INVESTIGATING THE INFLUENCE OF ACTN3 R577X POLYMORPHISM ON ANGULAR KINEMATICS USING MOTION CAPTURE TECHNOLOGY

Pongpanot Wongveerakul[1], Thet Hnin Moe[1], Vitoon Saengsirisuwan[1], Nongnapas Charoenpanich[2], and Ioannis Papadimitriou[1]

[1]Department of Physiology, Faculty of Science, Mahidol University

[2]Human Movement Performance Enhancement Research Unit, Faculty of Sports Science, Chulalongkorn University

Abstract

ACTN3 has been labeled as the 'gene for speed' due to the increased frequency of the R allele encoding the **α** – actinin - 3 protein in elite sprint athletes compared to the general population. In situ muscle fibers that express **α** – actinin - 3 protein can produce more force than the muscle fibers that are **α** – actinin - 3 deficient (*ACTN3 XX* genotype). In vivo, using single joint isokinetic dynamometry assessments individuals with *ACTN3 RR* genotype demonstrated higher peak torque at all angular speeds (30 - 180°/s) than those with *ACTN3 XX* genotype. However, no study so far has investigated the influence of the ACTN3 gene on angular velocity and acceleration during vertical jumps using motion capture technology. The aim of this study was to investigate the influence of the ACTN3 gene on Squat Jump (SJ), by comparing maximal angular velocity and acceleration in hip and knee joint between *ACTN3 RR* and *ACTN3 XX* homozygotes. The biomechanical data were obtained using Qualisys Track Manager (QTM) motion analysis system and the DNA was isolated from white blood cells. The 291 - bp PCR fragment was electrophoresed, visualized in UV light and finally digested with Hpy8 restriction endonuclease. No statistically significant differences were observed; However, *ACTN3 RR* homozygotes demonstrated a trend towards higher maximal angular velocity and acceleration at take-off during SJ jumps (RR 4533.61 ± 1067.62 deg/s^2 vs. XX 3183.53 ± 1695.99 deg/s^2) in hip joint compared to their *ACTN3 XX* counterparts. This study suggests that more participants are required to investigate the potential underlying ACTN3 gene effect on angular kinematics during explosive movements such as vertical jumps.

Keywords: Genetics, Biomechanics, Performance, Jumps, Velocity, Acceleration

Corresponding author: Dr. Ioannis Papadimitriou, Department of Physiology, Faculty of science, Mahidol university, Email: Ioannis.pap@mahidol.ac.th

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Introduction

In the last few decades, advances in genome sequencing has provided unprecedented access to the human genome and enabled the identification of specific sequence variations among individuals. Humans share 99.5% identical DNA implying that the resulting phenotypic diversity stems from the remaining 0.5% difference as well as epigenetic modifications (Robert, & Pelletier, 2018). Sequence differences arise due to the presence of insertion or deletion polymorphisms, and single-nucleotide polymorphisms (SNPs). Among SNPs, transitions (A ↔ G or C ↔ T) are more prevalent than transversions (A ↔ C or T; and G ↔ C or T). There are at least 10 million SNPs within the genome, occurring approximately every 100–300 base pairs making these by far the most common variant type in the human genome (Lander et al., 2001; Robert & Pelletier, 2018). Recently, there has been a great interest in genome-wide association studies (GWAS) where the prevalence of specific SNPs were linked to various phenotypes (Willems et al., 2017).

One of these SNPs with respect to athletic performance is the ACTN3 R577X single nucleotide polymorphism (North, Yang, Wattanasirichaigoon, Mills, Easteal, & Beggs, 1999). This null polymorphism (*R577X*) is a common transversion (C⟶T) in exon 15 of the ACTN3 gene that converts an arginine residue (R) to a stop codon (X) at amino acid position 577. Homozygosity for the *ACTN3 577X* variant (*ACTN3 577 XX*) is common (~18% of the world population). A recent study demonstrated a complete deficiency of **α** - actinin 3 protein in muscle biopsy samples of *ACTN3 XX* humans (Papadimitriou et al., 2019). An increased frequency of the *ACTN3 577R* variant (*ACTN3 577RR*) was initially detected in elite sprint/power Australian (Yang, MacArthur, Gulbin, Hahn, Beggs, Easteal, & North, 2003), Finish (Niemi, & Majamaa, 2005) and Greek (Papadimitriou, Papadopoulos, Kouvatsi, & Triantaphyllidis, 2008) elite athletes compared to the general population. Moreover, elite sprinters that carry at least one *R* allele of the ACTN3 gene can run faster compared to **α**-actinin-3 deficient *ACTN3 XX* homozygotes (Papadimitriou et al., 2016).

