Differential muscle damage response of ACTN3 R577X polymorphism in male adolescent badminton athletes

Respuesta diferencial al daño muscular del polimorfismo ACTN3 R577X en atletas adolescentes masculinos de bádminton

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Abstract

Background: Badminton's explosive movements carry a high risk of muscle injury. This study investigates the relationship between the ACTN3 R577X polymorphism and muscle damage biomarkers Creatine Kinase (CK) and myoglobin in male adolescent badminton athletes following the Badminton Fatigue Test (BFT).
Methods: This longitudinal observational study utilized a pre-post design involving 30 male athletes. Participants were categorized by ACTN3 genotype into RR (n=8), RX (n=13), and XX (n=9) groups before undergoing the BFT to induce muscle damage. Biomarker levels were evaluated at three time points: pre-test, 1-hour post-BFT, and 24-hours post-BFT.
Results: Repeated-measures ANOVA revealed a significant increase in CK and myoglobin ($p<0.001$) across all gene variants at both post-BFT time points. A two-way ANOVA showed no significant interaction between genotype and time for CK levels ($p=0.404$), but indicated a significant difference for myoglobin ($p=0.038$). Although CK differences lacked statistical significance, Cohen's d analysis demonstrated a moderate-to-large effect size (0.8–1.4), indicating higher CK and myoglobin responses in the XX variant (α-actinin-3 deficiency) compared to the other genotypes at both post-test points.
Conclusion: The absence of the α-actinin-3 protein (XX genotype) is associated with a substantially greater muscle damage response. This is of practical importance, as evidenced by the statistically significant increase in myoglobin levels following induced fatigue.

Keywords

ACTN3; ACTN3 R577X polymorphism; badminton; muscle damage.

Resumen

Introducción: Los movimientos explosivos del bádminton conllevan un alto riesgo de lesión muscular. Este estudio investiga la relación entre el polimorfismo ACTN3 R577X y los biomarcadores de daño muscular creatina quinasa (CK) y mioglobina, en atletas adolescentes masculinos de bádminton tras la Prueba de Fatiga de Bádminton (BFT, por sus siglas en inglés).
Metodología: Este estudio observacional longitudinal utilizó un diseño pre-post con la participación de 30 atletas masculinos. Los participantes fueron categorizados según su genotipo ACTN3 en los grupos RR (n=8), RX (n=13) y XX (n=9) antes de someterse a la BFT para inducir daño muscular. Los niveles de biomarcadores se evaluaron en tres momentos: pre-prueba, 1 hora post-BFT y 24 horas post-BFT.
Resultados: El ANOVA de medidas repetidas reveló un aumento significativo en la CK y la mioglobina ($p<0,001$) en todas las variantes genéticas en ambos puntos temporales post-BFT. Un ANOVA de dos vías no mostró una interacción significativa entre el genotipo y el tiempo para los niveles de CK ($p=0,404$), pero indicó una diferencia significativa para la mioglobina ($p=0,038$). Aunque las diferencias en la CK carecieron de significancia estadística, el análisis de la d de Cohen demostró un tamaño del efecto de moderado a grande (0,8–1,4), lo que indica mayores respuestas de CK y mioglobina en la variante XX (deficiencia de α-actinina-3) en comparación con los otros genotipos en ambas mediciones posteriores a la prueba.
Conclusiones: La ausencia de la proteína α-actinina-3 (genotipo XX) está asociada con una respuesta de daño muscular sustancialmente mayor. Esto es de importancia práctica, como lo demuestra el aumento estadísticamente significativo en los niveles de mioglobina tras la fatiga inducida.

Palabras clave

ACTN3; bádminton; daño muscular; polimorfismo ACTN3 R577X.

Introduction

Badminton is a sport that significantly depends on skill, fine motor coordination, and precision in racket swinging (Pardiwala et al., 2020). The sport requires intermittent physical activity and demands a combination of explosive strength, speed, and endurance, with movements include eccentric contractions such as lunges, landing after a jump, decelerating the arm after a smash, and thigh muscle movements during backpedaling which increase the risk of muscle injury (Phomsoupha & Laffaye, 2015; Pickering & Kiely, 2017). The change in the badminton scoring system by the Badminton World Federation (BWF) to rally points in 2006 led to longer match durations, significantly longer rallies, and an increased number of shots per rally (Iizuka et al., 2020). This shift contributed to the rise in acute injury among female badminton athletes (Iizuka et al., 2020). Therefore, the high physical demands of professional badminton showed the need for an effective recovery planning strategy to prevent fatigue buildup and reduce injury risk. To address these demands and ensure optimal recovery, systematic training load monitoring has become a crucial aspect of modern sports science to objectively assess athlete readiness and predict potential injury risks (Hasan et al., 2024).

