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Influence of ACTN3 R/X gene polymorphisms on racing strategy in rowing athletes

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ABSTRACT
This study compared the polymorphisms RR, XX and RX of the ACTN3 gene among rowers with different race strategies across competitive levels and weight categories. We evaluated 137 rowers divided into: male non-elite openweight ($n = 23$), male elite openweight ($n = 33$), male non-elite lightweight ($n = 31$), male elite lightweight ($n = 13$), female non-elite openweight ($n = 9$), female elite openweight ($n = 9$) and female non-elite lightweight ($n = 15$), female elite lightweight ($n = 4$). The main results indicated that rowers who adopted starting/early (within the initial 500 m) or finishing/late (within the final 500 m) strategies exhibited similar ACTN3 genotype frequencies, while fewer athletes who adopted moderate (within the middle 1000 m) pacing strategies were categorised with the ACTN3 XX (8%) genotype as compared to the RR (50%) and RX (42%) genotypes ($p = 0.002$). Elite openweight male rowers with the ACTN3 RR more frequently preferred moderate pacing strategies ($p = 0.006$), while non-elite lightweight male athletes with ACTN3 RR more frequently preferred starting/early pacing strategies ($p \leq 0.001$). Therefore, rowing athletes with specific ACTN3 genotypes may adopt unique race pacing strategies, while no differences were shown by competitive level or weight category.

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Endurance; genetics; performance; training

1. Introduction

Depending on the competitive level, weight category (lightweight or open) and number of athletes involved (from single skiff to an eight), official senior 2000 metre rowing races vary in time from approximately 5 min and 30 s to 8 min (Kölling et al., 2016). Gaining an early lead due to high-intensity efforts at the start of a race may confer tactical and psychological advantages with respect to performance (Garland & Atkinson, 2008). Achieving and maintaining this position during the race allows the rowers to monitor the position and

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strategic advances from competitors, and permits the avoidance of the wake generated by the other boats (Garland, 2005). Fast start strategies during 2000 metre races appear to be adopted by most athletes/teams, including men and women, regardless of the competitive result (Garland, 2005; Garland & Atkinson, 2008).

The sport of rowing requires strength endurance in order to produce enough sustainable force while stressing both the aerobic and anaerobic energy systems (primarily at the start and finish of the race) (Akca & Aras, 2015; Vaz, Picanço, & Del Vecchio, 2014). Therefore, rowing performance is affected by both individual/genetic (Bosnyák et al., 2015) and environmental factors, such as strategies during the race, training (Richer, Nolte, Bechard, & Belfry, 2016; Vaz et al., 2014), diet (Boegman & Dziedzic, 2016) and sociocultural factors (Frideres, Mottinger, & Palao, 2016; Kölling et al., 2016). However, knowledge of the influence of genetic aspects in rowing remains scarce. Although some studies have characterised the high-level openweight genotype (Cieszczyk et al., 2012; Maciejewska, Sawczuk, & Cięszczyk, 2011), the evaluation of gene polymorphism in elite and non-elite rowers has yet to be fully explored.

One of the most commonly investigated genetic polymorphisms in physical performance, including the predisposition to athletic capacity, involves the R/X alleles of the α-actinin-3 (ACTN3) gene (Baumert, Lake, Stewart, Drust, & Erskine, 2016; Mägi et al., 2016; Sarzynski et al., 2016; Yang et al., 2017). ACTN3 is the principal structural gene specific to skeletal muscle, via Z line function within the sarcomere and regulation of metabolism in type II fibres, that has been linked with physical performance (Ben-Zaken et al., 2015; Yang et al., 2003; Yang et al., 2007; Yang et al., 2011). The ACTN3 577R allele and 577RR genotype are related with elite level power performance in a wide array of sporting environments (Eynon et al., 2014; Papadimitriou et al., 2016), while the 577X allele and 577XX genotype have been reported to be associated with elite athletic status in endurance athletes (Ahmetov et al., 2010; Eynon et al., 2014; Kikuchi & Nakazato, 2015; Kikuchi et al., 2016; Mägi et al., 2016; Pasqua et al., 2016; Yang et al., 2007). However, few studies have been conducted in rowing athletes with whom the knowledge about polymorphisms might be useful for studying a wide range of optimisation scenarios with regard to physical conditioning and strength training (Chimera & Kremer, 2016).