In situ muscle fibers that express **α** – actinin - 3 protein can produce more force than muscle fibers that are **α** – actinin - 3 deficient (Broos et al., 2016). Furthermore, a recent study using isokinetic dynamometry assessments has shown that the individuals with the *ACTN3 XX* genotype demonstrated lower peak torque at all angular speeds (30 - 180 deg/s) in knee joint than those with the *ACTN3 RR* genotype (Walsh, Liu, Metter, Ferrucci, & Roth, 2008). Isokinetic dynamometry assessments are limited to single joint tasks with fixed angles of movement and no study so far has conducted to provide an extensive biomechanical analysis about the possible effect of the ACTN3 gene on angular velocity and acceleration during dynamic multi-joint movements.

Therefore, the aim of this study is to investigate the influence of the ACTN3 gene on jumping ability by comparing maximal angular velocity and acceleration during SJ jumps in hip and knee joint between individuals with *ACTN3 RR* and *ACTN3 XX* genotypes using motion capture technology.

Materials and methods

Subjects

More than 30 participants were recruited from the Greater Mekong Sub - region (GMS). Informed consent was obtained prior to a subject's participation in the research project. After screening for ACTN3 genotype, only the ACTN3 RR and the ACTN3 XX participants were included in this study to ensure we compared the two homozygotic genotypes (i.e., RR vs. XX) according to the study design described by Papadimitriou et al., (2019). Seven Thai and one from Myanmar (for ≥3 generations), with a Body Mass Index (BMI) mean 23.34 ± 2.44 $kg \bullet m^{-2}$, were included to this study to ensure both body composition and genetic homogeneity. The eight subjects were further divided into two groups. The first group included four participants with *ACTN3 RR* genotype (produce **α** - actinin3 protein) while the second group which served as control group included four participants with *ACTN3 XX* genotype that were completely **α** - actinin 3 deficient.

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 - 35 years, 2) BMI ranging from 18.5 to 30.6 kg/m^2 3) systolic blood pressure ranging from 104 - 137 mm/hg, 4) diastolic blood pressure ranging from 60 to 89 mm/hg. Professional power-oriented athletes, sprinters or individuals that performed regular sprint-power related exercise were excluded from our analysis. All procedures were approved by the ethical committee of the Mahidol University.

Research Tools

Preparation

The Physical - Activity Readiness Questionnaire (PAR - Q) developed by (Thomas, Reading, & Shephard, 1992) was used as an exclusion criterion to assess participants' ability to engage with the physical exercise and minimize the risk of injuries during exercise. Participants were asked to fill in the PAR - Q questionnaire before they had their body weight and height measured for the calculation of BMI.

Furthermore, a sphygmomanometer (Automatic Blood Pressure Monitor JPN1, made in Japan) was used for measuring participants' resting blood pressure levels while sitting on a chair.

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All participants' blood pressure was within normal range with the systolic ranging from 104 to 137 mm/hg and diastolic blood pressure ranging from 60 to 89 mm/hg.

Moreover, 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 the Australian Institute of Sport (AIS) guidelines (Burke et al., 2006) composed of mainly liquids and carbohydrates. Participants were also asked to refrain from caffeine and alcohol during and before all tests.

Finally, 50 - 200 ul of fingertip blood was obtained from each participant using capillary tubes. Immediately after the blood collection all samples were vortexed and stored on ice in EDTA blood collection tubes.