The average number of injuries in professional badminton athletes ranges between 1 and 4 per 1,000 hours of participation, with the vast majority (41-92%) occurring in the lower body (Stepper et al., 2025). These injuries primarily include muscle strains (11%-64%), ligament sprains, and tendinopathy, which are exacerbated by high competition levels and dense tournament schedules. Junior and elite athletes often compete in numerous tournaments annually, with matches played simultaneously every day for 4-5 days. While regular athletes may not experience significant muscle damage after a single match (Marchena-Rodriguez et al., 2020), the cumulative effect of eccentric loads over consecutive days without adequate recovery significantly elevates the risk of non-contact muscle injuries.

Beyond external workload, intrinsic genetic factors play a crucial role in an athlete's susceptibility to exercise-induced muscle damage. The ACTN3 R577X polymorphism, particularly the XX variant, has been identified as a significant factor in muscle performance and injury risk. This variant results in a premature stop codon, preventing the production of functional α-actinin-3 protein (Abián-Vicén et al., 2022). Normally expressed only in type II (fast-twitch) muscle fibers, this protein acts as an essential anchor at the Z-line of the sarcomere, maintaining structural and functional stability during intense and rapid muscle contractions. The deficiency of α-actinin-3 alters the protein makeup of the Z-line, forcing α-actinin-2 to compensate. Consequently, this structural alteration compromises the muscle's ability to withstand strong eccentric forces, making athletes with the XX variant more susceptible to microtrauma and severe muscle damage following intensive training and back-to-back matches.

The structural instability of the Z-line in α-actinin-3 deficient individuals directly impacts muscle cell integrity under mechanical stress. When the sarcomere is overstretched during explosive badminton movements, sarcolemma damage occurs, leading to a loss of cell membrane integrity (Stožer et al., 2020). This microtrauma provides a pathway for intracellular proteins, specifically Creatine Kinase (CK) and myoglobin, to leak into the bloodstream. Elevated levels of these biomarkers post-exercise serve as objective evidence of muscle fiber damage (Brancaccio et al., 2007, 2010). Despite evidence showing that none of the top ten professional badminton athletes in the 2018 European Championship possessed the XX variant (Abián-Vicén et al., 2022), suggesting a performance disadvantage and higher injury risk, no current study has analyzed the differential muscle damage responses among ACTN3 gene variants in male adolescent badminton athletes. Therefore, this study aimed to analyze the specific muscle damage response of the ACTN3 R577X polymorphism in this demographic. The induction of muscle damage was conducted using the Badminton Fatigue Test (BFT), adapted and modified from Huang (Huang et al., 2019), to provide critical insights for developing targeted, rapid muscle recovery strategies.

Method

This study used pre-post observational design to measure variables over a specific time scale (pre-post) and determine differences in muscle damage response between ACTN3 gene variants. The assessment

was conducted at three time points which were pre-, 1 hour, and 24 hours after BFT in male adolescent badminton athletes using a quantitative method.

Participants

A total of 30 male adolescent badminton athletes who regularly trained at PB Taqi Arena, Cimahi, Indonesia was selected as participants using purposive sampling based on specific criteria. The inclusion criteria were males, aged 14-17, registered as adolescent athletes with the Indonesian Badminton Association (PBSI), with professional training for more than 2 years, no injury in the past 3 months, and no use of medications or supplements to affect muscle strength. The characteristics of the participants were measured as mean ± SD and presented in Table 1. Written informed consent was obtained from all participants before participation in this study. The ACTN3 gene variants were determined using a genetic test in a standardized genetics laboratory. The results showed RR (n=8; 15.5±1.6), RX (n=13; 15.6±1.3), and XX variant groups (n=9; 14.8±1.1). All the variant groups were induced to muscle damage using a BFT protocol adapted from Huang (Huang et al., 2019). Moreover, ethics approval was granted by the Research Ethics Committee of Aissyiyah University, Bandung, West Java, Indonesia, No. 1178/KEP.01/UNISA-BANDUNG/III/2025 in Bandung on March 12, 2025.

Table 1. Anthropometric Characteristics, Experience, and Training Duration of Participants Based on ACTN3 Gene Variants

Variable (unit)	RR	RX	XX	p *Value*
	Average DT	Average DT	Average DT	
n	8	13	9	-
Age (years)	15.5 ± 1.6	15.6 ± 1.3	14.8 ± 1.1	NS
Height (cm)	166.8 ± 7.2	163.8 ± 4.9	166.1 ± 2.1	NS
Weight (kg)	56.5 ± 11.4	55.5 ± 5.2	58.1 ± 7.3	NS
BMI (kg/m2)	20.1 ± 2.5	20.6 ± 1.0	21.3 ± 2.3	NS
Badminton Experience (years)	7.8 ± 1.7	7.9 ± 2.0	6.6 ± 1.5	NS
Number of Training per Week (days)	6.0 ± 0.0	6.0 ± 0.0	6.0 ± 0.0	NS
Total Training per Week (hours)	27.0 ± 0.0	27.0 ± 0.0	27.0 ± 0.0	NS

NS = Not Significant (p-value > 0.05), n = Number. BMI: Body Mass Index

Procedure

Study Time and Place

The study was conducted at the PB Taqi Arena Academy in Cimahi, West Java, Indonesia, from April 20, 2025, to June 10, 2025

Data Collection

Anthropometric Data, Experience, Training, and Medical History

All participants completed a questionnaire, which was reconfirmed through interviews and direct physical examinations. The data collected included age, gender, weight, height, badminton experience, training duration, medical history, medication or supplement consumption history, and injury history.