To the authors' knowledge, no studies have examined race strategy during rowing with consideration for genetic polymorphisms. Furthermore, the potential significance of the ACTN3 genotype in aerobic sports may be more clearly identified through an analysis of the pacing strategies adopted by rowers. A well-established experimental paradigm in which to precisely investigate the influence of gene polymorphisms in rowers on pacing strategy is required. Additionally, moderating factors, such as competitive level and weight category, may play a role in these relationships. Therefore, the aim of the current study was to compare genotype (RR, XX and RX) frequencies of the ACTN3 polymorphism among rowers with different preferential race strategies across competitive levels and weight categories. We hypothesised that rowing athletes with different race strategy preferences would possess unique ACTN3 genotypes.

2. Methods

2.1. Experimental approach

This study investigated the genotype frequency of ACTN3 gene polymorphisms in rowing athletes with consideration for racing strategy, gender, competitive level and weight category. To accomplish this objective, a wide range of male and female rowing athletes from both the lightweight (below ~57 kg in women; below ~70 kg in men) and openweight categories were surveyed to determine their competitive level and racing strategy preferences, and subsequently completed genetic testing to determine their ACTN3 polymorphisms (RR vs. RX vs. XX genotypes). Elite rowing athletes were defined as those who had represented their country at least twice at the international level, while non-elite rowing athletes were defined as those who had represented their club at least twice at the regional or national level.

2.2. Participants

The participants in this study included 137 scullers, rowing athletes (age range = 26–38 years). The sample of athletes was divided into 8 groups: male non-elite openweight (n = 23), male elite openweight (n = 33), male non-elite lightweight (n = 31), male elite lightweight (n = 13), female non-elite openweight (n = 9), female elite openweight (n = 9) and female non-elite lightweight (n = 15), female elite lightweight (n = 4). The participants were not provided information about the purpose of the study until after they completed the experiment. The Ethics Committee of the Local University approved the study protocol before the commencement of the assessments. Each participant signed an informed consent prior to taking part in the study.

2.3. Genotyping

Genetic analyses were performed at the Nutrigenomic Laboratory of the University where the study was conducted. All participants in this study gave informed consent to genotyping with the understanding that procedures were anonymous and obtained results would have confidential status. Genomic DNA was isolated from buccal cells and the ACTN3 R/X polymorphisms (RR vs. RX vs. XX genotypes) were determined as previously described (Schadock et al., 2015). The Sequences for Allele-Specific and C-R- and T-X-Specific Primers are presented below:

(a) Name: *hACTN3f*, Sequence: *5¢-CGCCCTTCAACAACTGGCTGGA-3¢*, Product size: *690 bp with hACTN3r;*
(b) Name: *hACTN3r*, Sequence: *5¢-GATGAGCCCGAGACAGGCAAGG-3¢*, Product size: *690 bp with hACTN3f;*
(c) Name: *hACTN3Tif*, Sequence: *5¢-CAACACTGCCCGAGGCTGACTG-3¢*, Product size: *318 bp with hACTN3r;*
(d) Name: *hACTN3Cir*, Sequence: *5¢-CATGATGGCACCTCGCTCTCGG-3¢*, Product size: *413 bp with hACTN3f.*

Briefly, primers at 5 μM were mixed in one tube adding 4 volumes of each external primer (hACTN3f and hACTN3r), 1 volume of forward internal primer (hACTN3Tif) and 2 volumes of reverse internal primer (hACTN3Cir). Five μL of the primer mix was added to 10 μL of 2x GoTaq® Green Master Mix (PROMEGA, cat M7122) and 5 μL of DNA sample, for a reaction of 20 μL. The PCR conditions were: 95 °C for 2 min; 35 cycles at 95 °C for 10 s, 68 °C for 10 s, 72 °C 45 s; and a final step of 72 °C for 2 min. PCR products were analysed in 2% agarose gel with 1:10,000 SYBR® Safe DNA Gel Stain (Invitrogen, U.S.A.) compared to a 100 bp length marker (100 bp DNA Ladder, Invitrogen, U.S.A.).