Familiarization

The warm-up was consisted of three minutes jogging followed a followed by dynamic stretching. After that participants familiarized themselves with Squat jump (SJ), a form of vertical jump from 90° angle and 11 reflective markers were attached on certain anatomical locations of their lower limps as shown in Figure 2, with adhesive tape. Finally, the participants rested for 15 - 20 minutes before the actual testing phase.

Vertical Jumps

The five SJ jumps were executed starting from a parallel 90° feet alignment with simultaneous take - off from two legs and proper amortization when landing while recorded using Qualisys Track Manager (QTS) motion analysis system. All participants were instructed to perform five trials for each jump. The starting position of the knee angle was measured with a handheld goniometer, followed by a restricted arm motion jump as it is demonstrated in Figure 2, according to the procedure described by (Papadopoulos et al., 2009). The SJ jump with the highest linear velocity at the propulsion phase was further analyzed for angular kinematics (velocity & acceleration) in hip and knee joints using QTM.

QTM Analysis

The SJ jump with the highest velocity at take-off was further analyzed for angular kinematics (velocity & acceleration) in hip and knee joints using QTM Track Manager Software for analyzing maximal velocity and acceleration were obtained and analyzed using QTM motion analysis system. The QTM was used to collect and process motion data captured from nine cameras ensuring high accuracy and minimal latency, with at least two cameras covering each marker. The reflection of eleven markers was received and analyzed during jumping by the software. QTM is designed to work seamlessly nine cameras in real-time ensuring fast and precise data collection. After the recording was finished, the 3D angular kinematic data from hip and knee

joint 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.

Genotyping

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 Anza™ 114 Hpy8 restriction endonuclease **(**Thermo Fisher Scientific, CA, USA) 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 as shown in Figure 3. Anza™ 114 Hpy8 restriction endonuclease that can cleave the DNA specific C-T site of the amplified PCR product enable us to identify the three possible genotypes of ACTN3 gene. Representative images of ACTN3 RR genotype (2 bands), ACTN3 RX genotype (4 bands) and ACTN3 XX genotype (3 bands) are shown in Figure 3.

Procedures

As shown in Figure 1 the participants initially were asked to fill in the PAR - Q questionnaire, their blood pressure, and body weight & height were measured as well as they were asked to consume an energy drink (1 to 1.5 g•kg−1 BM) before 50 - 200 ul of fingertip blood was obtained from each participant. Prior to the exercise intervention phase, all participants completed a general warm up for 15 minutes and familiarized themselves with vertical jumps by practicing squat jumps (SJ) from a 90^0 fixed starting position. After that, the participants were asked to rest for around 15 - 20 minutes before they proceeded with the actual five recorded SJ jumps trials. After data collection the highest take - off velocity jump was further analyzed for angular kinematics, and the extracted DNA was stored in -20 $^{\circ}$C for the subsequent genetic analysis as described above.