ACTN3 Gene Data

DNA/RNA Shield™ Collection Tubes (Zymo Research) were used to collect buccal swab samples from all participants for subsequent examination at the laboratory of PT. Genomik Solidaritas Indonesia (GSI Lab) in Jakarta. The ACTN3 gene was analyzed using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method through the four stages of DNA extraction, PCR amplification, restriction enzyme digestion, and electrophoresis visualization or TapeStation (Ghatak et al., 2013).

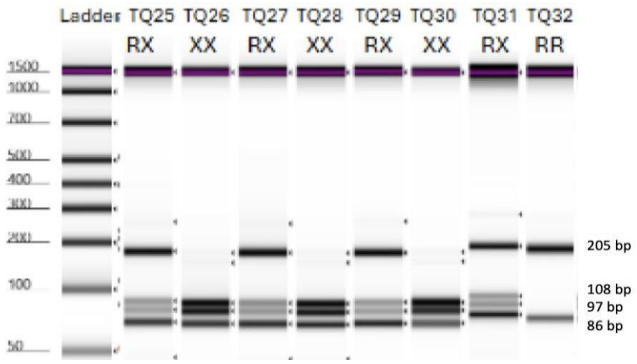
The ACTN3 gene PCR-RFLP analysis was initiated with the centrifugation of the buccal swab preservation solution to separate the supernatant from the pellet. DNA extraction was conducted through the Zymo Research Quick DNA Miniprep Kit using the Biological Fluids and Cell protocol. The next stage after DNA elution was quality control (QC) which was performed using Qubit dsDNA in order to determine the concentration of the extracted product. The amplification process was conducted on a PCR Exicycler 96 Bioneer ® machine with the composition of MyTaq HS Red Mix (12,520 µl), 20 µM Forward Primer (0.5 µl), 20 µM Reverse Primer (0.5 µl), DNA Template (100 ng), and Nuclease-Free Water (up

to 25 µl). The PCR reaction required specific primers from INVITROGEN (5'-CTGTTGCCTGTGG-TAAGTGGG-3' and 5'-TGGTCACAGTATGCAGGAGGG-3').

PCR amplification was conducted using a Biocycler® thermal cycler with an initial denaturation program of 95 °C (1 minute), followed by 95 °C (15 seconds), annealing at 58 °C (15 seconds), and extension at 72 °C (10 seconds) with the process repeated for 35 cycles. The concentration of the PCR amplicon was rechecked using Qubit dsDNA. The restriction process continued after amplification by mixing a maximum of 10 µL of PCR product with the restriction enzyme HpyF3I (Ddel) Thermo Fisher® and incubating for 16 hours at 37 °C. The composition of the master mix for DNA restriction included PCR reaction (250 ng), 10x Buffer Tango (2 µl), NFW (up to 32 µl), and HpyF3I/DdeI (1 µl). The restriction DNA fragments were measured using the Agilent Screentape D1000 TapeStation (AG 5067-5582) Z® which was an automated system often used to analyze the quantity of DNA samples. In this process, the sample was mixed with buffer in a strip tube followed by the insertion of the strip tube containing the master mix and screen tape into the Agilent machine. The results were the visualizations of the fragment sizes from the restriction. The fragment patterns formed were subsequently compared to the reference for each ACTN3 gene.

The DNA band segment patterns from the TapeStation were used to differentiate gene variants in the participants. The R allele band produced fragments of 205 and 86 base pairs while the X allele had 108, 97, and 86 base pairs as presented in Figure 1. The combination of the bands was used to identify the RR, RX, or XX variants of the ACTN3 gene in all participants.

