INVESTIGATING THE INFLUENCE OF ACTN3 R577X POLYMORPHISM ON SPEED - POWER RELATED PARAMETERS USING MOTION CAPTURE TECHNOLOGY

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Abstract

ACTN3 gene has been labeled as the 'gene of speed' due to the increased frequency of the R allele in various cohorts of elite sprint athletes compared to the general population. The R allele of the gene encodes the sarcomeric protein **α**-actinin-3 almost exclusively restricted to fast, glycolytic, type 2X fibers which are responsible for producing 'explosive', powerful contractions. Elite athletes who expressed **α**-actinin-3 protein (*ACTN3* RR genotype) have demonstrated faster sprint times compared to those who do not express it (*ACTN3* XX genotype). The present study aimed to provide an extensive biomechanical analysis to investigate the influence of *ACTN3* gene on, Squat Jump (SJ), Counter Movement Jump (CMJ), Drop Jump (DJ), and 5 m sprints by comparing maximal performance, velocity and acceleration, in jumps and sprints between Thai individuals with different *ACTN3* genotypes. Participants had a mean age of 24.2 years ± 3.86 years, a mean BMI of 23.53 ± 2.87 kg/m^2 and a mean systolic blood pressure of 121 ± 12.20 mmHg. The biomechanical data were obtained using a QTM motion analysis system and the genetic analysis was conducted with MiniAmp™ Cycler. Our results showed no statistically significant differences between ACTN3 genotypes. DJ height performance for the participants with *ACTN3 RR* genotype was 0.38 ± 0.08 m compared to 0.37±0.06 m for their *ACTN3 XX* counterparts. Our preliminary findings indicated a considerable variability in velocity and acceleration between participants. More participants are required to allow us to investigate the potential underlying *ACTN3* gene influence responsible for this variability.

Keywords: Genetics, Biomechanics, Performance, Jumps, Sprinting

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Introduction

For many years, scientists debated whether genes or environment were more important in a person's physical performance. In the early '70s, research into the effect of heredity on athletic performance was mostly conducted via the twin studies model (Klissouras, 1971). Based on this model genetic factors seem to be an important cause of the observed phenotypic variability in vertical jump performance with heritability estimates ranged between 60.8% and 87.3% for males and between 76.5% and 88.6% for females (Peeters et al., 2005). In other words, genetics could explain as much as 87.3% of the phenotypic variation in vertical jump performance and various other explosive speed related phenotypes. However, this approach was unable to identify specific genes that influence human performance variability. The discovery of polymerase chain reaction (PCR) in early '90s and the completion of the human genome project (HGP) in 2003 facilitated the identification of genetic variants associated with athletic performance.

One of these genes concerning athletic performance is the ACTN3 gene (North et al., 1999). This common null polymorphism (R577X) results in replacement of an arginine (R) residue with a premature stop codon (X) at amino acid 577. The ACTN3 gene is located on the long arm of chromosome 11(11q13-q14) and encodes for the **α**-actinin-3 protein (Beggs et al., 1992). This protein is present at Z line of sarcomeres of fast twitch muscle fibers. Homozygosity for the *ACTN3 R577X* variant (*ACTN3 577XX*) is common and results in complete deficiency of **α**-actinin-3 protein. An estimated 18% of the world population (~1.5 billion people) is completely deficient in **α**-actinin-3 protein. Case: control studies showed a higher frequency of *ACTN3 577RR* genotype in elite sprint/power athletes (Yang et al., 2003; Niemi and Majamaa, 2005; Papadimitriou et al., 2008) compared to the general population, suggesting that the production of the **α**-actinin-3 protein could be linked with higher levels of power/sprinting performance. Some recent cross: sectional studies have shown further evidence that **α**-actinin-3 deficiency (*ACTN3 577XX* genotype) is associated with lower athletic performance in elite power-oriented athletes (Papadimitriou et al., 2016) while others not (Ruiz et al., 2011). All these studies collected their data without the use of high precision laboratory equipment and showed contradictory results. Here, we sought to address these limitations and provide deeper insights into the influence of the ACTN3 R577X polymorphism on sprint performance by using accuracy motion capture technology.

No studies so far have provided an extensive biomechanical analysis to investigate the influence of ACTN3 gene on certain biomechanical parameters. Therefore, the objective of this study is to compare maximal velocity, acceleration and performance between *ACTN3 RR* vs. *ACTN3 XX* on moderately active South East Asians, during explosive jumps and sprints using motion analysis technology.

