantly lower in superior Japanese wrestlers (I-W group) than in controls and that there is a linear relationship between XX genotype frequency and athletic status in wrestlers.

Wrestling requires high absolute and relative values of maximal strength, muscle power, and anaerobic metabolism. Garcia-Pallares et al1 reported that elite wrestlers were older and had more extensive training, fat-free mass, absolute and relative maximum strength and power, and vertical-jump height and power and higher crank-arm Wingate peaks and greater mean power than amateur wrestlers. The researchers suggested that the higher absolute and relative values of these parameters give elite wrestlers a clear advantage during the most frequently used techniques in Olympic wrestling.1 Furthermore, adult elite wrestlers had a greater percentage of the fiber types IIa and IIax and higher mean fiber-type IIa and IIax areas than did adolescent elite wrestlers.23 Long-term and intensive training of wrestling may bring about...
such adaptations based on mechanical and biochemical requirements; in other words, Olympic wrestling must demand more remarkable innate and adaptable muscle strength and power.

The possible mechanisms underlying the associations between the ACTN3 R577X polymorphism and power performance have been discussed in detail elsewhere, and recent findings have indicated that the percentage of surface coverage and number of type IIX fibers were greater in the RR genotype group than in the XX genotype group in athletes and nonathletes. One explanation for the relationship between the XX genotype and type II fibers could be the fact that α-actinin interact with signaling proteins for muscle protein metabolism. Calcinurin also plays a key role in determining oxidative muscle-fiber type. A mechanistic link in the association between the ACTN3 genotype and muscle characteristics has also been proposed in several animal models. MacArthur et al. reported that the loss of α-actinin-3 expression in a knockout-mouse model shifted muscle metabolism toward the more efficient aerobic pathway and increased intrinsic endurance.

Another explanation is the structural strength of skeletal muscles. As mentioned previously, wrestlers experience high internal and external forces during practice and games. Vincent et al. described the protective role of the α-actinin-3 protein (complete deficiency in XX individuals) in muscle damage after eccentric training. In addition, the XX individuals exhibited higher creatine kinase activity, α-actin concentration, and levels of cortisol than RR individuals among professional soccer players. Thus, individuals with the XX genotype, being completely deficient in α-actinin-3 protein, would have inferior function of skeletal muscles during the force generation of contraction or a low ability to recover from high-intensity intermittent exercise. Above all, these factors might determine some aspects of performance in wrestling matches.

Our data indicated that the I-W group was older and had more athletic experience than the N-W group. This might suggest that performance in wrestling games is individually influenced by age and athletic experience independent of genetic factors such as the ACTN3 genotype. To test this hypothesis, we must investigate these interactions in longitudinal studies.

Practical Applications and Conclusions

In summary, we found an inverse linear correlation between the frequency of the ACTN3 XX genotype and level of athletic performance. The data indicated that ACTN3 RR or RX genotypes are strongly correlated with athletic status of elite wrestlers in Japan.

In this study, however, we only examined the relationship between a genetic factor and athletic status in elite Japanese wrestlers. A recent report described the relationship between a combination of several polymorphisms and elite performance. Further studies will be required to clarify the possible role of other polymorphisms and the combination of ACTN3 and other polymorphisms and/or environmental factors in determining elite athletic performance across ethnic groups. We conclude that the assessment of several polymorphisms such as ACTN3 R577X can provide useful information (eg, talent selection and genotype-based customization of training) for athletes and coaches. Above all, XX individuals would have a lower ability to recover from high-intensity intermittent training than individuals with the R allele. Thus, it may be necessary for XX individuals to set up more recovery time between heavy training sessions, and in case of fatigue, ease the distribution of training protocols according to circumstances with the aim of avoiding overtraining or muscle injury.

Acknowledgments

The authors acknowledge the invaluable assistance of the Japan Olympic Committee (JOC), Japan Wrestling Federation (JWF), and wrestling team of Nippon Sport Science University. The cooperation of all the subjects and athlete coaches, and managers is greatly appreciated.

Reference


ACTN3 Genotype in Japanese Elite Wrestlers