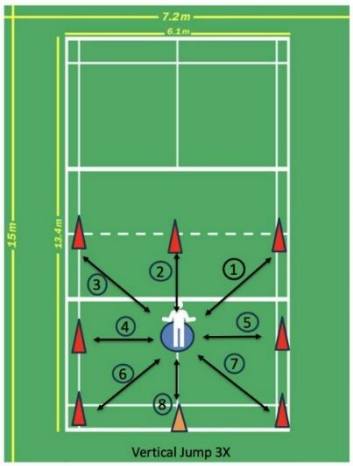
Figure 1. Visualization of ACTN3 Gene TapeStation Results



Badminton Fatigue Protocol (BFT)

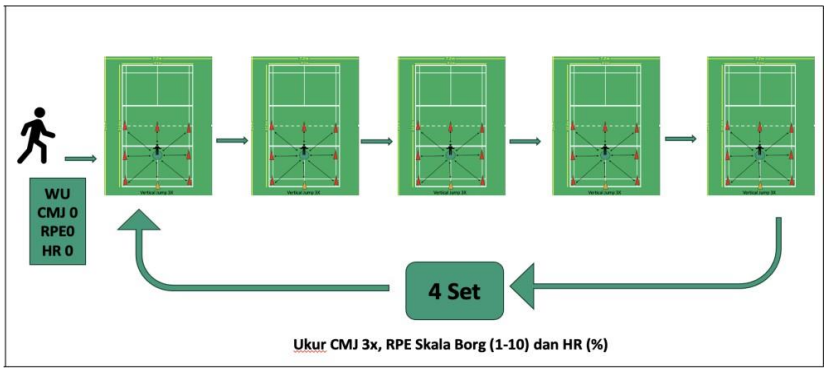
The BFT adapted from Huang et al., (2019) and modified was administered to all participants to induce muscle damage as presented in Figure 1. The test was designed with the basic principle that all participants received the same treatment in terms of difficulty level and training volume. In one BFT repetition, participants started from the center of the court and moved sequentially in order as presented in Figure 2. The initial movement was initiated at the front right corner, front center, front left, front right, center left, back right corner, back left corner, and finally, back center corner followed by three vertical jumps. Each repetition returned to and started at the center of the court. Participants were required to complete each set as quickly as possible by touching the cones at each designated corner.

Figure 2. BFT Protocol (Huang et al., 2019)



All participants performed a light warm-up for 10 minutes followed by a pre-test consisting of Counter Movement Jump (CMJ), a Borg Rating of Perceived Exertion (RPE) scale of 1-10, and heart rate (HR) measurements using Polar® before the BFT. A total of twenty repetitions were performed in four sets of five each and the CMJ, RPE, and HR were measured after each set as shown in Figure 2. All the measurements were recorded followed by the comparison of only the pre- and post-BFT values.

Figure 3. BFT Scheme



Description: WU: Warm-up, CMJ: Counter-Movement Jump, RPE: Rating of Perceived Exertion, HR: Heart Rate, 1 set consisted of 5 repetitions.

CK and Myoglobin Tests

Blood samples for CK and myoglobin tests were collected before the BFT as a baseline and at 1 and 24 hours after by laboratory analysts at Selasih Medika Hospital, Bekasi City. A maximum of 3 ml venous blood was drawn from each participant using a 3 ml syringe and placed in a Blood Collection Tube for centrifugation to obtain blood serum. The collected serum was divided into two microtubes with each containing 1 ml for the CK and myoglobin tests respectively. All serum samples were labeled according to the ACTN3 gene test label. The CK test was conducted at the Bekasi City Hospital laboratory while the myoglobin test was at the LPPOM MUI Bogor City laboratory with both facilities confirmed to be standardized and certified.

Data analysis

Statistical analysis was initiated with the presentation of descriptive statistics including the mean and standard deviation for each group. A normality test was also conducted using the Shapiro-Wilk test. Important statistical assumptions were verified before the inferential test using the Levene Test to assess homogeneity of variances across groups with a significance level of $p < 0.05$. Subsequently, two

types of Analysis of Variance (ANOVA) tests were performed. These included repeated measures ANOVA to compare CK and myoglobin levels at the three time points. A two-way ANOVA was also conducted to assess the differences in CK and myoglobin levels among ACTN3 gene variant groups at each time point, based on mean values, and the interaction between gene and time. Finally, Cohen's d effect size test was applied to compare the magnitude of differences in muscle damage, CK, and myoglobin between ACTN3 gene variants at post 1 and post 24 hours.

Results

BFT Results

The BFT showed that all ACTN3 gene variant groups exhibited similar fatigue responses. There was also a significant increase in RPE and HR but not in CMJ as presented in Table 2.

Table 2. CMJ, RPE, and HR Pre-Post BFT Results Between ACTN3 Gene Variants

Variables	RR (n=8)		RX (n=13)		XX (n=9)	
(units)	Pre	Post	Pre	Post	Pre	Post
CMJ (cm)	59,0 ± 5,8	60,5 ± 5,1	55,8 ± 4,7	58,2 ± 5,1	57,7 ± 3,7	57,0 ± 6,2
RPE (1-10)	3,2 ± 1,0	9,6 ± 0,5 *	3,2 ± 1,0	9,6 ± 0,5 *	3,4 ± 0,9	9,8 ± 0,4 *
HR (%)	56,1 ± 8,1	88,8 ± 6,4 *	57,6 ± 12,2	91,3 ± 6,3 *	56,2 ± 10,8	88,1 ± 10,0 *

Data are presented as means and standard deviations, CMJ: Counter Movement Jump, RPE: Rating of Perceived Exertion, HR: Heart Rate, *: p 0.000 (highly significant), Paired T-test pre-post

The Influence of ACTN3 R577X Polymorphism on CK and Myoglobin

Statistical calculations and data analysis were conducted in line with the objectives using data obtained during the study period. The data were analyzed through comparison and Cohen's d effect size tests to determine differences in muscle damage in response to the ACTN3 R557X polymorphism in male adolescent badminton athletes after BFT.