2.4. Competition

Preferential racing strategy was determined with a survey based on official results of the main regattas from the last two years (2014–2015). 500 metre split times were obtained for each athlete in every openweight and lightweight race by official data from World Rowing Federation, Brazilian Rowing Confederation and State Rowing Federation of São Paulo. After that, each participant confirmed the preferential tactical time to overtake other boats during the race split times (Garland, 2005) and were separated into starting (within the first 500 m), moderate (within the middle 1000 m) and finishing (1500–2000 m) strategy groups. Additional training data, including rowing-specific training, conditioning/resistance training and overall experience, was recorded via questionnaire completed by the athletes along interviews with coaches during competitive events. The Photo-Finish Timing System for Rowing & Paddling (Lynx, U.S.A.) that is used during all official championships was also used to verify the competitive level of the athletes.

2.5. Statistical analysis

The descriptive data are presented as mean ± standard deviation (SD) or percentages according to genotype occurrence. Three-way (sex × weight category × competitive level) analysis of variance was used to evaluate between group differences in anthropometric/demographic values and training data. Chi-squared tests were conducted to compare ACTN3 genotypes (*RR* vs. *RX*; *RX* vs. *XX; XX* vs. *RR*) by competitive level (elite vs. non-elite) and weight category (lightweight vs. openweight), and to examine differences according to race pace strategies (starting vs. moderate; moderate vs. finishing; starting vs. finishing). A significance level of $p \le 0.05$ was used. All analyses were conducted using SPSS 20.0 for Windows.

3. Results

3.1. Description of sample and genotyping profile

Descriptive data from the sample are presented in Table 1. Significant differences in body mass between groups were found ($F_{2,134} = 25.017$; $p \le 0.001$; $\eta^2 = 0.293$), with female and male openweight rowing athletes having higher body mass values than female and male lightweight rowing athletes, respectively ($p \le 0.001$ for all comparisons). No effects were observed for any of the other anthropometric/demographic values or training data ($p > 0.05$ for all comparisons).

The ACTN3 genotype frequency data according to sex, competitive level and weight category is presented in Table 2. Significant differences in ACTN3 genotype frequency were found for the elite lightweight male rowers ($X^2 = 9.500$, $p = 0.009$, df 2), where the RX

Table 1. Descriptive data from the sample of rowing athletes (mean ± Standard Deviation).

	Female				Male			
	Openweight		Lightweight		Openweight		Lightweight	
Profile	Non-elite	Elite	Non-elite	Elite	Non-elite	Elite	Non-elite	Elite
Age (yrs.)	33.0 ± 6	35.6 ± 15.7	40.7 ± 25.5	31.0 ± 1.5	37.9 ± 19.4	35.0 ± 9.1	22.5 ± 8.3	30.5 ± 3.5
Age Start Rowing (yrs.)	29.0 ± 5.2	28.4 ± 12.8	38.3 ± 23.2	28.5 ± 0.7	22.4 ± 7.5	17.6 ± 5.3	20.3 ± 6.1	29.0 ± 5.7
Body Mass (kg)	68.0 ± 10.8*#	68.4 ± 6.8*#	56.3 ± 6.4*	57.0 ± 1.4*	85.3 ± 10.7#	80.9 ± 8.0#	72.5 ± 17.2	70.0 ± 0.1
Height (cm)	171.0 ± 3.0	173.8 ± 6.5	161.3 ± 8.3	169.0 ± 1.4	185.2 ± 6.2	181.9 ± 10.6	174.2 ± 10.6	174.5 ± 9.2
Rowing training session/week (frequency)	5.3 ± 1.5	5.6 ± 0.9	5.7 ± 0.6	6.5 ± 0.7	4.3 ± 1.8	7.9 ± 2.7	4.0 ± 2.8	4.7 ± 2.1
Rowing training session/week (min)	90.0 ± 30.0	132.0 ± 50.2	123.3 ± 30.6	150.0 ± 42.4	104.3 ± 44.7	117.9 ± 28.0	110.0 ± 62.4	146.2 ± 56.5
Conditioning and strength training/week (frequency)	3.3 ± 1.5	3.4 ± 1.7	3.3 ± 1.5	4.5 ± 3.5	4.0 ± 1.3	3.6 ± 1.9	3.3 ± 1.8	4.5 ± 2.1
Conditioning and strength training time (min)	50.0 ± 17.3	72.0 ± 16.4	140.0 ± 45.8	112.5 ± 10.6	60.7 ± 28.3	91.4 ± 40.2	112.5 ± 62.7	75.0 ± 21.2
Training experience (month)	47.3 ± 37.0	65.4 ± 45.3	25.7 ± 29.9	24.0 ± 17.0	174.7 ± 241.6	194.6 ± 144.4	19.3 ± 23.8	24.0 ± 17.0