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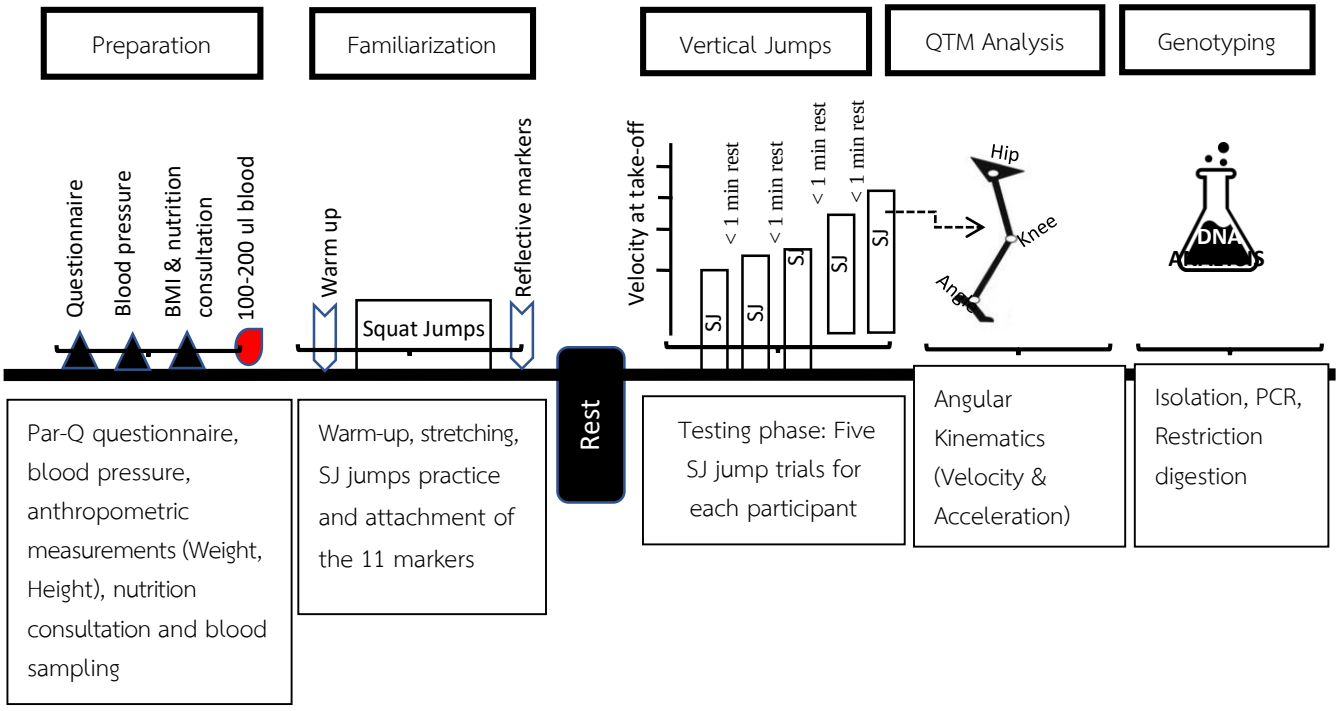