Differences in CK Levels with ACTN3 Gene Variants

Table 3 shows a significant increase in CK levels after 1 hour and 24 hours in all ACTN3 gene variants ($p < 0.001$). There was also no significant interaction between genotype and time ($p = 0.404$) and this showed that the pattern of CK level changed over time without any substantial difference among the gene groups.

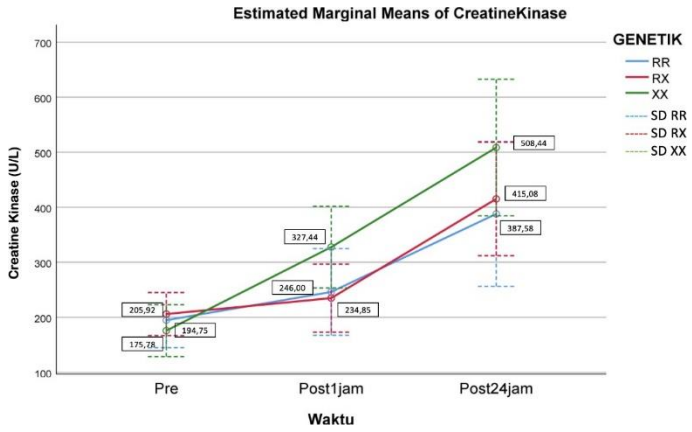
Table 3. Differences in CK Levels by Time

Gen ACTN3	Pretest	Post 1 Hour	Post 24 Hours	p #	p^
	Mean±SD	Mean±SD	Mean±SD		
RR (n=8)	194,75 ± 59,78	246,00 ± 55,88*	387,75 ± 166,78*	0,000	
RX (n=13)	205,92 ± 75,53	234,85 ± 83,50*	415,08 ± 212,99*	0,000	0,404
XX (n=9)	175,78 ± 65,57	327,44 ± 162,80*	508,44 ± 136,81*	0,000	

Data in the form of mean±SD, #Repeated ANOVA test, units U/L, *Significant compared to the pretest CK value of each variant group, ^Two Way ANOVA Test, interaction of gene and time

Figure 3 is a graph of estimated marginal means of CK to show the changes in response to ACTN3 gene variants over time. The data are presented as means and standard deviations. The visual and numerical observations in Figure 3 showed an interesting pattern where the XX variant consistently exhibited significantly higher mean CK values than the RR and RX variants both at 1-hour and 24-hour post-BFT measurements.

Figure 3: Graph of CK Response Between ACTN3 Gene Variants Based on Time



Differences in Myoglobin Levels with ACTN3 Gene Variants

Table 4 shows the differences in myoglobin levels in male adolescent badminton athletes. The results of the repeated ANOVA test showed a p-value of 0.000 for all ACTN3 gene groups to reflect the existence of statistically significant differences in myoglobin levels over time for each gene group. Another important observation was that the Two-Way ANOVA reported a significant interaction between genotype and time (p=0.038). The significance of this interaction showed that the pattern of change in myoglobin levels over time differed significantly across ACTN3 gene variant groups. Therefore, the response of myoglobin levels to activity over time was different across ACTN3 gene variant groups.

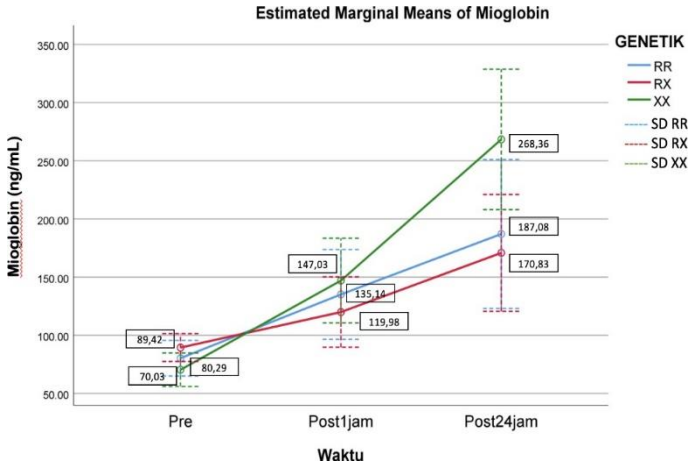
Table 4. Differences in Myoglobin Levels Based on Time