Notes: *different from male athletes; #different from lightweight athletes of the same gender and competitive level, $p \leq 0.05$.

Table 2. ACTN3 genotype frequencies in rowing athletes.

Groups			Polymorphism		
			ACTN3 RR (%)	ACTN3 RX (%)	ACTN3 XX (%)
Female	Openweight	Non-elite	66.7	33.3	10.0
		Elite	22.2	44.4	33.3
	Lightweight	Non-elite	33.3	44.4	22.2
		Elite	25.0	50.0	25.0
Male	Openweight	Non-elite	44.4	38.9	16.7
		Elite	41.7	45.8	12.5
	Lightweight	Non-elite	33.3	37.0	37.0
		Elite	8.3*	75.0	16.7

Notes: *significantly different from RX and XX, $p \leq 0.05$.

genotype frequency was greater than the RR and XX frequencies. No other effects were observed ($p > 0.05$ for all comparisons).

3.2. Racing strategy

The occurrence of ACTN3 genotypes separated by pacing strategy preference is presented in Figure 1. For the overall group, significant differences in ACTN3 genotype frequency were found for the moderate pacing strategy athletes ($X^2 = 15.333$, $p = 0.002$, df = 3), where the XX genotype frequency (8%) was lower than the RR (42%) and RX (50%) genotype frequencies. No significant effects were observed in the starting ($p = 0.115$) and finishing ($p = 0.158$) strategy athletes.

The ACTN3 genotype frequencies of each pacing strategy group are presented in Table 3. Not enough valid cases were available for processing comparisons in non-elite female openweight ACTN3 RX athletes and all genotypes of elite female lightweight athletes. However, significant differences in pacing strategy preference were found for the non-elite openweight male rowing athletes ($X^2 = 10.160$, df = 2, $p = 0.006$) having the RR genotype, with a greater frequency preferring a moderate pacing strategy as compared to those preferring starting and finishing strategies.

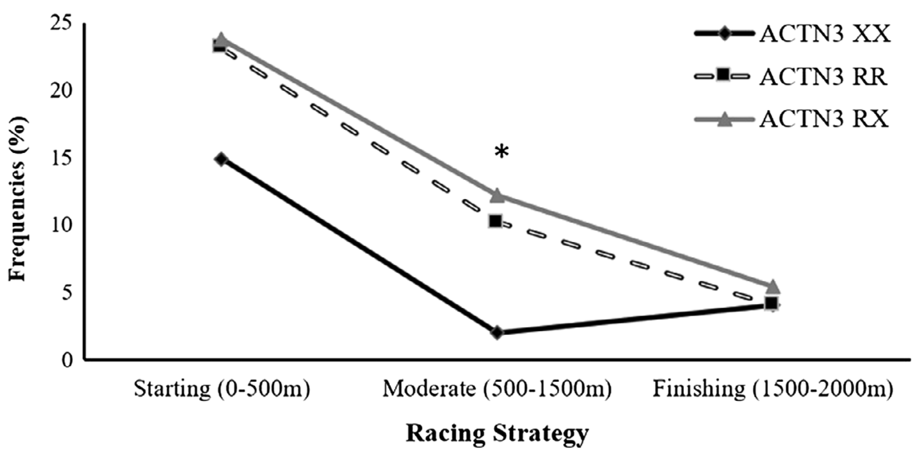

Figure 1. Racing strategy preference for each genotype. *significantly different between XX and both RR and RX genotypes, $p \leq 0.05$.