Figure 1 *Study overview*

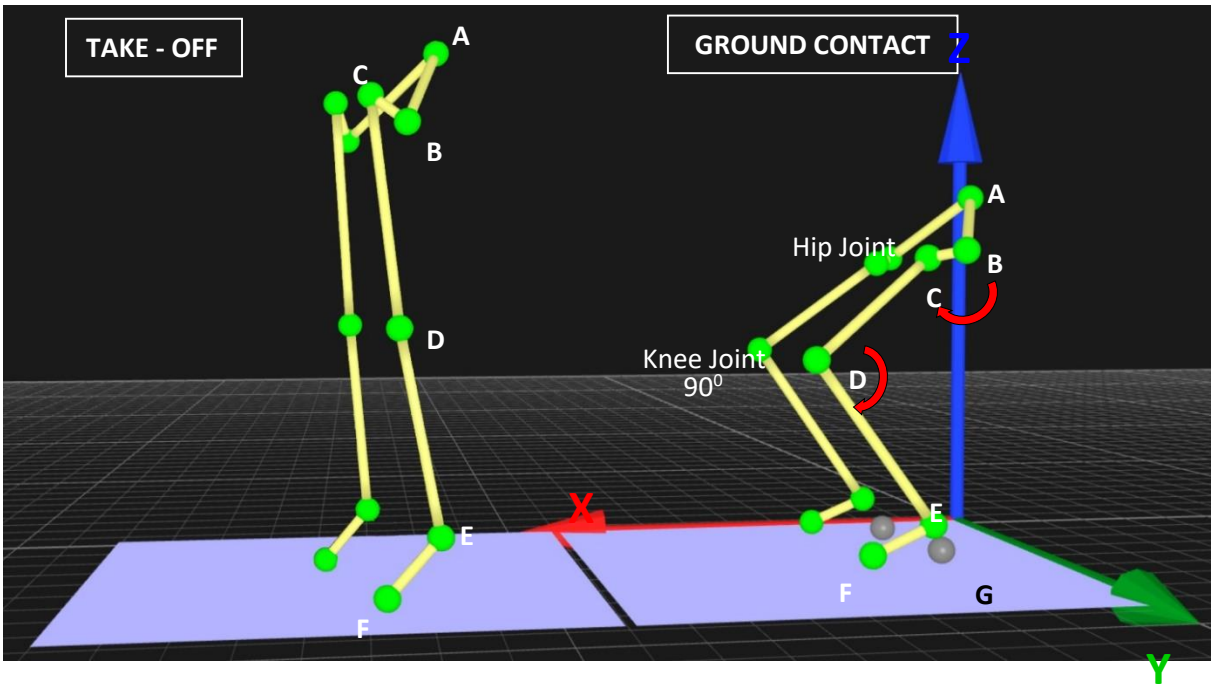

Figure 2 *An overview of the 11 skin-marker model: (A) Sacrum, (B) Ilium - Anterior Superior Iliac Spine, (C) Femur - Greater Trochanter, (D) Femur – Lateral condyle, (E) Fibula - Apex of the Lateral Malleolus and (F) Distal Foot - 5th Metatarsal. Point (G) demonstrates heel ground contact. Maximal acceleration and velocity at take-off in hip (BCD) and knee (CDE) joints were calculated by QTM.*

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

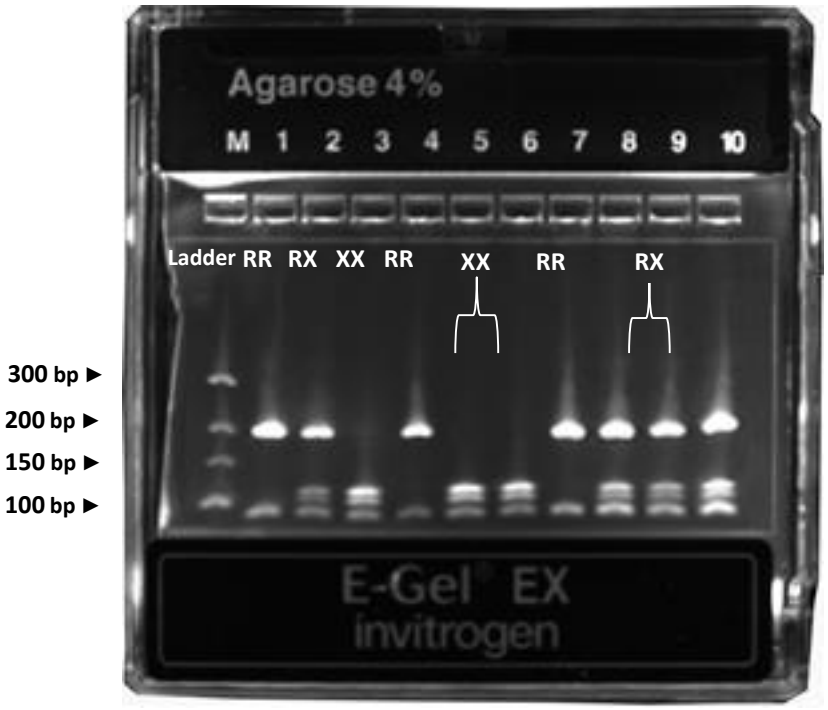

Figure 3 *A representative image of digested PCR products indicating the three possible ACTN3 genotypes.*

Figure 3 shows that the genotype frequencies were 50% for the *ACTN3 RX* genotype, 25 % for the *ACTN3 XX* genotype and 25 % for the RR genotype.