Gen ACTN3	Pretest	Post 1 Hour	Post 24 Hours	p#	p^
	Mean±SD	Mean±SD	Mean±SD		
RR (n=8)	80,29 ± 23,74	135,14 ± 56,88*	187,08 ± 104,50*	0,000	
RX (n=13)	89,42 ± 23,58	119,98 ± 55,00*	170,83 ± 71,07*	0,000	0,038
XX (n=9)	70,37 ± 12,88	147,03 ± 46,63*	268,36 ± 95,53*	0,000	

Data are showed as mean±SD, myoglobin unit ng/mL, #Repeated ANOVA test, * Significant compared to the pretest myoglobin value of each variant group, ^Two Way ANOVA Test, gene and time interaction

Figure 4 is a graph of the Estimated Marginal Means for Myoglobin which shows the change in myoglobin response across ACTN3 gene variants over time. The data are presented as means and standard deviations with the graph considered important for understanding the significant interaction effect between gene and time identified in the previous Two-Way ANOVA analysis.

Figure 4. Graph of Myoglobin Response Between ACTN3 Gene Variants Based on Time



CK and Myoglobin Response Profiles Between ACTN3 Genes Based on Cohen's d Effect Size

The Cohen's d values for the changes in CK and myoglobin levels of the XX variant of the ACTN3 gene compared to RR and RX in 1 hour and 24 hours after BFT ranged between 0.4 and 1.4 as presented in Table 5. The comparison of the RR and RX variants also produced Cohen's d values that ranged from 0.1 to 0.5 as shown in Table 6.

Table 5. Comparison of Effect Size of XX Variants of the ACTN3 Gene in CK and Myoglobin Against RR and RX

XX	RR		RX	
	Post 1 Hour	Post 24 Hours	Post 1 Hour	Post 24 Hours
	Cohen's d	Cohen's d	Cohen's d	Cohen's d
ΔCK	0,8	0,9	1,1	0,9
ΔMyoglobin	0,4	0,9	0,8	1,4

Interpretation of Cohen's d: 0.2 (trivial), 0.2-0.6 (small), 0.6-1.2 (moderate), 1.2-2.0 (large), 2.0-4.0 (very large),>4.0 (extremely large)

Table 6. Comparison of Effect Size of RR Variants of the ACTN3 Gene in CK and Myoglobin Against RX Variants

RR	RX	
	Post 1 Hour	Post 24 Hours
	Cohen's d	Cohen's d
ΔCK	0,5	0,1
ΔMyoglobin	0,4	0,3

Interpretation of Cohen's d: 0,2 (trivial), 0,2-0,6 (small), 0,6-1,2 (moderate), 1,2-2,0 (large), 2,0-4,0 (very large),>4,0 (extremely large)

Discussion

This study aimed to analyze differences in the muscle damage response between the ACTN3 R577X XX variant and the RR and RX variants in adolescent male badminton athletes. Genetic tests were conducted using PCR RFLP before the experiments to determine ACTN3 gene variants. The banding patterns of DNA segments from the tapestry were used to determine the ACTN3 gene alleles and variants of the participants. The R allele produced fragments of 205 and 86 base pairs while the X allele had 108, 97, and 86 base pairs. The combination of these bands identified the RR, RX, or XX variants of the ACTN3 gene in all participants. Moreover, the examination showed the RR variant in eight participants, the RX in 13, and the XX in nine. The characteristics of these participants including ACTN3 gene variants, anthropometric data, training history, and experience are presented in Table 1.

BFT was conducted to induce muscle damage, and all participants received the same treatment. The three parameters measured were the CMJ, RPE on a Borg Scale of 1-10, and HR. The measurement and calculation were conducted before and after BFT with the results presented in Table 2. CMJ was used to determine the explosive strength and lower leg power(Markovic et al., 2004). This was based on the criterion that a decrease in jump height reflected neuromuscular fatigue or reduced lower leg muscle power from training. The results showed that there was no significant change in CMJ across all ACTN3 gene variant groups. This reflected that the BFT performed was not strenuous enough to cause a significant decrease in lower leg power. It was reported that 160-240 lunges in badminton matches significantly decreased neuromuscular leg power function (Lin et al., 2023). However, the BFT protocol used in this study required performing approximately 140 lunges which could be the reason for the lack of a significant CMJ reduction. Another possible reason was that the participants were trained athletes and there could be a post-activation potentiation effect (Blazevich & Babault, 2019). This study did not limit the protocol to eccentric contractions which were the primary cause of muscle damage(Vincent et al., 2010). The others included were calf and thigh muscle contractions upon landing after a jump, arm contractions after a smash, and thigh muscle movements during backpedaling (Phomsoupha & Laffaye, 2015).