Table 3. Racing strategy preference frequencies separated by competitive level, weight category and genotype.

	Female				Male			
	Openweight		Lightweight		Openweight		Lightweight	
Racing strategy	Non-elite (%)	Elite (%)	Non-elite (%)	Elite (%)	Non-elite (%)	Elite (%)	Non-elite (%)	Elite (%)
Starting (0–500 m)								
ACTN3 RR	100	50	100	100	50	20	88.9*	100
ACTN3 RX	100	0	75	0	42.9	36.4	80	77.8
ACTN3 XX	100	33	100	0	33.3	33.3	75	50
Moderate (500–1500 m)								
ACTN3 RR	0	50	0	0	50	70*	11.1	0
ACTN3 RX	0	25	0	50	57.1	54.5	20	22.2
ACTN3 XX	0	33	0	0	0	0	0	0
Finishing (1500–2000 m)								
ACTN3 RR	0	0	0	0	0	10	0	0
ACTN3 RX	0	75	25	50	0	9.1	0	0
ACTN3 XX	0	33	0	100	66.7	66.7	25	50

Notes: *significantly different from other genotypes in the same group profile, $p \leq 0.05$.

Significant differences in pacing strategy preference were found for the non-elite lightweight athletes ($X^2 = 43.257$, df = 2, $p \leq 0.001$) having the RR genotype with a greater frequency preferring a starting strategy as compared to those preferring moderate and finishing strategies. Significant differences in pacing strategy preference were found for the elite lightweight athletes ($X^2 = 6.500$, df = 2, $p = 0.039$) having the RR genotype with a greater frequency preferring a starting strategy as compared to those preferring moderate and finishing strategies.

4. Discussion

Several genes influence endurance capabilities from both a physiological and a psychological perspective (Ahmetov et al., 2009, 2010; Bosnyák et al., 2015; Fedotovskaya, Mustafina, Popov, Vinogradova, & Ahmetov, 2014; Znazen et al., 2017). Our study is the first to have examined the potential influence of ACTN3 genotypes on pacing strategies in 2000 m rowing events lasting between 5 min 30 s and 8 min. The main results suggest that rowing athletes with specific ACTN3 genotypes may adopt unique race pacing strategies, while no differences were shown in ACTN3 genotypes frequencies when examined by competitive level or weight category.

Competitive 2000 m rowing races last approximately 330–460 s and generally feature greatest power and velocity values at the beginning of the event with a potential increase at the end of the event (Garland, 2005; Garland & Atkinson, 2008). Analysis of race strategy during the Sydney Olympics revealed patterns during the finals reflecting + 2.8% for the initial 500 m, −1.2% for the 2nd 500 m, −1.3% for the 3rd 500 m and −0.1% for the final 500 m of the average speed over 2000 m race, while medal winners had 0.6% slower times for the initial 500 m but appeared be to 0.6% faster during final 500 m compared to non-medal winners (Kleshnev, 2001; Renfree, Martin, Richards, & Gibson, 2012). Specifically, competitive rowers who adopted starting/fast (within the initial 500 m) or finishing/late (within the final 500 m) strategies exhibited similar ACTN3 genotype frequencies, while fewer athletes who adopted moderate (within the middle 1000 m) pacing strategies were categorised with the ACTN3 XX (8%) genotype as compared to the RR (50%) and RX (42%) genotypes.