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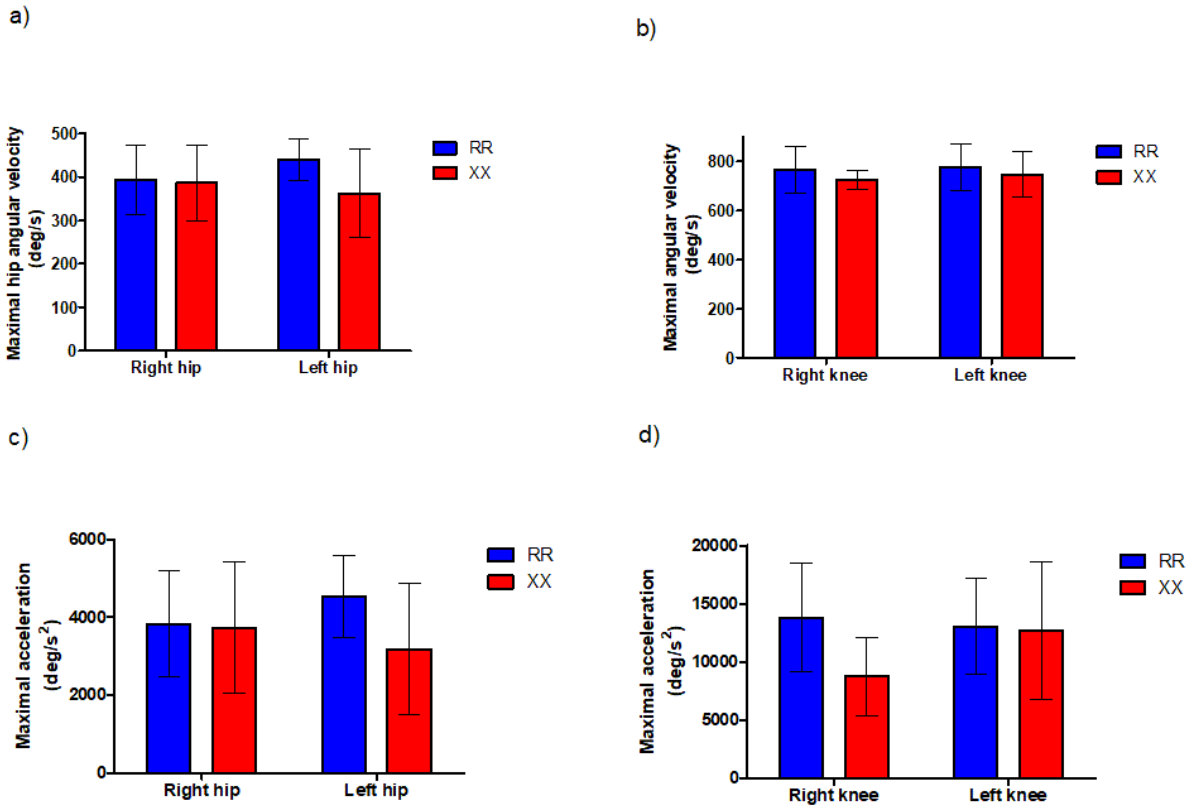

Figure 4 ***a)*** *Right and left hip maximal angular velocity between ACTN3 RR and ACTN3 XX participants achieved during the propulsion phase of SJ jumps.* ***b)*** *Right and left knee maximal angular velocity between ACTN3 RR and ACTN3 XX participants achieved during the propulsion phase of SJ jumps.* ***c)*** *Right and left hip maximal angular acceleration between ACTN3 RR and ACTN3 XX participants achieved during the propulsion phase of SJ jumps.* ***d)*** *Right and left knee maximal angular acceleration between ACTN3 RR and ACTN3 XX participants achieved during the propulsion phase of SJ jumps.*

Figure 4 shows no statistically significant differences between *ACTN3 RR* and *ACTN3 XX* genotypes. However, participants with the *ACTN3 RR* genotype demonstrated a trend towards higher maximal angular velocity and acceleration at take - off during SJ jumps (RR 4533.61 ± 1067.62 deg/s2 vs. XX 3183.53 ± 1695.99 deg/s2) in left hip joint compared to their *ACTN3 XX* counterparts.