RPE is a subjective scale that measures how difficult or easy it is for an individual to perform physical activity for a specific period after maximum effort (Eston, 2012). The increase in RPE from pre- to post-BFT shows an increase in perceived fatigue and the intensity of effort. A higher RPE reflects more tiredness or harder effort. The results showed that all ACTN3 gene variants experienced a very significant increase in RPE ($p < 0.001$). The average pre-RPE was estimated at 3 classified as very light while the post-RPE increased to 9.6-9.8 categorized as very hard. The data clearly showed that BFT caused severe

subjective fatigue across all ACTN3 gene variant groups. A similar trend was observed in HR which increased significantly from pre to post ($p < 0.001$) at a mean of 56-57% to 88-91%. This showed an increase in exercise intensity as well as cardiovascular and metabolic physiological load. HR is a measure of the cardio response but a large increase in the value can be associated with fatigue. This is possible because fatigued muscles require greater effort from the cardiovascular system to maintain output. The results for the three parameters in Table 2 showed that all participant groups received similar treatment and responded similarly to BFT inducing muscle damage. The increase in RPE and HR across all ACTN3 gene groups consistently reflected significant fatigue both subjectively and physiologically. The sharp spikes strongly showed a substantial fatigue response while CMJ had a less consistent response in lower limb power after BFT.

Table 3 and Figure 3 show the response pattern of CK levels for ACTN3 gene variants before and after BFT. The RR variant (n=8) specifically experienced a gradual increase in the mean CK level from 194.75 ± 59.78 U/L at pretest to 246.00 ± 55.88 U/L at 1 hour posttest and 387.75 ± 166.78 U/L at 24 hours posttest. Similarly, the RX variant (n=13) showed an increase in CK levels from 205.92 ± 75.53 U/L to 234.85 ± 83.50 U/L and 415.08 ± 212.99 U/L respectively. The XX variant (n=9) showed the most striking increase in CK levels, which started from a relatively lower pretest average of 175.78 ± 65.57 U/L but increased sharply to 327.44 ± 162.80 U/L at post 1 hour and finally reached the highest among all groups at post 24 hours with 508.44 ± 136.81 U/L. The trends signaled a significant increase in CK levels at pre, 1 hour, and 24 hours in all ACTN3 gene variants ($p < 0.001$).

The results of the Two-Way ANOVA test showed no significant interaction effect between genotype and time ($p = 0.404$). This was a sign that the pattern of change in CK levels over time was not significantly different between the gene groups. The CK levels changed significantly over time, but the pattern was similar across all gene variant groups. The statistical analysis did not show a significant difference but visual and numerical observations in Figure 3 signaled an interesting pattern. The XX variant consistently had a mean CK value that tended to be higher than the RR and RX at both 1 hour and 24 hours after BFT.

Table 4 presents the results of the test to determine the differences in myoglobin levels across the three measurement times. The repeated ANOVA test showed a p-value of 0.000 for all ACTN3 gene groups at both 1 and 24 hours after BFT. The trend reflected a statistically significant difference in myoglobin levels over time across gene groups. This showed that the exercise or activity conducted caused a significant change in myoglobin levels in the bodies of the participants. The comprehensive results showed that the mean level for the RR variant (n = 8) increased from 80.29 ± 23.74 ng/mL in the pretest to 135.14 ± 56.88 ng/mL in the post 1 hour and continued to 187.08 ± 104.50 ng/mL in the post 24 hours. The RX variant (n=13) had a similar pattern from 89.42 ± 23.58 ng/mL to 119.98 ± 55.00 ng/mL and 170.83 ± 71.07 ng/mL respectively. Meanwhile, the XX variant (n=9) started with the lowest at 70.37 ± 12.88 ng/mL for the pretest but showed the most drastic increase to reach the highest level among all genes at 147.03 ± 46.63 ng/mL for post 1 hour and 268.36 ± 95.53 ng/mL for post 24 hours.

This increase generally signaled a normal physiological response, but the XX variant showed a different pattern compared to the others. Silva et al. Be explained that the deficiency of α-actinin-3 protein in the XX variant could cause greater susceptibility to muscle damage compared to the RR and RX variants with the component (Seto et al., 2011; Silva et al., 2025). The trend is in line with the report of a previous study that the absence of the α-actinin-3 protein classified as the main structural component supporting the Z line in the sarcomere together with α-actinin-2 can increase the susceptibility to muscle damage (Lee et al., 2016; Vincent et al., 2010).

The Estimated Marginal Means of Myoglobin graph in Figure 4 visualizes the changes in mean myoglobin levels in all ACTN3 gene variant groups at the three points. The graph is important to understand the significant difference identified between gene and time in the Two-Way ANOVA analysis with a value of $p = 0.0038$ ($p < 0.005$). This showed that the pattern of changes in myoglobin levels between ACTN3 variant groups was different over time. The trend reflected the influence of ACTN3 gene variants on the response of myoglobin to BFT based on time. The highly significant increase in CK and myoglobin levels across all the variants at both 1 hour and 24 hours post-BFT was a physiological response to fatigue-inducing exercises. This was in line with the report that the CK and myoglobin increased immediately after exercise and reached a peak at 24, 48, and even 72 hours post-exercise (SAITA et al., 2023; Twist

& Eston, 2005). The trend led to the inference that BFT was successful in inducing muscle damage in male adolescent badminton athletes.