Regarding athletes and their respective genotype, elite openweight male rowers with the ACTN3 RR genotype more frequently preferred moderate pacing strategies, while non-elite lightweight male athletes with ACTN3 RR genotype more frequently preferred starting/early pacing strategies. The current findings may be explained by the structural nature of α-actinin-3 protein, encoded by the ACTN3 gene, as the primary component of the Z line and its interaction with actin filaments, which is known to influence the expression of muscular strength in type II fibres (Yang et al., 2017). More specifically, individuals with the ACTN3 XX genotype are deficient in α-actinin-3 and tend to excel in endurance activities, perhaps related to the finishing strategy outlined in the current investigation, whereas those with the RR genotype may have an advantage when producing forceful muscle contractions at high velocities (Zanoteli et al., 2003), such as those required by rowing athletes when attempting to pass competing boats and during starting/fast pacing strategies.

With the exception of openweight males, rowing athletes with the ACTN3 RR genotype most frequently preferred starting/early pacing strategies (50–100%), while those with the ACTN3 RX genotype did not show a particular pacing strategy preference. Furthermore, with the exception of openweight males, primarily the elite, as opposed to the non-elite, rowing athletes with the ACTN3 XX genotype preferred late pacing strategies (33–100%). When compared to other athletes, Hungarian rowers were recently shown to possess similar ACTN3 R/X polymorphism frequencies (Bosnyák et al., 2015). However, a genetic association was shown between ACTN3 genotype and the long-distance (6 km) results of 54 athletes at the Russian Cup Rowing Tournament with male rowers exhibiting the ACTN3 577RR genotype yielding faster times than those exhibiting the 577RX or 577XXgenotypes (Ahmetov et al., 2010).

Though there are important strategic and psychological reasons for starting fast, the genetic and physiological explanations for adopting these strategies by specific genotypes are unclear. Probable mechanisms for increasing performance by utilising a rapid start include accelerated increase in blood flow (Faull, Cotter, & Lucas, 2015), ventilation (Mazic et al., 2015) and/or oxygen uptake (Murray, McCrudden, Murias, Nolte, & Belfry, 2016). Assuming the responsive to training is associated with genotype, these factors would likely result in reduced fatigue later in the race. Nonetheless, the ability to speculate using the current data is limited because these factors were not directly investigated. Present article indicates as a limitation the lack of comparisons of genotypes of athletes who competed in different boat classes. This can be explored in future research to improve rowing training and to detect talents, directing them to the boats possibly associated with ACTN3 genotypes.

We observed that starting/fast pacing strategies were adopted by the majority of the rowers regardless of ACTN3 genotype (RR, RX and XX). A lack of attention to specific genotype by coaches may be problematic as a fast start in extended duration events, such as these rowing races, with higher initial power outputs would lead to more rapid metabolic acidosis via the anaerobic energy system (Garland & Atkinson, 2008). Depending on the genotype and training, this strategy could inhibit anaerobic glycolysis and muscle contraction, and subsequently result in a reduction in maximal power output late in the race coupled with difficulty maintaining effective technique (Garland & Atkinson, 2008). The present results have practical implications in strength and conditioning for rowing. Genotype combinations may be reflective of individual metabolic capabilities while providing insight into the selection and/or development of elite rowing athletes (Ulucan, Sercan, & Biyikli, 2015).

5. Conclusion

Present observed the influence of ACTN3 genotypes on pacing strategies in 2000 m rowing events, these data can contribute to talent detection. Results indicated that rowing athletes with specific ACTN3 genotypes may adopt unique race pacing strategies, significant differences in pacing strategy preference were found for the non-elite openweight male rowing athletes having the RR genotype with a greater frequency preferring a moderate pacing strategy as compared to those preferring starting and finishing strategies. In addition, non-elite lightweight athletes having the RR genotype with a greater frequency preferring a starting strategy as compared to those preferring moderate and finishing strategies, while elite lightweight athletes having the RR genotype with a greater frequency preferring a starting strategy as compared to those preferring moderate and finishing strategies. Practitioners should note that these findings also highlight the essential need to incorporate pacing strategies into rowing race training. In addition, it is advised that rowers take full advantage of the knowledge of individual genotypes during training and competition to enhance performance.

Disclosure statement

No potential conflict of interest was reported by the authors.

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