Discussion

The present study was to investigate the influence of *ACTN3* gene on jumping ability, by comparing maximal angular velocity and acceleration in hip and knee joints, during the propulsion phase of SJ jumps. As for results, demonstrate no statistically significant differences in maximal

angular velocity or acceleration, between South East Asians with different *ACTN3* genotypes. However, *ACTN3 RR* homozygotes showed a trend towards higher maximal angular velocity and acceleration at the propulsion phase of SJ jumps compared to *ACTN3XX* counterparts. This observation is consistent with recent studies results that showed that *ACTN3 RR* individuals demonstrate higher peak torque at higher angular speeds (30 - 180 deg/s) in knee joint compared to those with the *ACTN3 XX* genotype using isokinetic dynamometry assessments (Walsh et al., 2008). Isokinetic dynamometers are devices which resist applied forces and control the angular velocity of a specific joint at a predetermined rate. Thus, assessments using isokinetic dynamometry are limited to single joint tasks with fixed angle of movement. On the other hand, our methodological approach using motion analysis technology allows us to assess various angular kinematic characteristics such as velocity and acceleration during multi - joint type of movements such as vertical jumps. Given the fact that SJ jump is considered a functional indicator for explosive muscle power of leg extensor muscles, this detected trend allows us to hypothesize a probable prominent effect of **α** – actinin - 3 protein in extensor muscle group kinematic chain during vertical jumps. However, more participants are required to give us the statistical power to investigate this hypothesis.

Furthermore, recent evidence indicates an influence of ACTN3 gene on the preservation of force with increasing muscle contraction velocity rather than on force itself in vivo (Broos, Van Leemputte, Deldicque, & Thomis, 2015). Based on ground contact time, SJ jumps have been described as slow > 250 milliseconds while Drop Jumps (DJ) as fast < 250 milliseconds, respectively (Papadopoulos et al., 2009). To perform a DJ jump you stand on a box, step off, hit the ground, and immediately jump up as high as possible at a very short ground contact time and increased muscle contraction velocity combining a rapid coupling between an eccentric and concentric muscle action, commonly referred as stretch-shortening cycle (SSC). Taken together, these findings lead us to hypothesize that the ACTN3 R577X genotype could have a more pronounced effect on jumps that take place at higher muscle contraction velocity such as DJ jumps compared to slower jumps such as SJ jumps analyzed in this study. Consistent with this hypothesis our preliminary unpublished data show that participants with *ACTN3 RR* genotype demonstrate higher angular velocity values in DJ jumps compared to their *ACTN3 XX* counterparts indicating that further research towards this direction is required.

Variables such as maximal angular velocity and acceleration during jumps that are considered key factors for achieving high levels of power performance have not been analyzed in the previous studies. To date, in humans the influence of specific genetic polymorphisms on angular velocity has been studied only using isokinetic dynamometry assessments (Walsh et al., 2008) or in terms of maximal performance in jumps (Ruiz et al., 2011); (Broos et al., 2015; Garatachea et al., 2014) or sprints (Papadimitriou et al., 2016). All these studies have either been based on a single - joint

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analysis or on data collected without the use of precision laboratory equipment and have had mixed results. Some studies have found a possible link between *ACTN3 RR* genotype and jumping ability, while others have not. Our multi - joint 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 angular velocity and acceleration during vertical jumps in South East Asians.

Conclusion

Our preliminary results based on eight samples, suggest that the influence of the ACTN3 gene on angular kinematics using motion capture technology merits further study. We highlight that larger cohorts are urgently needed for adequate genetics - biomechanics interaction assessments. We are currently recruiting more participants and analyzing more data, including various other phenotypes such as DJ jumps in order to increase the phenotypic specificity and statistical power of our cohort.

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References

Broos, S., Malisoux, L., Theisen, D., van Thienen, R., Ramaekers, M., Jamart, C., Deldicque, L., Thomis, M. A., & Francaux, M. (2016). Evidence for ACTN3 as a speed gene in isolated human muscle fibers. *PLoS One, 11*(3). doi:10.1371/journal.pone.0150594

Broos, S., Van Leemputte, M., Deldicque, L., & Thomis, M. A. (2015). History - dependent force, angular velocity and muscular endurance in ACTN3 genotypes. *Eur J Appl Physiol, 115*(8), 1637 - 1643. doi:10.1007/s00421-015-3144-6

Burke, L., Cort, M., Cox, G., Crawford, R., Desbrow, B., Farthing, L., Minehan, M., Shaw, N., & Warnes, O. (2006). *Supplements and Sports Foods* (3rd ed.). Sydney, Australia: McGraw-Hill Education.