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Materials and methods

Subjects

Twenty healthy South East Asians (for ≥3 generations), with a Body Mass Index (BMI) between 18.5 and 30.6 kg/m^2, were included in the study to ensure both body composition and genetic homogeneity. Participants that answered 'yes' to any of the eight PAR-Q questions or suffered from medical conditions interfering with their ability to exercise such as high blood pressure, medical conditions that might indicate high risk of acute or chronic musculoskeletal injuries were excluded from the study. The mean values of the inclusion criteria of the participants were: 1) age ranging from 18-33 years, 2) BMI ranging from 18.5 to 30.6 kg/m^2 3) systolic blood pressure ranging from 104-137 mmHg, 4) diastolic blood pressure ranging from 60 to 89 mmHg. Professional power-oriented athletes, sprinters or individuals that performed regular sprint-power related exercise were excluded from our analysis. Only the *ACTN3 RR* and the *ACTN3 XX*, participants were analyzed in this study to ensure we compared the two homozygotic genotypes (i.e., RR vs. XX). All procedures were approved by the ethical committee of the Mahidol University.

Research Tools

Physical activity screening

The physical activity screening was used to determine if there were any fluctuations in habitual physical activity between participants, one week before commencing the study the participants' activity levels were accessed using the GPPAQ questionnaire.

The GPPAQ questionnaire

The General Practice Physical Activity Questionnaire (GPPAQ) was developed by Chatterjee, Chapman, Brannan, & Varney, (2017), it was used to examine physical activity fluctuations, for physical activity screening purposes and it provides four level of physical activity levels inactive, moderately inactive, active and moderate active.

The PAR-Q questionnaire

The Physical-Activity Readiness Questionnaire (PAR-Q) was developed by Thomas, Reading, & Shephard, (1992) was used as an exclusion criterion assessing the participants ability to engage with the physical exercise and the risk of injuries during exercise.

Nutrition consultation

To ensure adequate access to carbohydrate energy stores, participants were asked to consume an energy drink (1 to 1.5 g•kg^{-1} BM) before the commencement of the testing session, according to Australian Institute of Sport (AIS) guidelines (https://www.ausport.gov.

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au/ais/sports_nutrition), composed of mainly liquids and carbohydrates. Participants were also asked to refrain from caffeine and alcohol during and before all tests.

Procedures

The participants were asked to fill in the questionnaires and then they had their blood pressure, body weight and height measured. 100 - 200 ul of fingertip blood was obtained from each participant for the genetic analysis. Prior to the exercise intervention phase, all participants completed a general warm up for 5 minutes and dynamic stretching for 10 minutes. Then, the participants were asked to practice all types of jumps (SJ, CMJ & DJ) and sprints to familiarize themselves. After that, the participants were asked to rest for around 35 - 45 minutes before they proceeded with actual tests (as shown in Fig. 1).