The magnitude of the difference in the response to muscle damage by the variants containing and lacking α-actinin-3 protein was determined using Cohen's d effect size. The ACTN3 R577X polymorphism in the XX variant lacks α-actinin-3 protein while RR and RX have the component. The comparison of the CK levels of the XX variant with the RR 1 hour post BFT showed a Cohen's d value of 0.8 which was classified as a moderate impact. A similar trend was also observed at 24 hours post BFT with 0.9 and the results showed that the XX variant had a different and longer-lasting CK recovery pattern compared to the RR. The comparison between the XX and RX variants also showed a significant effect with the value reaching 1.1 at 1 hour post BFT, which was classified as a strong moderate. The value recorded at 24 hours post BFT was 0.9, which was classified as moderate and the trend showed the CK response in individuals with the XX variant differed significantly from those with the RR and RX.

The comparison of the myoglobin in XX and RR variants showed interesting dynamics. The difference at 1 hour post BFT was relatively small with a Cohen's d value of 0.4 but increased to 0.9 classified as moderate at 24 hours post BFT. This showed that the effect of the absence of α-actinin-3 protein in the XX variant on myoglobin was observed in the longer recovery phase compared to the RR. A more striking comparison was identified between the XX and RX variants with a moderate increase in myoglobin levels 1-hour post-BFT at a Cohen's d value of 0.8 which increased to a large effect of 1.4 at 24 hours post-BFT. This was the strongest sign that the XX variant of the ACTN3 gene had a very different and clinically significant myoglobin response compared to the RX specifically at 24 hours after BFT.

Table 6 shows the effect sizes of the RR on RX and Cohen's d value is in the range of 0.1 (trivial) to 0.5 (small). This showed that the variant groups containing α-actinin-3 protein including RR and XX provided almost the same response to changes in CK and myoglobin levels after induction of muscle damage. The result was different from the moderate to large effect size observed when the RR and RX variant groups were compared with the XX which was without α-actinin-3 protein in Table 7.

Sullivan & Feinn (2012) previously identified effect size as an important result to be reported in a quantitative study in addition to the P value. This was due to the role of effect size as the substantive significance while the P value was identified as the statistical significance. The P value shows the existence of a relationship while the effect size reflects the magnitude. Cohen's d effect size is widely used in sports science studies (Fröhlich et al., 2009). Therefore, it is very relevant for making inferences about the effect of the ACTN3 R577X polymorphism on CK and myoglobin levels as biomarkers of muscle damage compared to the other ACTN3 gene variants.

The Two-Way ANOVA statistical test only showed a significant change in myoglobin levels based on the interaction of gene and time at $p = 0.038$ in Table 5 but the Cohen's d effect size analysis reflected the practically important and relevant results. The comparison of the XX variant with RR and RX for the CK showed a moderate effect size with a Cohen's d value of 0.8-1.1 at 1 hour and 24 hours post BFT. Furthermore, the myoglobin levels of XX were compared with RX and the results showed a large effect size with Cohen's d value of 1.4 24 hours post BFT. This showed that the XX variant of the ACTN3 gene had a substantially different but statistically insignificant response to CK and myoglobin levels after BFT compared to the RR and RX. The trend provided in-depth information regarding the impact of the ACTN3 R577X polymorphism on muscle damage.

The hypothesis that the increase in CK and myoglobin levels as biomarkers of muscle damage after BFT in the XX variant (ACTN3 R577X polymorphism) was greater than in the RR and RX variants was strongly supported by Cohen's d effect size data. Therefore, it was inferred that there were differences in muscle damage response between ACTN3 gene variants in male adolescent badminton athletes after BFT. The XX variant showed a greater muscle damage response than the RR and RX variants. Some limitations were identified in this study in relation to the methods applied and the experiments. For example, the study had a limited sample size, which may have influenced these findings. Therefore, future studies should use larger sample sizes and integrate more intensive eccentric training with the BFT protocol to increase statistical power and confirm the observed muscle damage response pattern in the XX variant.

Conclusions

The results show that the ACTN3 R577X polymorphism in the XX variant has a greater muscle damage response than the RR and RX. This reflects the need to identify the ACTN3 gene variants in male adolescent badminton athletes to serve as a guide for personalized training and post-match recovery strategies. Moreover, the recovery strategies for male adolescent badminton athletes with the XX variant who are at higher risk of muscle damage due to α-actinin-3 protein deficiency should be structured and aggressive to ensure readiness for intense competition.

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