Garatachea, N., Verde, Z., Santos-Lozano, A., Yvert, T., Rodriguez-Romo, G., Sarasa, F. J., Hernandez-Sanchez, S., Santiago, C., & Lucia, A. (2014). ACTN3 R577X polymorphism and explosive leg-muscle power in elite basketball players. *Int J Sports Physiol Perform, 9*(2), 226 - 232. doi:10.1123/ijspp.2012-0331

Lander, E. S., et al. (2001). Initial sequencing and analysis of the human genome. *Nature, 409*.

Niemi, A.-K., & Majamaa, K. J. E. J. o. H. G. (2005). Mitochondrial DNA and ACTN3 genotypes in Finnish elite endurance and sprint athletes. *Eur J Hum Genet, 13*(8), 965 - 969.

North, K. N., Yang, N., Wattanasirichaigoon, D., Mills, M., Easteal, S., & Beggs, A. H. J. N. g. (1999). A common nonsense mutation results in α-actinin-3 deficiency in the general population. *Nat Genet, 21*(4), 353 - 354.

Papadimitriou, I. D., Eynon, N., Yan, X., Munson, F., Jacques, M., Kuang, J., Voisin, S., North, K. N., & Bishop, D. J. (2019). A "human knockout" model to investigate the influence of the alpha-actinin-3 protein on exercise-induced mitochondrial adaptations. *Sci Rep, 9*(1), 12688. doi:10.1038/s41598-019-49042-y

Papadimitriou, I. D., et al. (2016). ACTN3 R577X and ACE I/D gene variants influence performance in elite sprinters: A multi-cohort study. *BMC Genomics, 17*, 285. doi:10.1186/s12864-016-2462-3

Papadimitriou, I. D., Papadopoulos, C., Kouvatsi, A., & Triantaphyllidis, C. (2008). The ACTN3 gene in elite Greek track and field athletes. *Int J Sports Med, 29*(4), 352 - 355. doi:10.1055/s-2007-965339

Papadopoulos, C., Sambanis, M., Gissis, I., Noussios, G., Gandiraga, E., Manolopoulos, E., & Papadimitriou, D. I. (2009). Evaluation of force and vertical jump performance in young swimmers with different force-time curve characteristics. *Biology of Sport, 26*(4), 301 - 307. doi:10.5604/20831862.901135

Robert, F., & Pelletier, J. (2018). Exploring the impact of single - nucleotide polymorphisms on translation. *Front Genet, 9*, 507. doi:10.3389/fgene.2018.00507

Ruiz, J. R., Fernandez del Valle, M., Verde, Z., Diez-Vega, I., Santiago, C., Yvert, T., Rodriguez -Romo, G., Gomez-Gallego, F., Molina, J. J., & Lucia, A. (2011). ACTN3 R577X polymorphism does not influence explosive leg muscle power in elite volleyball players. *Scand J Med Sci Sports, 21*(6), e34 - e41. doi:10.1111/j.1600-0838.2010.01134.x

Thomas, S., Reading, J., & Shephard, R. J. (1992). Revision of the physical activity readiness questionnaire (PAR - Q). *Can J Sport Sci, 17*(4), 338 - 345.

Walsh, S., Liu, D., Metter, E. J., Ferrucci, L., & Roth, S. M. (2008). ACTN3 genotype is associated with muscle phenotypes in women across the adult age span. *J Appl Physiol (1985), 105*(5), 1486 - 1491. doi:10.1152/japplphysiol.90856.2008

ISSN 2673-0952 (PRINT)
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Willems, S. M., et al. (2017). Large-scale GWAS identifies multiple loci for hand grip strength providing biological insights into muscular fitness. *Nat Commun, 8*, 16015. doi:10.1038/ncomms16015

Yang, N., MacArthur, D. G., Gulbin, J. P., Hahn, A. G., Beggs, A. H., Easteal, S., & North, K. J. T. A. J. o. H. G. (2003). ACTN3 genotype is associated with human elite athletic performance. *Am J Hum Genet, 73*(3), 627 - 631.

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