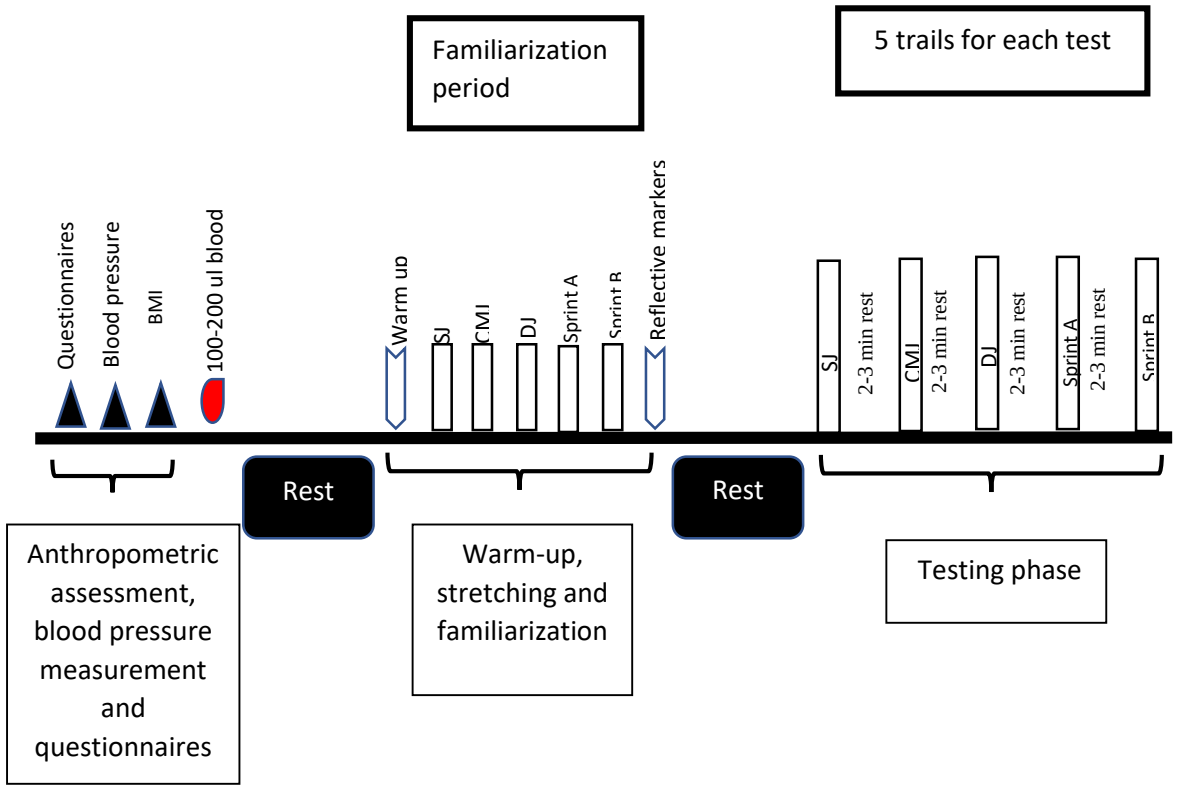

Figure 1 *Study overview – The participants were asked to fill in the PAR-Q & GPPAQ questionnaire and then they had their blood pressure, body weight and height measured for the calculation of BMI. 100 - 200 ul of fingertip blood was obtained from each participant. SJ: Squat jump; CMJ: countermovement jump; DJ: Drop jump; Sprint A: 5m sprint from the static initial position; Sprint B: 10 m sprint with velocity, acceleration and sprint time captured with the QTM motion analysis system at last 5 m of the sprint. After that, a warm up session took place with dynamic stretching followed by the familiarization phase which consisted of three types of jumps and two different types of sprints. Two reflective markers were attached with adhesive tape on anterior Superior Iliac Spine of left and right Ilium and*

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one reflective marker on sacrum. After that the participants rested for 35 - 40 minutes before the actual testing phase which was recorded with the QTS motion analysis system when the participants conducted five trails for each jump SJ, CMJ, DJ and sprints. Between each jump, 2 - 3 minutes rest was allowed for the participants

Two reflective markers were set on anterior Superior Iliac Spine of left and right Ilium and one reflective marker on sacrum. The maximal velocity, acceleration, jump height and sprint times were obtained and analyzed using Qualisys Track Manager (QTM) a motion analysis system. The QTM was used to collect and process motion data captured from eight cameras ensuring high accuracy and minimal latency, with at least two cameras covering each marker. QTM is designed to work seamlessly eight cameras in real - time ensuring fast and precise data collection. After the recording was finished, the 3D data were analyzed manually and exported for further statistical analysis. For the calibration a stationary object was used as a reference while a wand was moved around in the volume to define and coordinate the system for the capture.

Vertical Jumps

The vertical jumps were performed with simultaneous take - off from two legs and proper amortization when landing. All participants were instructed to complete three different types of vertical jumps and asked to perform 5 trials for each jump.

i. Squat jump (SJ) SJ were executed starting from a parallel 90° feet alignment. The starting position of the knee angle was measured with a handheld goniometer, followed by a restricted arm motion jump according to the procedure described by Papadopoulos et al., (2009). On completion of the SJ trials, the participants had five minutes recovery before moving into CMJ and DJ trials.

ii. Counter Movement Jump (CMJ) For CMJ trails, the participants started from the upright standing position performing a preliminary fast downward movement by flexing their knees and hips, then immediately extended their knees and hips again to jump vertically up off the ground (Papadopoulos et al., 2009).

iii. Drop Jump (DJ) For DJ trails, the participants had to stand on a 20 cm box and then they had to step off from the box and immediately jump up vertically after touching the ground (Papadopoulos et al., 2009).

After that, the participants were asked to rest around 30 - 45 seconds between each trail for the ATP-PCr system and glycolysis system to re-synthase the ATP (as shown in Fig. 1), before they were asked to perform 5 trials for two different types of sprints.

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Sprints

i. Sprint (A) The participants were asked to run with their maximal speed for 5 meters between two marker gates placed on the ground while being captured by QTM cameras.

ii. Sprint (B) The participants were asked to run at maximal effort for 10 meters sprints but only the last 5 meters of the sprint was captured by the cameras and further analyzed.

After testing, the participants were instructed to perform static stretching to cool down for about 10 minutes.

Genetic analysis

Genomic DNA was extracted from fingertip blood samples obtained from using the Biospin® Genomic Whole Blood DNA Purification Kit. The 291 - bp PCR fragment that contains the R577X polymorphism was amplified using MiniAmp™ Cycler, electrophoresed with E-Gel Electrophoresis System and visualized in UV light. The PCR conditions and the primers used as previously described (Papadimitriou et al., 2008). The amplified PCR fragments were subsequently digested with Invitrogen™ Anza™ 114 Hpy8 restriction endonuclease and the 577R and 577X alleles were distinguished by the presence (577R) or absence (577X) restriction site of 114 Hpy8 restriction site Digested products were then electrophoresed in 4% agarose gels (Figure 2).

Statistical analysis

The mean value and standard deviation were used for descriptive statistics. The comparison between the two groups (*ACTN3 RR* vs. *ACTN3 XX*) was performed with an independent T-test using SPSS 18. A value of $P< 0.05$ was considered statistically significant.

Results

A representative image of digested PCR products is indicating the three possible genotypes of the ACTN3 gene (Figure 2). Digestion of PCR products of the 577X allele yields bands of 108, 97 and 86 bp, whereas digestion of PCR products of the 577R allele gene yields bands of 205 and 86 bp.

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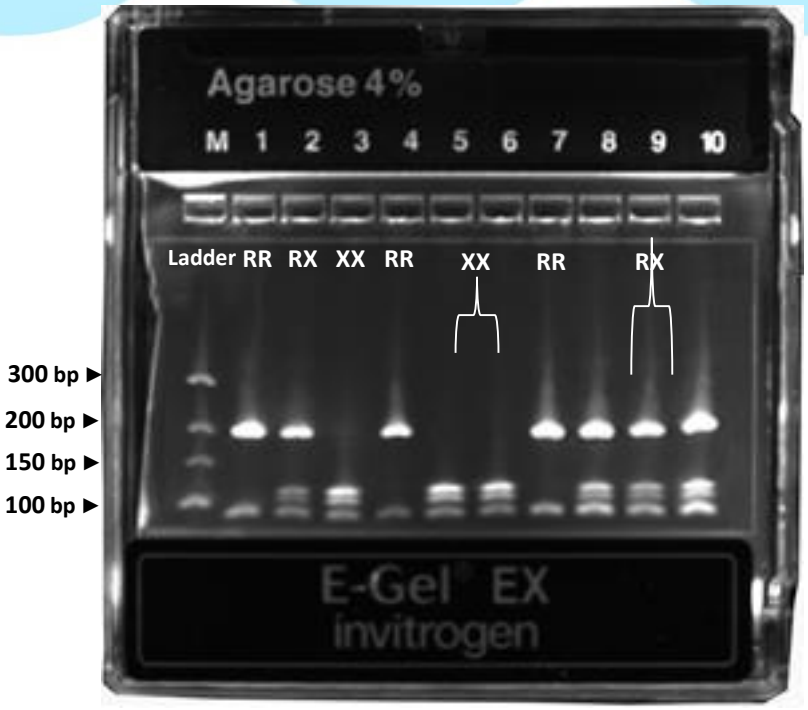

Figure 2 *A representative image of digested PCR products indicating the three possible ACTN3 genotypes.*

Our results showed that the genotype frequencies were 50% for the RX genotype, 25 % for the XX genotype and 25 % for the RR genotype.

Table 1. Biomechanical power-related parameters measured in the ACTN3 RR participants: maximal velocity, acceleration, jump height and running time. For the jumps, were measured maximal vertical velocity and acceleration at take - off while for the sprints, were measured the maximal horizontal running velocity achieved during the sprints. Sprint A was a 5m sprint from the static initial position while the Sprint B was a 10 m sprint but velocity, acceleration and sprint time captured with the QTS motion analysis system at last 5 m of the sprint.

	SJ	DJ	CMJ	Sprint A	Sprint B
Maximal Velocity	2.51 ± 0.56 m/s	2.57 ± 0.55 m/s	2.68± 0.43 m/s	5.18 ± 0.55 m/s	6.03 ± 0.49 m/s
Maximal Acceleration	28.62 ± 7.96 m/s^2	27.94 ± 7.95 m/s^2	28.37± 6.93 m/s^2	26.92 ± 6.86 m/ s^2	27.65 ±5.78 m/ s^2
Jump Height	0.35 ± 0.09 m	0.38 ± 0.08 m	0.39 ± 0.07 m/s^2	-	-
Running time	-	-		1.84 ± 0.08 sec	0.86 ± 0.08 sec

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Table 2. *Biomechanical power-related parameters measured in ACTN3 XX participants: maximal velocity, acceleration, jump height and running time. For the jumps were measured the maximal vertical velocity and acceleration at take - off while for the sprints, were measured the maximal horizontal running velocity achieved during the sprints. Sprint A was a 5m sprint from the static initial position while the Sprint B was a 10 m sprint but velocity, acceleration and sprint time captured with the QTS motion analysis system at last 5 m of the sprint.*

	SJ	DJ	CMJ	Sprint A	Sprint B
Maximal Velocity	2.48 ± 0.38 m/s	2.57 ± 0.48 m/s	2.74 ± 0.42 m/s	5.29 ± 0.36 m/s	6.15 ± 0.33 m/s
Maximal Acceleration	26.05 ± 4.33 m/s^2	26.97 ± 6.35 m/s^2	30.65 ± 3.72 m/s^2	30.21 ± 6.52 m/s^2	34.44± 7.09 m/s^2
Jump Height	0.35± 0.05 m	0.37± 0.06 m	0.40 ± 0.06 m	-	-
Running time	-	-	-	1.41 ± 0.47 sec	0.85 ± 0.06 sec

Individuals with *ACTN3 RR* genotype showed no statistically significant difference compared to *ACTN3 XX* counterparts in any of the tested variables. Our results show that those with *ACTN3 RR* genotype demonstrate higher maximal velocity at take - off in SJ (2.51 ± 0.56 m/s vs. 2.48 ± 0.38 m/s) and DJ height (0.38 ± 0.08 m vs. 0.37± 0.06 m) compared to their *ACTN3 XX* counterparts (Table 1 & 2).

Discussion

The present study was to provide an extensive biomechanical analysis to investigate the influence of *ACTN3* gene on, Squat Jump (SJ), Counter Movement Jump (CMJ), Drop Jump (DJ), and 5 m sprints by comparing maximal performance, velocity and acceleration, in jumps and sprints between Thai individuals with different *ACTN3* genotypes. As for results, demonstrate no statistically significant differences between jump height or sprint time performance between ACTN3 genotypes. This finding is consistent with what some other studies have found (Ruiz et al., 2011). Moreover, there were no detected statistically significant differences in maximal velocity and acceleration, between ACTN3 genotypes. Even though our study was tightly controlled and high accuracy motion capture technology was utilized for the data collection we still observe significant variability in all biomechanical parameters (Table 1 & 2). More participants are required to give us the statistical power to

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investigate our hypothesis about a possible influence of the ACTN3 gene in power-related biomechanical parameters such as maximal velocity and acceleration.

Furthermore, although there was no statistically significant jump height difference in CMJ, SJ and DJ between participants with different ACTN3 genotypes, those with the *ACTN3 RR* genotype demonstrated higher DJ compared to their *ACTN3 XX* counterparts (Table 1 & 2). The reason for this finding could be the large time differences in stretch - shortening cycle SSC between CMJ and SJ, > 250 milliseconds vs. DJ < 250 milliseconds, respectively (Papadopoulos et al., 2009). *ACTN3 RR* genotype has been related to speed in previous studies (Moran et al., 2007; Papadimitriou et al., 2016) thus; this particular genotype is expected to have a more pronounced effect on jumps at higher speed such as DJ compared to slower speed jumps such as CMJ or SJ. Consistent with this observation participants with *ACTN3 RR* genotype showed higher values in maximal velocity at take-off during SJ compared to their *ACTN3 XX* counterparts indicating that further research to test this hypothesis is required.

Variables such as maximal velocity and acceleration that are considered key factors for achieving high levels of sprint/power performance have not been analyzed in the previous studies. To date, the influence of specific genetic polymorphisms on jumping and sprinting performance has been studied only in terms of maximal performance in jump height (Ruiz et al., 2011; Broos et al., 2015; Garatachea et al., 2014) or running times in adolescents (Moran et al., 2007), moderately trained individuals (Santiago et al., 2010) or elite sprinters (Papadimitriou et al., 2016). All these studies collected their data without the use of high precision laboratory equipment and showed contradictory results. Our approach using high accuracy motion analysis techniques has the potential to clarify and further investigate the influence of the ACTN3 gene on specific biomechanical parameters such as maximal velocity, acceleration in South East Asians.

Conclusion

Our preliminary results based on twenty samples, show promising evidence and suggest that the influence of the ACTN3 gene on certain biomechanical parameters using motion capture technology merits further study. We highlight that larger cohorts are urgently needed for adequate genetics - biomechanics interaction assessments. We are currently analyzing more data, in order to increase the statistical power of our cohort.

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